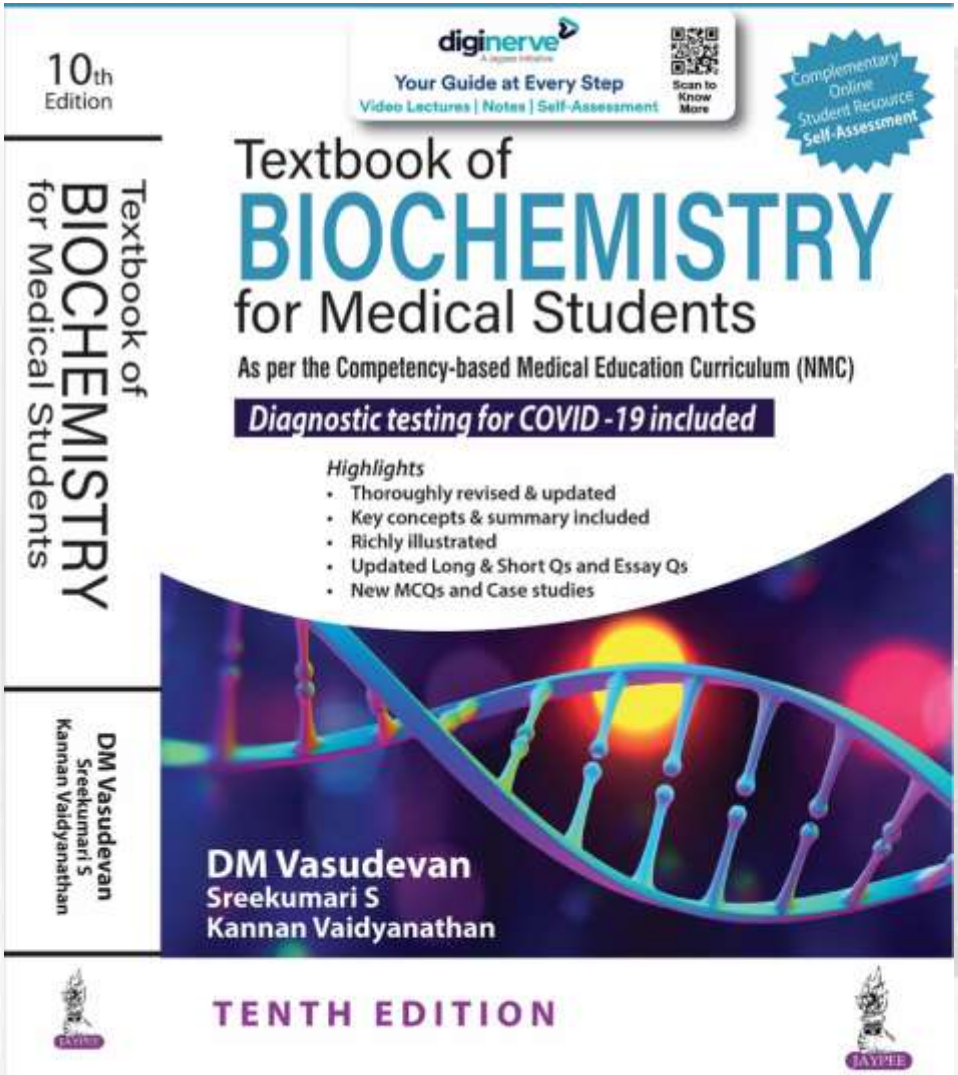


Chapter 2B: Transport Mechanisms

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

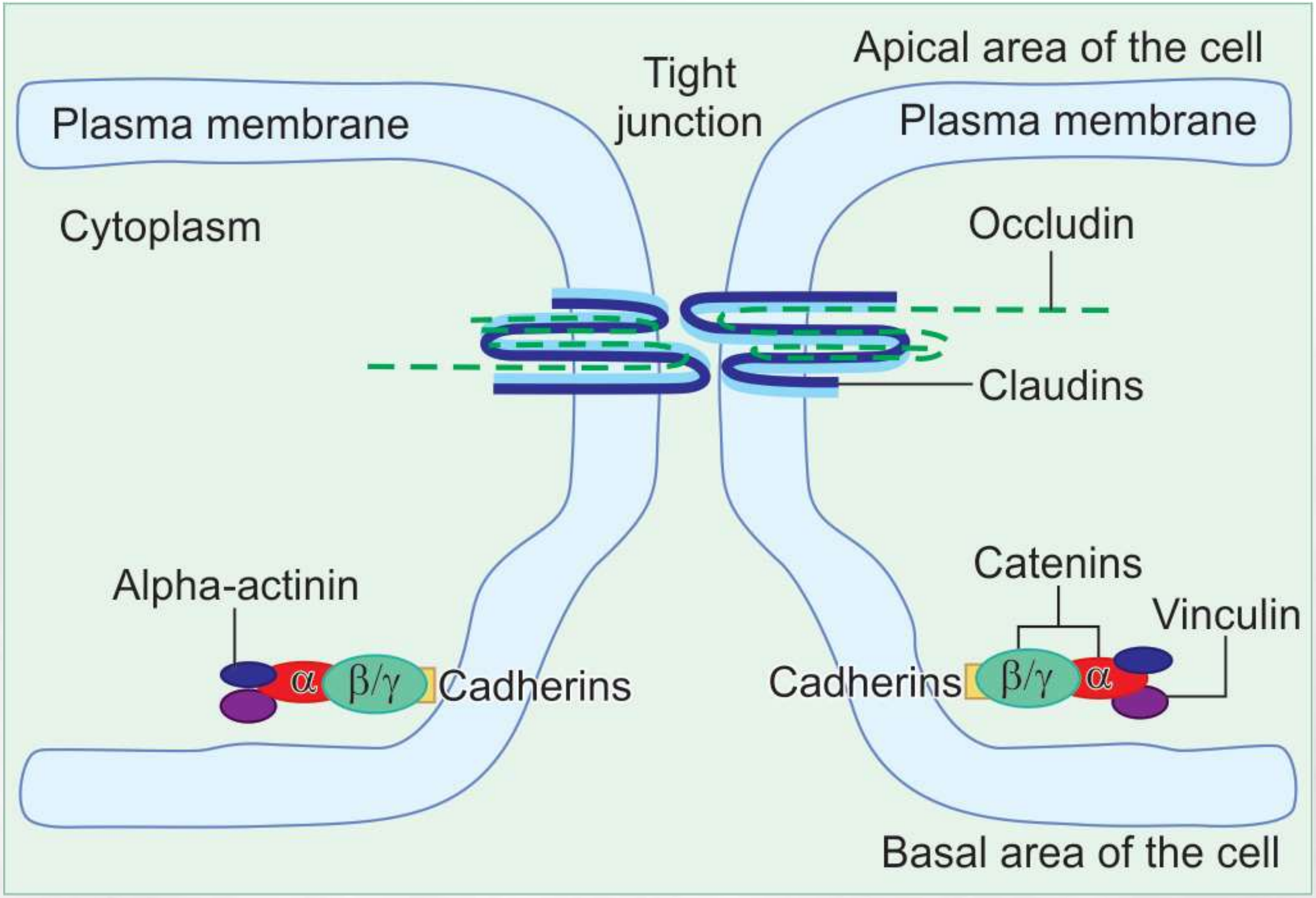
TENTH EDITION

Specialized Membrane Structures

- Tight junction – Allows communication between cells. Seals off sub-epithelial spaces of organs from lumen.
- Myelin sheath – Made of membrane of Shcwann cells. Increases speed of nerve conduction.
- Microvilli – Formed due to fluid nature of membrane. Involved in locomotion.
- Cytoskeleton and molecular motors

Tight Junction

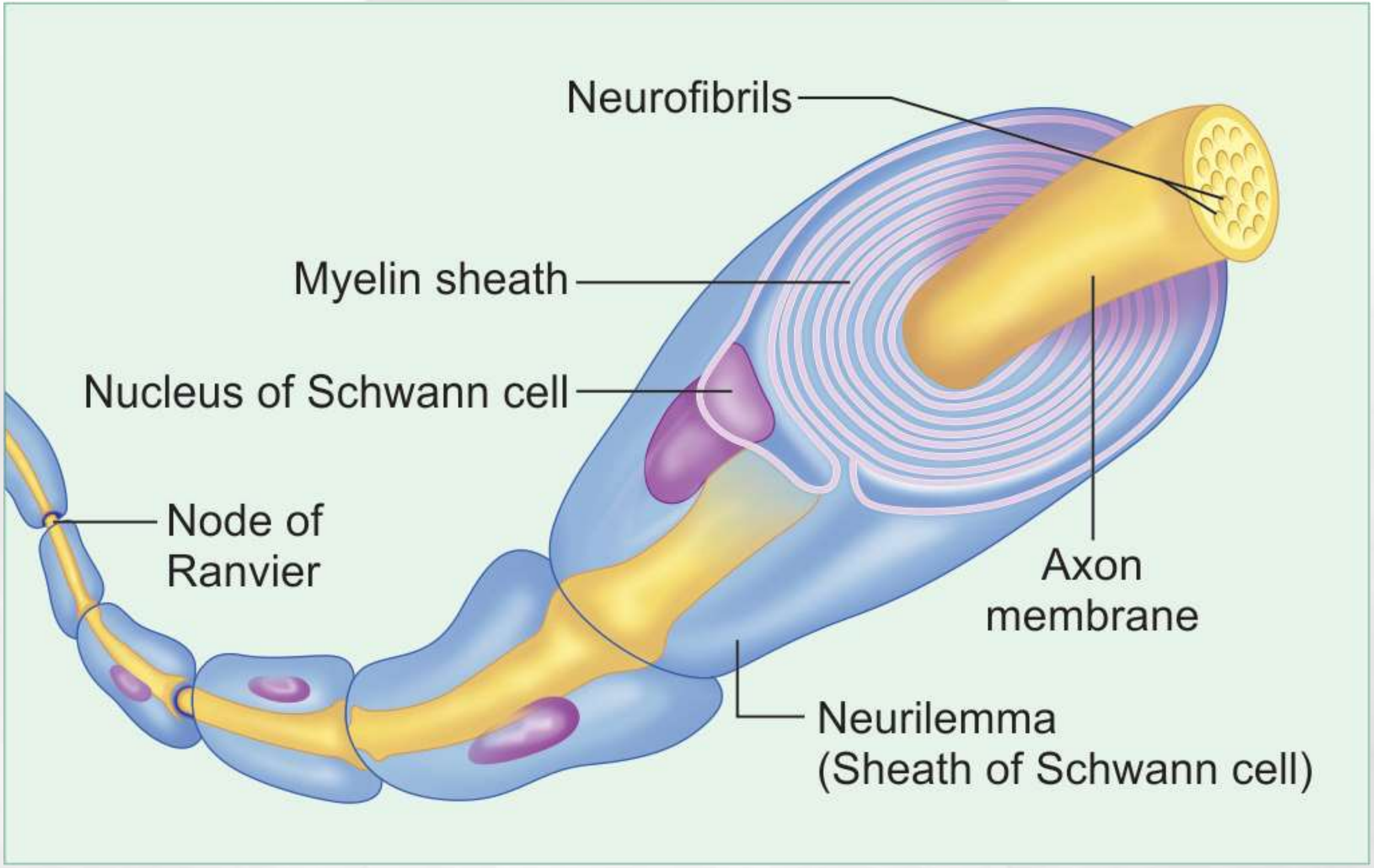
When two cells are in close approximation, in certain areas, instead of 4 layers, only 3 layers of plasma membranes are seen. This tight junction permits calcium and other small molecules to pass through from one cell to another, via narrow hydrophilic pores. They contain specialized proteins such as **occludin**, **claudins** and also adhesion molecules such as **Cadherins, Catenins and Vinculin**


Apical area of the cell
Tight junction
Plasma membrane
Plasma membrane
Cytoplasm
Occludin
Claudins
Alpha-actinin
Catenins
Vinculin
α
β/γ
Cadherins
Cadherins
β/γ
α
Basal area of the cell

Myelin Sheath

It is made up of the membrane of **Schwann cells** condensed and spiraled many times around the central axon. The cytoplasm of Schwann cells is squeezed to one side of the cell. Myelin is composed of sphingomyelin, cholesterol and cerebroside. Myelin sheaths thin out in certain regions, these are called **Nodes of Ranvier**. Due to this arrangement, the propagation of nerve impulse is wave-like; and the speed of propagation is also increased.

In multiple sclerosis, demyelination occurs at discrete areas, velocity of nerve impulse is reduced, leading to motor and sensory deficits.

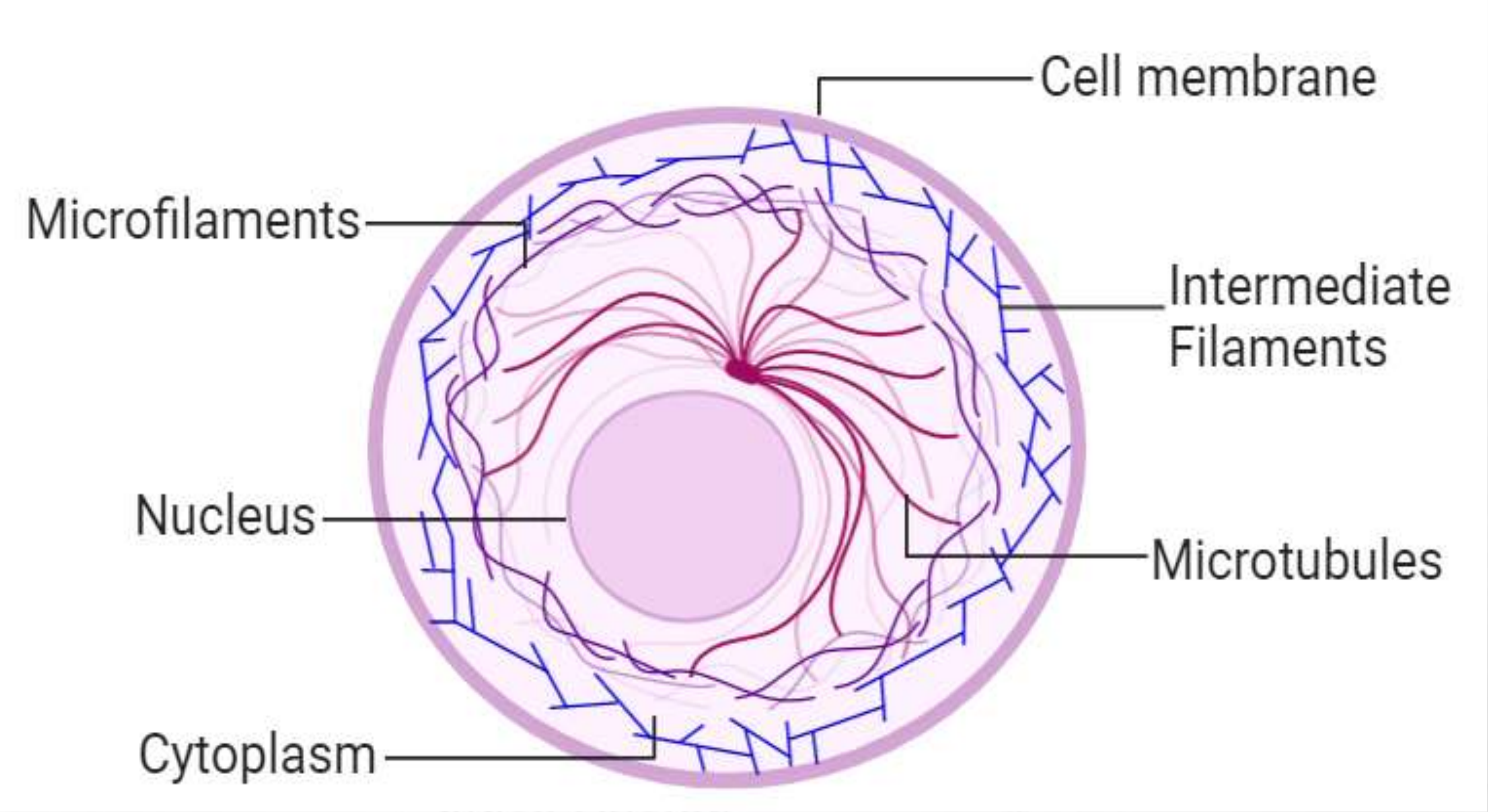


Neurofibrils
Myelin sheath
Nucleus of Schwann cell
Node of
Ranvier
Axon
membrane
Neurilemma
(Sheath of Schwann cell)

Cytoskeleton Organizes the Intracellular Contents

- Cellular morphology
- Intracellular transport
- Cell motility
- Cell division

Cytoskeleton



Cytoskeleton

The human body is supported by the skeletal system; similarly, the structure of a cell is maintained by the cytoskeleton present underneath the plasma membrane.

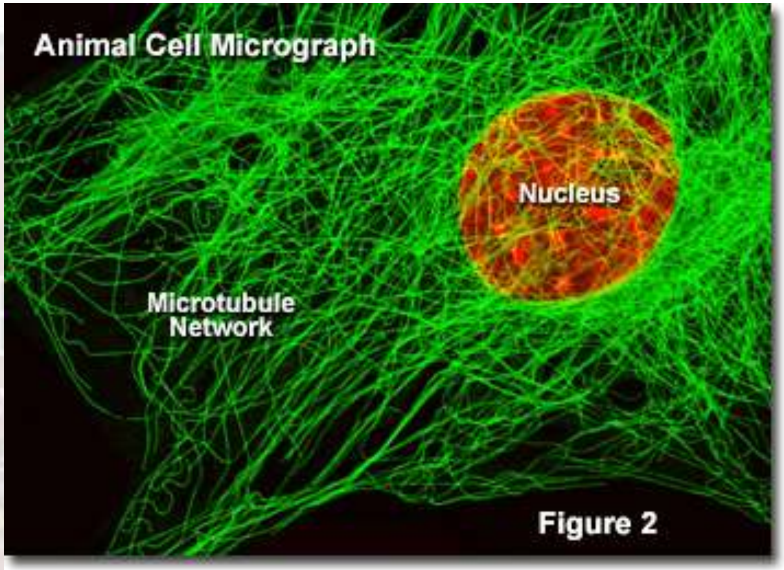
The cytoskeleton is responsible for the shape of the cell, its motility and chromosomal movements during cell division.

The cytoskeleton is made up of a network of microtubules and microfilaments, which contain the proteins spectrin and ankyrin.

Tubules consist of polymers of tubulin.

Microtubules

- Long unbranched slender cylindrical structures
- Diameter ~ 25nm



- Heterodimer of 'Tubulin'
- Associates with the inner face of plasma membrane

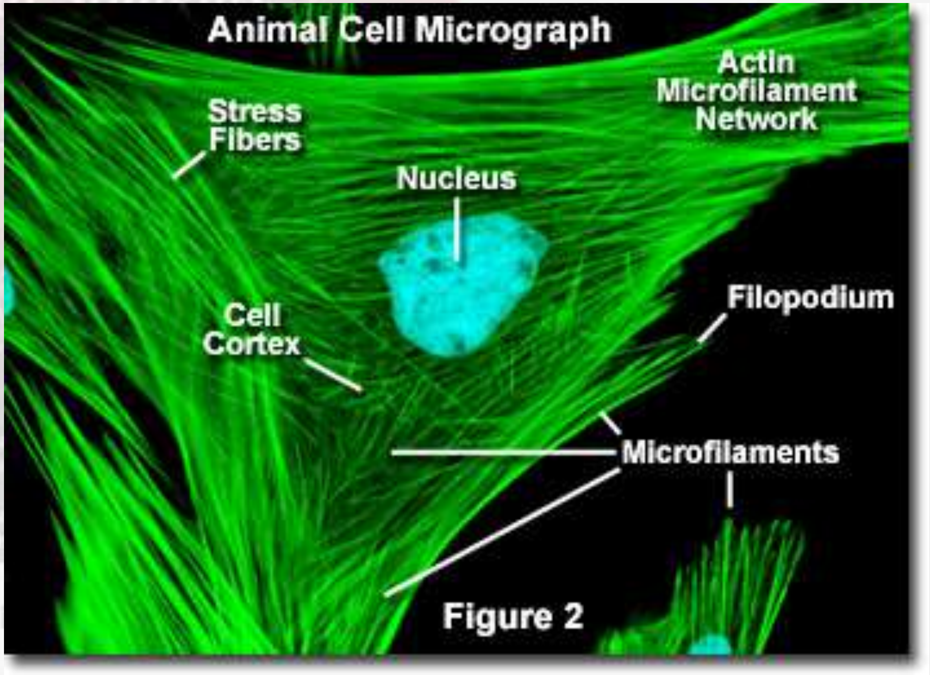
Microtubules

Functions:

- Assembly and disassembly of the spindle structures during mitosis.
- Provide internal structure to the cell and helps in maintenance of shape of eukaryotic cell.
- May be involved in trans membrane signals.

Microfilaments – Motor Units

- Slender cylindrical structures
- Made up of contractile proteins 'Actin'
- Linked to the inner surface of plasma membrane
- Internal cell motion.



Microtrabeculae

- Fragile tubes that form a transient network in cytosol
- Function not clearly defined
- Cytosolic enzymes are associated or clustered with these structures to form unstable multi enzyme complexes.

Transport Mechanisms

- Permeability of substances dependent on lipid solubility, and not on molecular size.
- Water soluble compounds are generally impermeable and require carrier mediated transport.
- One important function of membrane – Withhold unwanted molecules.
- Passive (simple and facilitated diffusion) and active transport mechanisms present.

Transport mechanisms are classified into

1. **Passive** transport,
    - (a) simple diffusion,
    - (b) facilitated diffusion and
    - (c) Ion channel (specialized carrier systems)
2. **Active** transport and
3. **Pumps** that can drive molecules against the gradient using energy.

Simple Diffusion

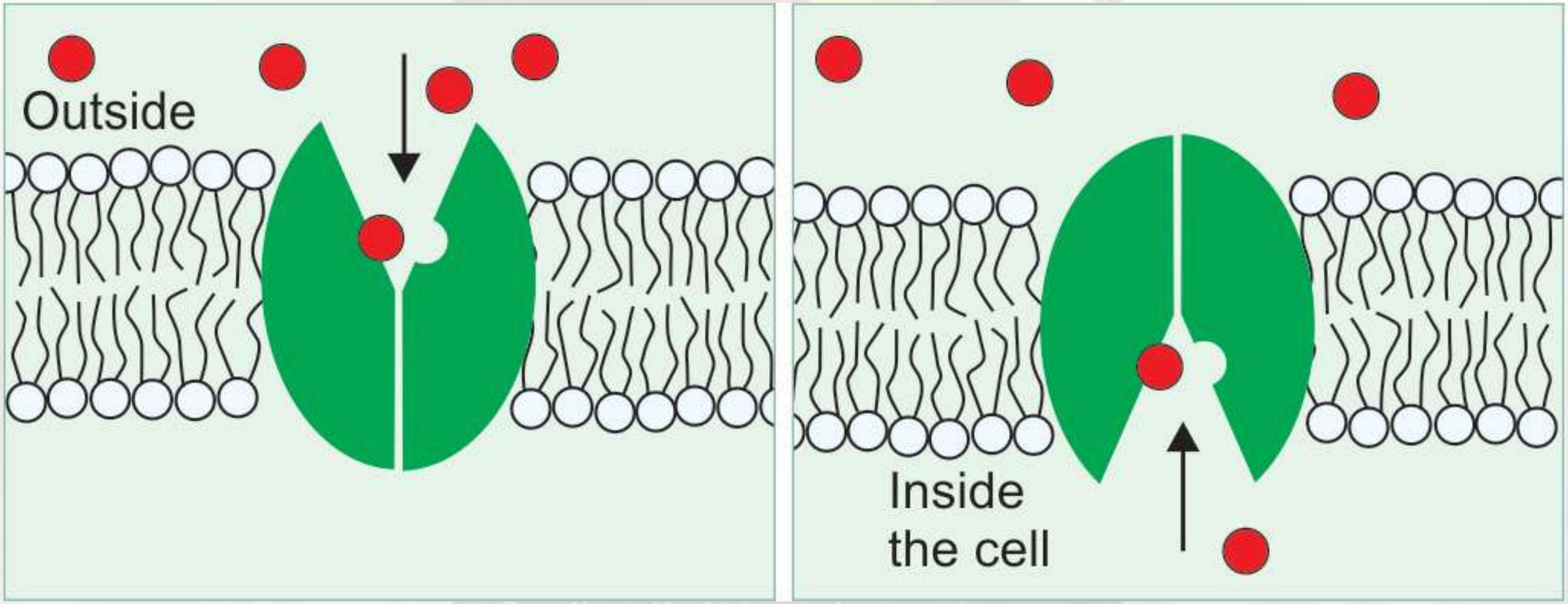
- Solutes and gases enter into cells passively.
- Driven by concentration gradient.
- Occurs from higher to lower concentration.
- Rate of entry proportional to solubility of solute in the hydrophobic core of membrane.
- Does not require energy.
- Very slow process.

Facilitated Diffusion

- Carrier mediated process.

1. Carrier mechanism could be saturated, similar to Vmax of enzymes.
2. Structurally similar solutes can competitively inhibit solute entry.
3. Can operate bi-directionally.
4. Does not require energy, but rate of transport is more than simple diffusion.
5. Dependent on concentration gradient.

Facilitated Diffusion



The carrier molecules exist in two conformations

- Carrier molecules can exist in two states – Ping and pong states.
- Pong state – Active sites exposed to outer side of membrane, solute binds. Binding leads to conformational changes.
- Ping state – Active sites face interior, releases solute molecules into the cell. Reverts proteins back to pong state.
- E.g. Glucose and amino acid transport.

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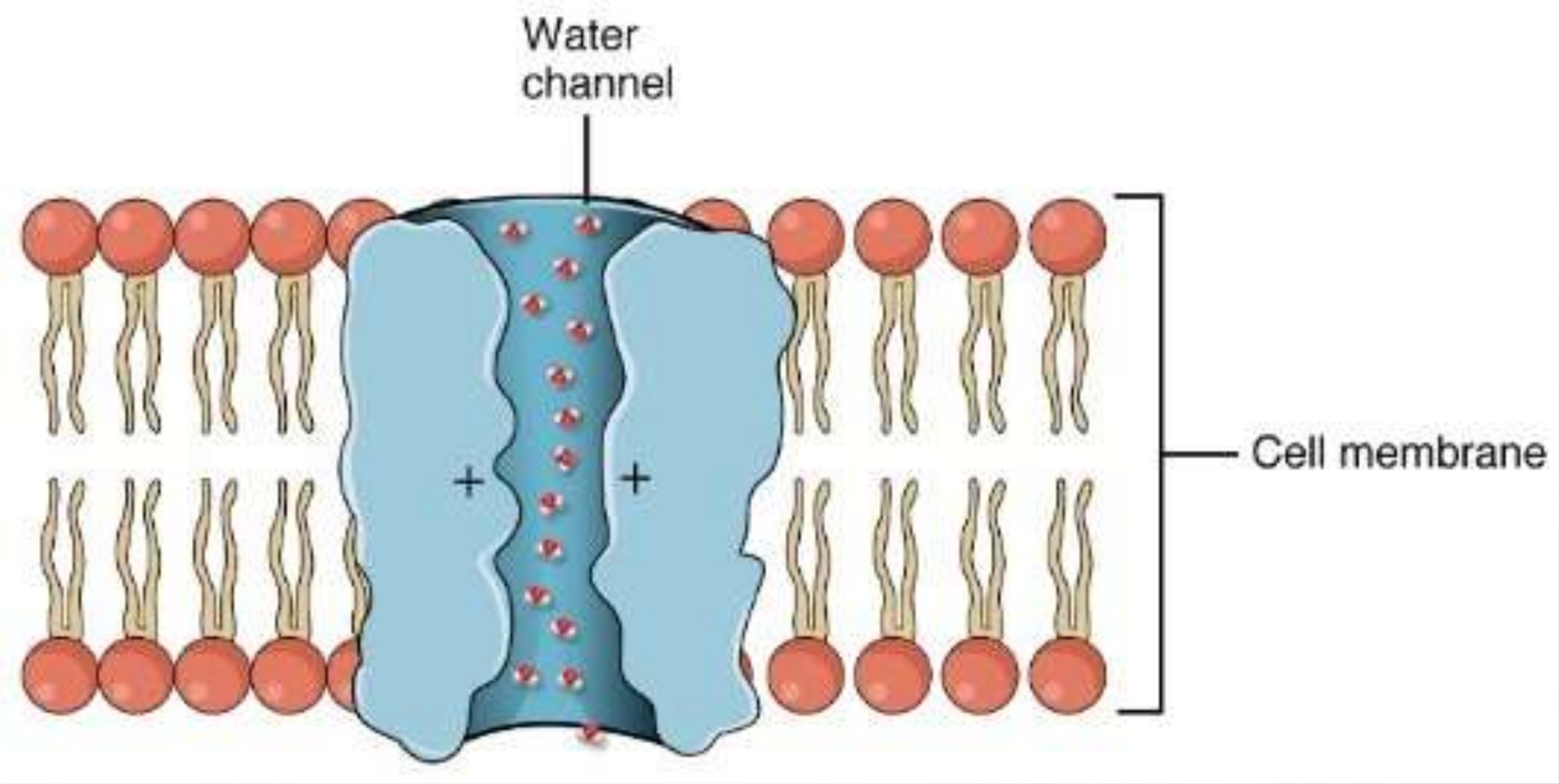
Aquaporins

They are water channels that serve as selective pores, through which water crosses the plasma membranes of cells. They form tetramers in the cell membrane and facilitate the transport of water. They control the water content of cells.

Diseases such as nephrogenic diabetes insipidus is due to impaired function of these channels.

Channelopathies are a group of disorders that result from abnormalities in the proteins forming the ion pores or channels. A few examples are cystic fibrosis (chloride channel), Liddle's syndrome (sodium channel) and periodic paralysis (potassium channel).

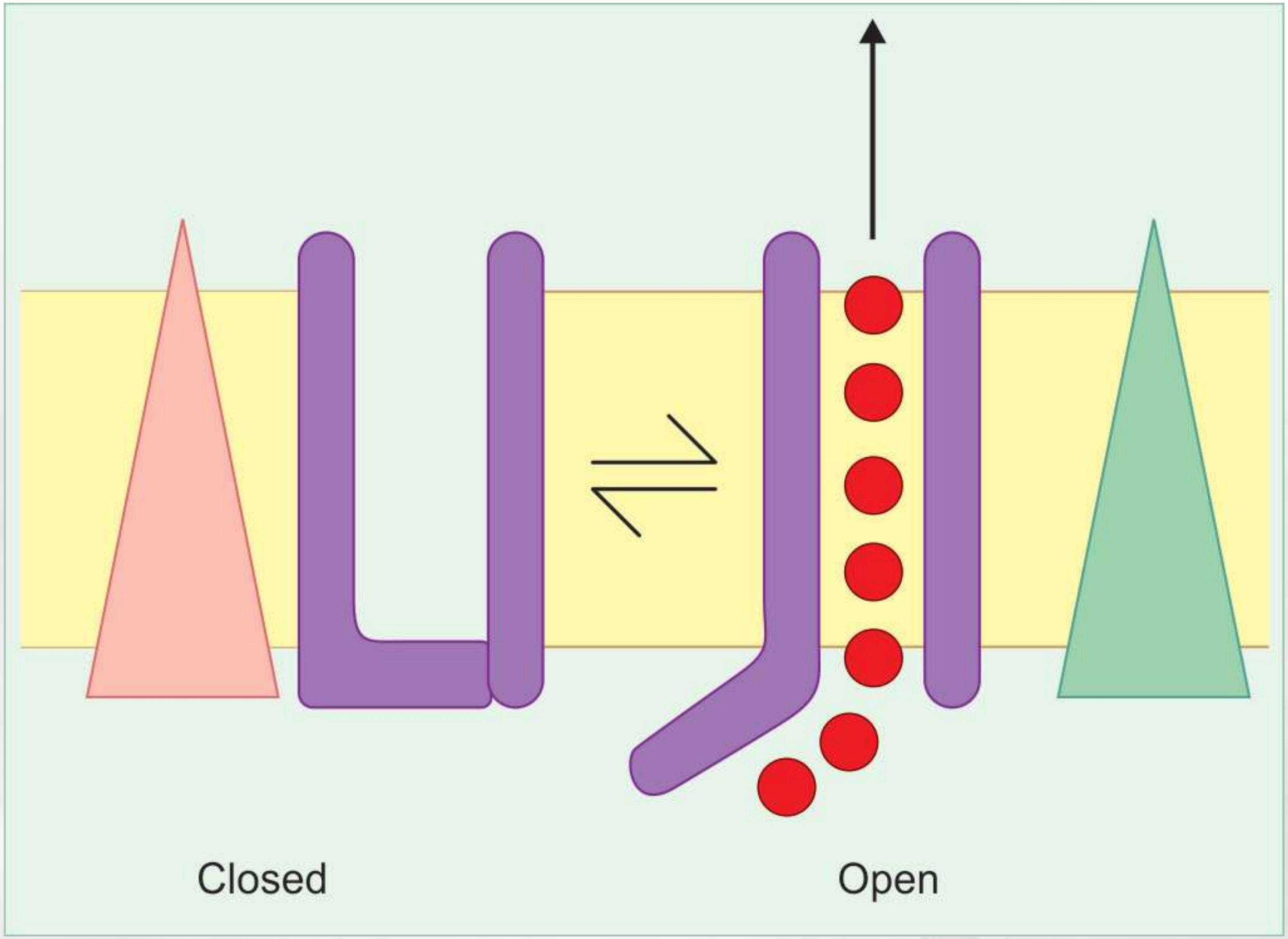
Water Channel or Aquaporin.



Ion Channels

- For quick transport of electrolytes like Ca, K, Na and Cl.
- Selective ion conductance pores.
- Span the membrane.
- 3 states – Open, closed and inactivated.
- Important for nerve conduction, synaptic transmission and secretion of biologically active substances.

Ion Channels

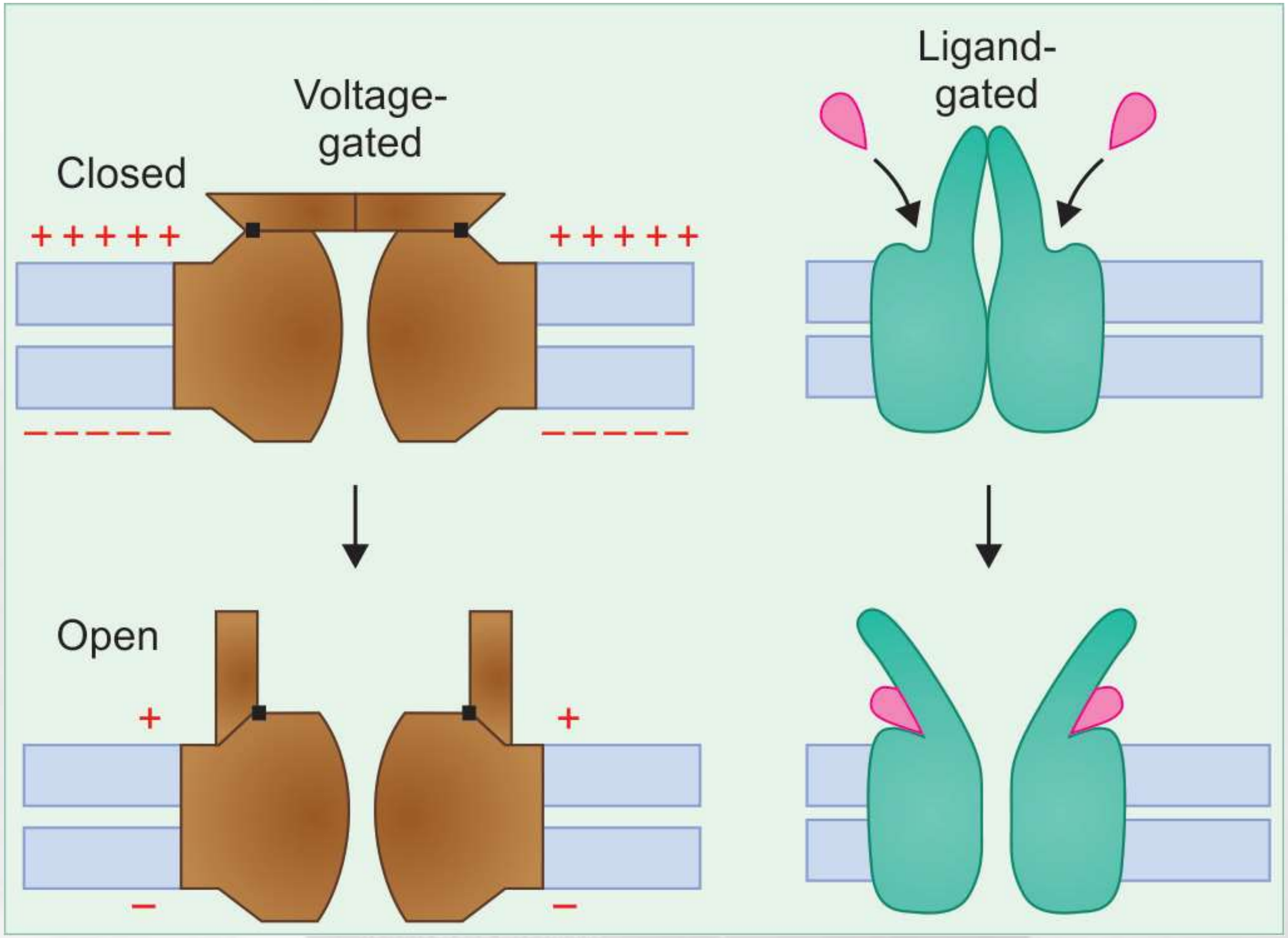


Salient Features of ion Channels

1. They are transmembrane proteins.
2. Selective transport for one particular ion.
3. Regulation of activity is done by voltage-gated, ligand-gated or mechanically gated processes.
4. Different channels are available for Na^+, K^+, Ca^{++} and $Cl^–$.
5. Transport through the channel is very quick.

Cation Conductive Channels

- Generally remains closed, but in response to stimulus, they open allowing rapid influx of ions down the gradient.
- Hence the name "gated".
- Based on nature of stimuli that trigger opening of gate, known as 'voltage gated' and 'ligand gated'.
- Voltage gated – Opened by membrane depolarisation.
- Ligand gated – Opened by binding of effectors.



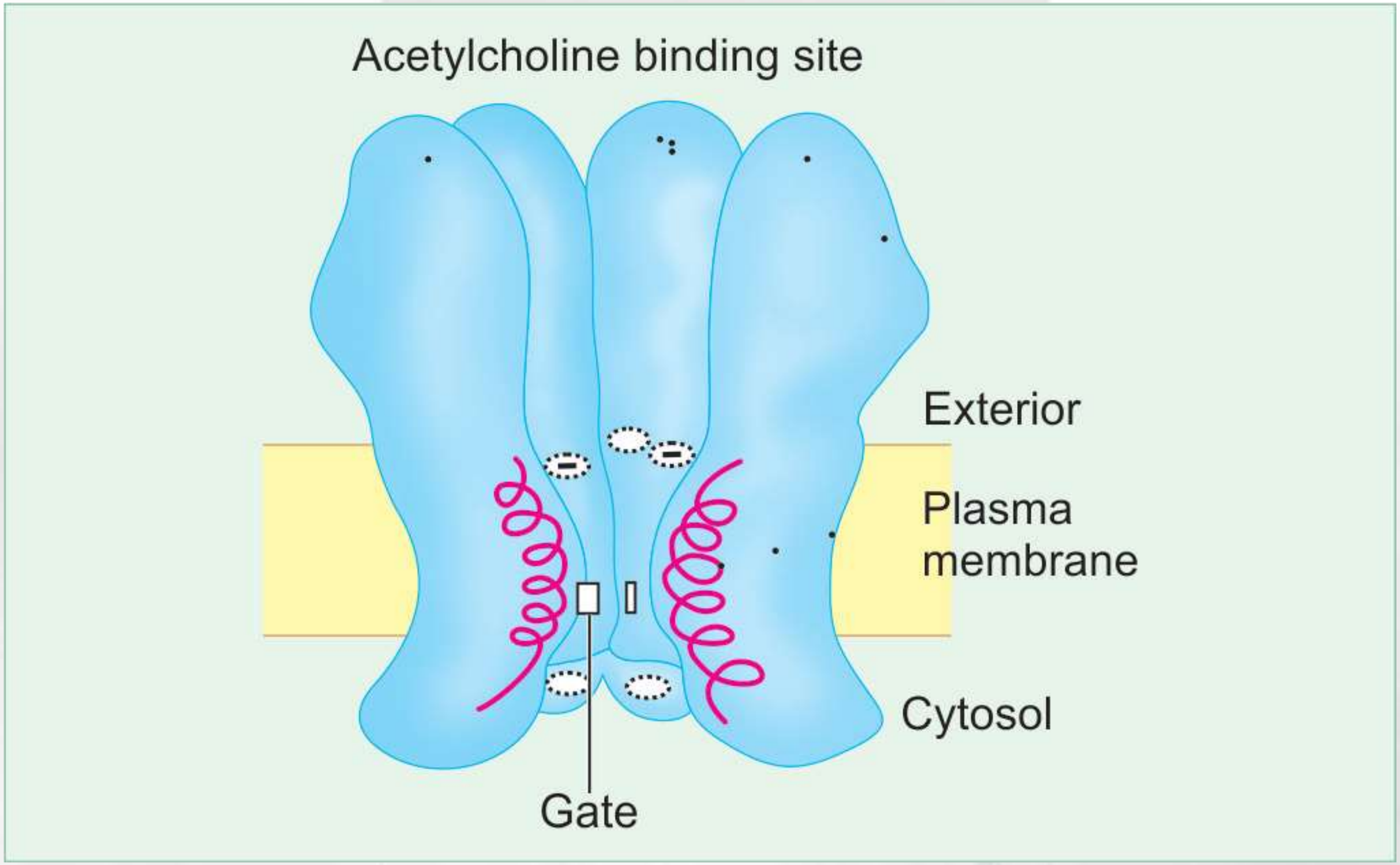
Left side, voltage-gated channels; on the right side, ligand gated channels; closed and open positions.

- Have 4-6 hydrophobic trans-membrane segments.
- Have the following properties –
  - Ion selectivity – Change of one amino acid can change sodium channel to potassium channel.
  - Voltage sensitivity
  - Inactivation

Ligand Gated Channels

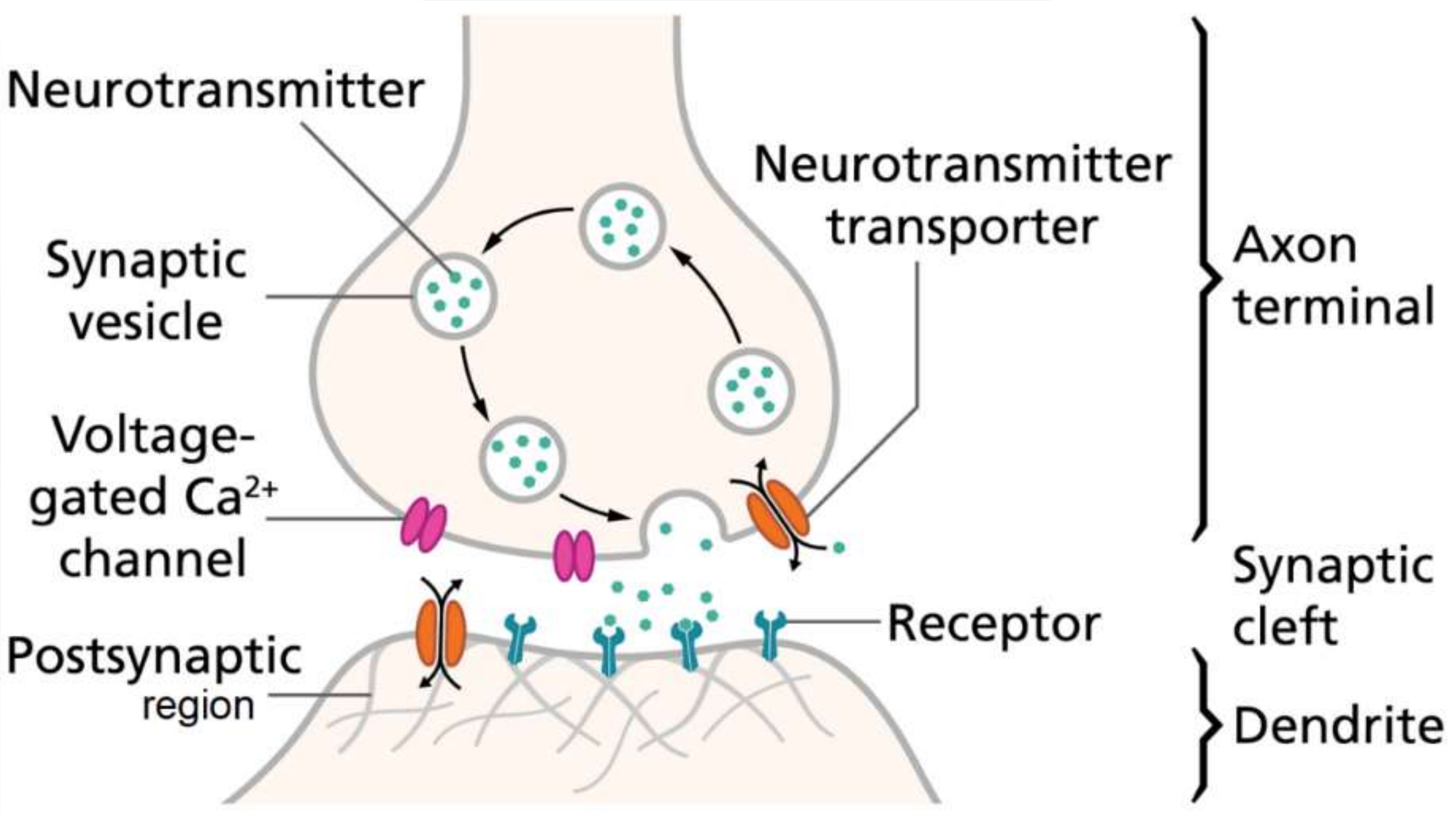
- Acetyl choline receptor
- Calcium channels
- Amelogenin

Acetyl Choline Receptor



Acetylcholine receptor is the best example for ligand-gated ion channel.

- It is present in postsynaptic membrane.
- It has 5 subunits, consisting of acetylcholine binding site and the ion channel.
- Acetylcholine released from the presynaptic region binds with the receptors on the postsynaptic region, which triggers
- Opening of the channel and influx of Na+. This generates an action potential in the postsynaptic nerve.


Neurotransmitter
Synaptic vesicle
Voltage-gated Ca^{2+} channel
Postsynaptic region
Neurotransmitter transporter
Receptor
Axon terminal
Synaptic cleft
Dendrite

Calcium channels:

In the muscle cells, under appropriate stimuli, calcium channels are opened in the sarcoplasmic reticulum membrane, leading to an elevated calcium level in the cytosol.

Calcium channel blockers are therefore widely used in the management of hypertension.

Amelogenin, a protein present in enamel of teeth has hydrophobic residues on the outside.

A 27 amino acid portion of amelogenin functions as a calcium channel.

Phosphorylation of a serine residue of the protein opens the calcium channel, through which calcium ions zoom through and are funneled to the mineralization front.

The amelogenin is used for the formation of calcium hydroxyapatite crystals.

Voltage Gated Channels

- Sodium channels (Voltage gated)
- Potassium channels (Voltage gated)
- Chloride channels

Voltage-gated Channels

Voltage-gated channels are opened by membrane depolarization. The channel is usually closed in the ground state. The membrane potential change (voltage difference) switches the ion channel to open, lasting less than 25 milliseconds.

In voltage-gated channels, the channels open or close in response to changes in membrane potential. They pass from closed through open to inactivated state on depolarization. Once in the inactivated state, a channel cannot re-open until it has been reprimed by repolarization of the membrane.

Voltage-gated Channels

Voltage-gated **sodium channels** and voltage-gated potassium channels are the common examples.

These are seen in nerve cells and are involved in the conduction of nerve impulses. Ion channels allow passage of molecules in accordance with the concentration gradient.

Ion pumps can transport molecules against the gradient.

Ionophores

- An ionophore is a molecule that reversibly binds ions.
- Many ionophores are lipid-soluble entities that transport ions across a cell membrane.
- They are membrane shuttles for specific ions. They transport antibiotics.
- Ionophores increase the permeability of membrane to ions by acting as channel formers. The two types of ionophores are; mobile ion carriers (e.g. **Valinomycin**) and channel formers (e.g. **Gramicidin**).

Clinical applications of channels

1. **Sodium channels:** Local anesthetics such as procaine act on sodium channels as blockers and on gating mechanisms. Point mutation in sodium channel leads to myotonia, characterized by increased muscle excitability and contractility.
2. In **Liddle's disease**, the sodium channels in the renal epithelium are mutated, resulting in excessive sodium reabsorption, water retention and elevated blood pressure.
3. **Potassium channel** mutations in **"Long QT syndrome"** leads to inherited cardiac arrythmia, where repolarization of the ventricle is delayed, resulting in prolonged QT intervals in ECG.

Clinical applications of channels

4. **Chloride channels:** The role of GABA and glycine as inhibitory neurotransmitters is attributed to their ability to open the chloride channels at the postsynaptic membranes.

5. **Cystic fibrosis:** It is due to certain mutations in the CFTR gene (cystic fibrosis transmembrane regulator protein), which is a chloride transporter.

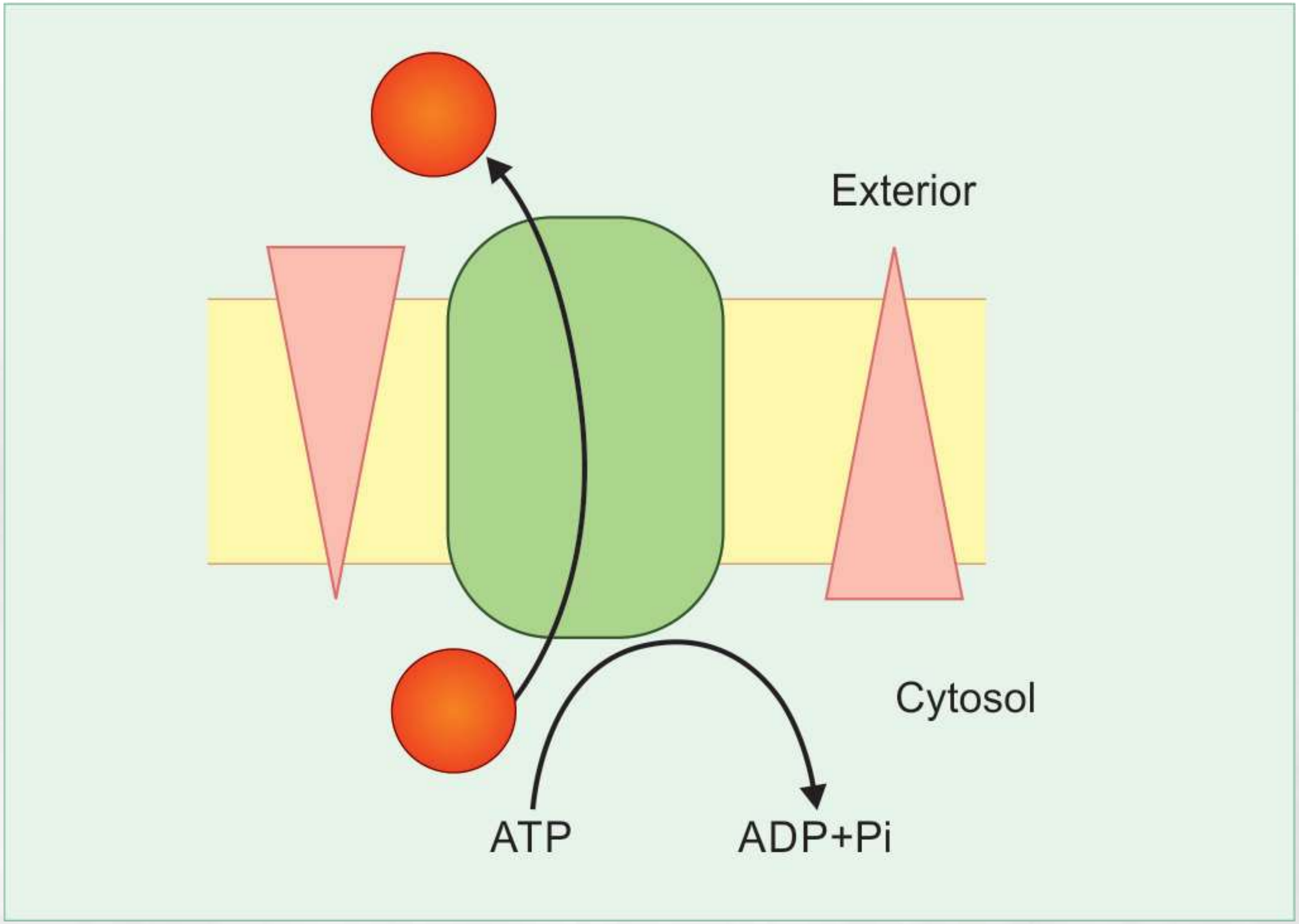
6. **Retina:** The excitation of retinal rods by a photon is by closing of cation specific channels resulting in hyperpolarization of the rod cell membrane. This light induced hyperpolarization is the major event in visual excitation.

Active Transport

- Salient features of active transport are –

1. Requires energy
2. Uni-directional
3. Requires specialized integral proteins called transporters
4. Saturated at higher concentration of solutes
5. Susceptible to inhibition

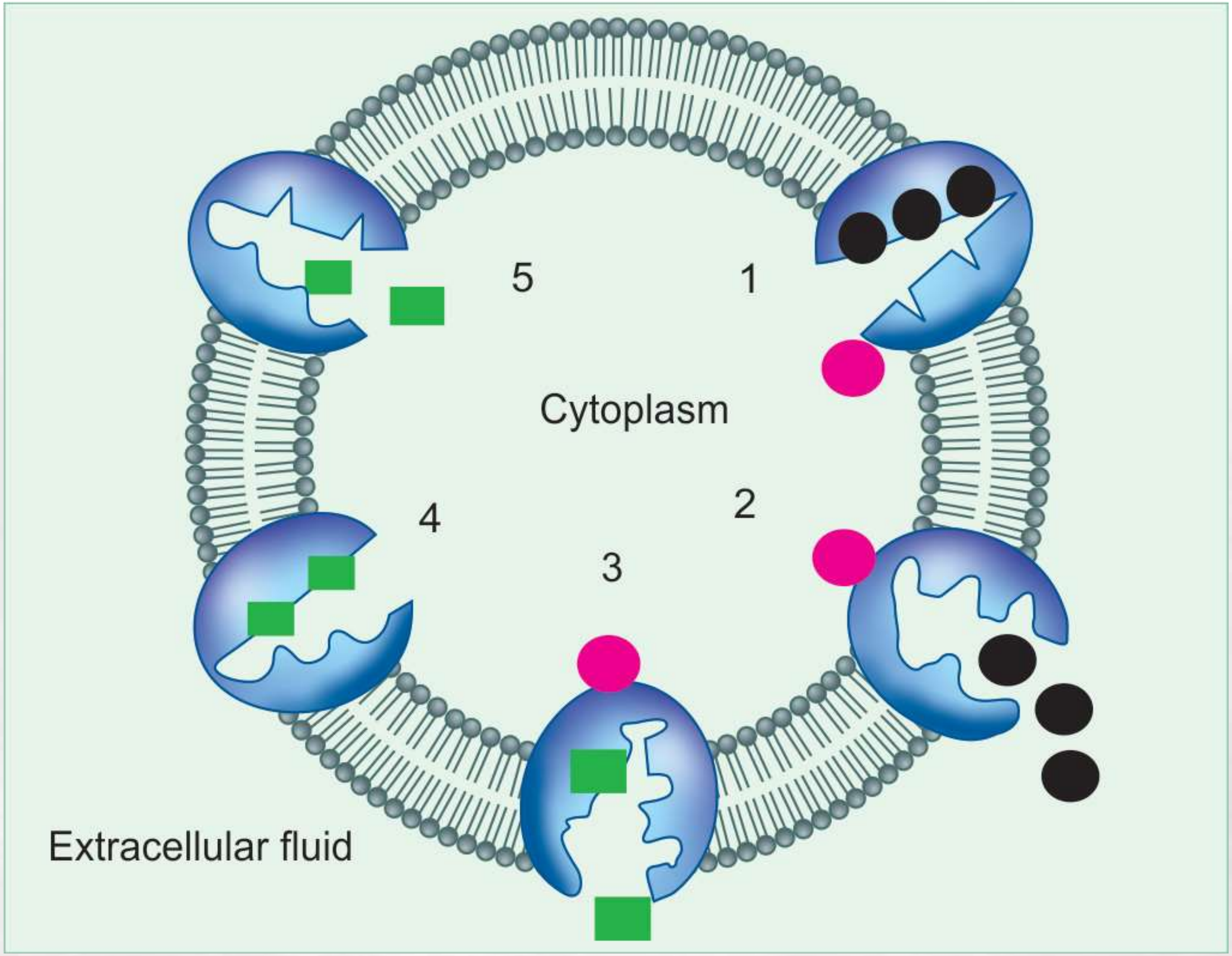
Active Transport



Sodium Pump

- Active transport
- Cell has low intra-cellular sodium and high potassium.
- Maintained by sodium-potassium activated ATPase or sodium pump.
- Integral membrane protein
- Binding sites for ATP and sodium inside and potassium outside.

- Hydrolysis of one ATP leads to expulsion of 3 Na and influx of 2 K ions.
- Sodium binds to pump, leading to phosphorylation by ATP and release of Na.
- K binding removes phosphate and reverts it back and K is released.
- Ion transport and ATP hydrolysis are tightly coupled.
- Digoxin and ouabain inhibits pump.


5
1
Cytoplasm
4
2
3
Extracellular fluid

Clinical applications of Sodium Pump

- Cardiotonic drugs like **digoxin** bind to the alpha subunit and act as competitive inhibitor of potassium ion binding to the pump.
- Inhibition of the pump leads to an increase in Na+
- level inside the cell and extrusion of Ca++ from the myocardial cell.
- This would enhance the contractility of the cardiac muscle and so improve the function of the heart. These drugs are not in common use now.
- Calcium channel blockers and potassium channel
- openers are now widely used in treatment of cardiac dysfunction.

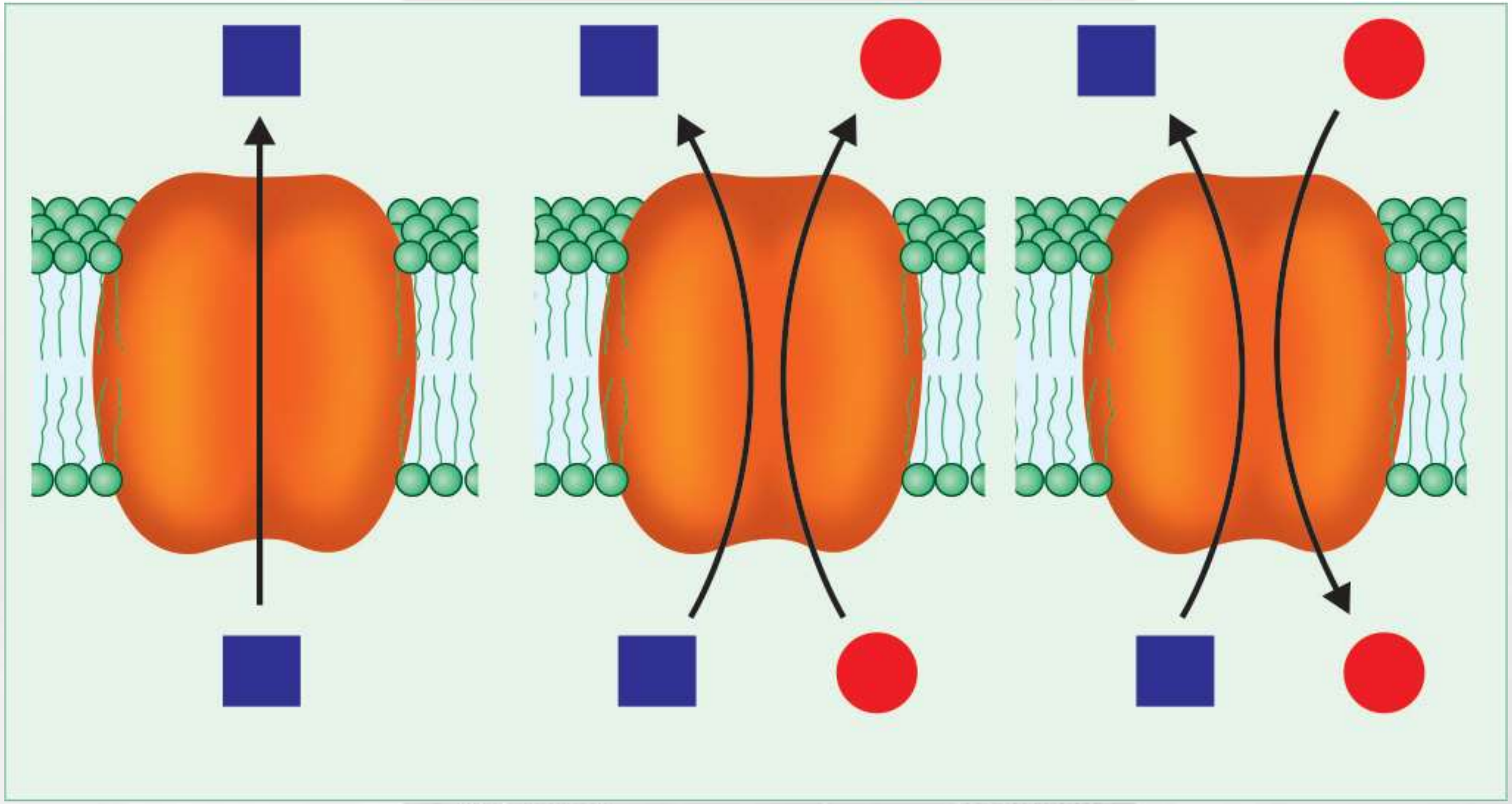
Calcium Pump

- An ATP dependent calcium pump also functions to regulate muscle contraction.
- A specialized membrane system called sarcoplasmic reticulum is found in skeletal muscles which regulates the Ca^{++} concentration around muscle fibers.
- In resting muscle the concentration of Ca^{++} around muscle fibers is low.
- But stimulation by a nerve impulse results in a sudden release of large amounts of Ca^{++}. This would trigger muscle contraction.
- For each ATP hydrolyzed, 2 Ca^{++} ions are transported.

Uniport, Symport, Antiport

- Uniport – Carries single solute across membrane. E.g. Calcium pump, glucose transporter.
- Co-transport – If transport depends on simultaneous or sequential transfer of another molecule.
- Active transport may be coupled with energy indirectly.

- Co-transport may be symport or antiport.
- Symport – If transporter carries two solutes in the same direction across the membrane. E.g. Sodium dependent glucose transport (inhibited by phlorizin), amino acid transport.
- Antiport – Carries two solutes or ions in opposite direction. E.g. Sodium pump, chloride – bicarbonate exchange in RBC.

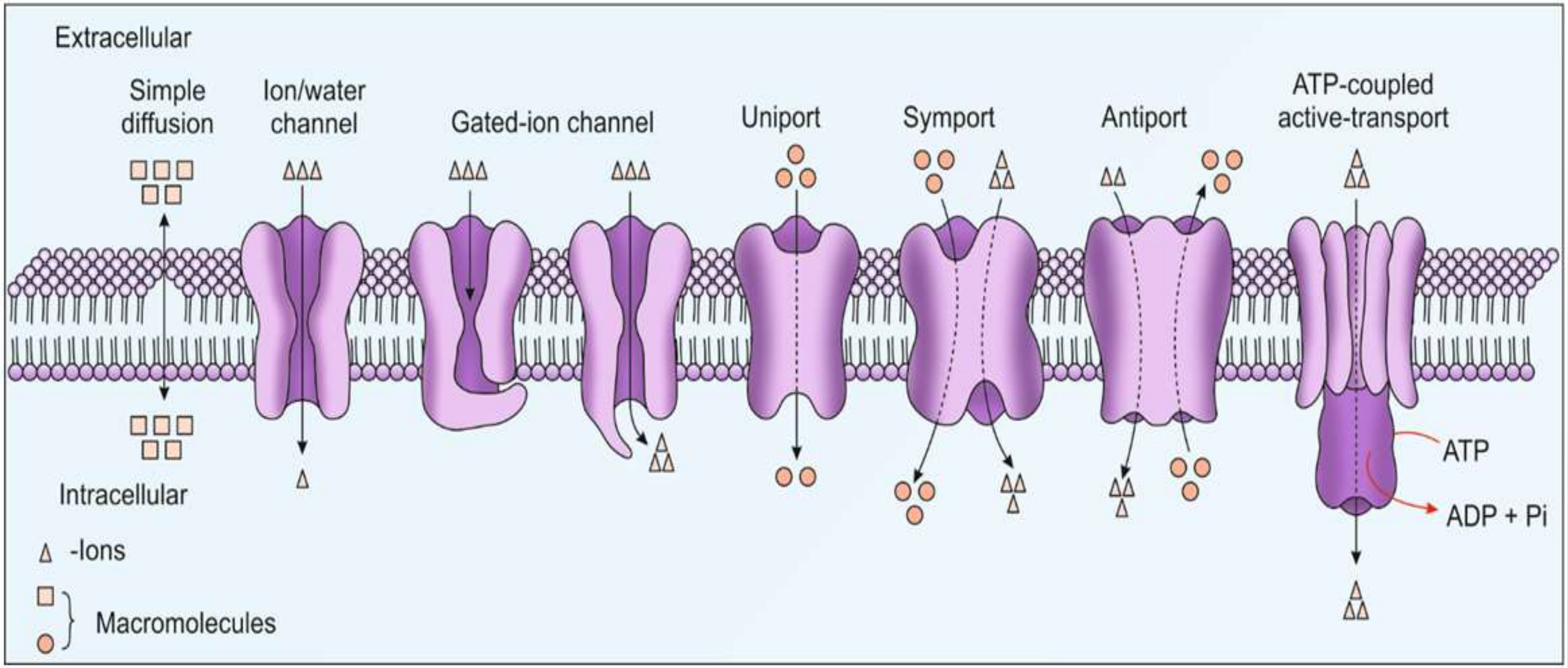


(A) Uniport; (B) Symport and (C) Antiport transport systems.

Types of Transport Mechanisms

	Carrier	Against gradient	Energy required	Examples
Simple diffusion	no	no	nil	water
Facilitated diffusion	yes	no	nil	glucose to RBCs
Primary active	yes	yes	directly	sodium pump
Secondary active	yes	yes	indirect	glucose to intestine
Ion channels	yes	no	no	sodium channel

Summary of different types of transport systems



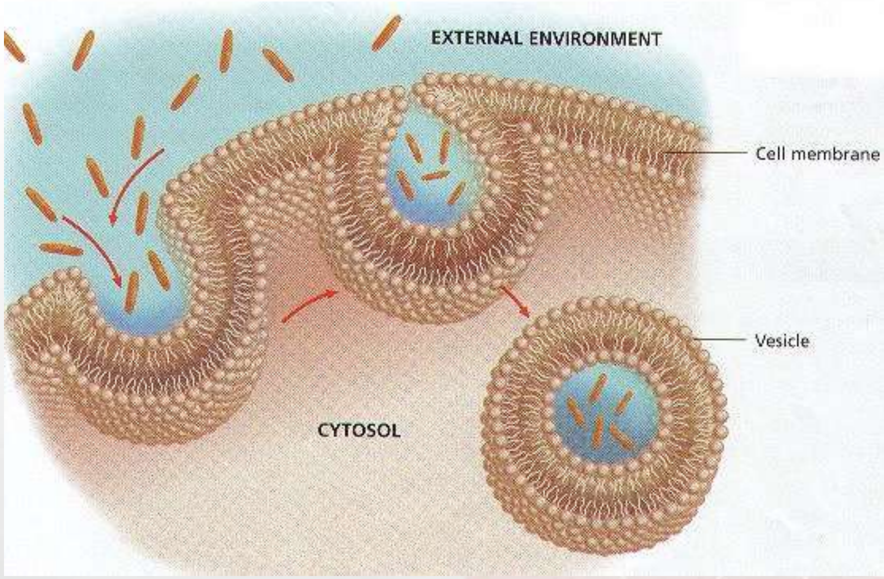
Diseases due to defective transport systems

Name of Disease	Solute transported	Defect
Hartnup's disease	Neutral amino acids like tryptophan	Defective absorption from the intestine and renal tubules.
Cystinuria	Cysteine and Cystine	Renal tubular reabsorption is defective
Vitamin D resistant rickets	Phosphate	Reabsorption of phosphate from renal tubules
Familial hyper-cholesterolemia	LDL particles	Receptor defect leading to defective uptake of LDL by cells; Receptor mediated endocytosis
Cystic fibrosis	Chloride ions	Chloride channel protein which is an ABC protein
Wilson’s disease	Copper	Copper transporting ATPase –copper toxicity
Congenital long QT syndrome	Potassium	Defective potassium channel causing a delay in repolarization of heart

Exocytosis, Endocytosis

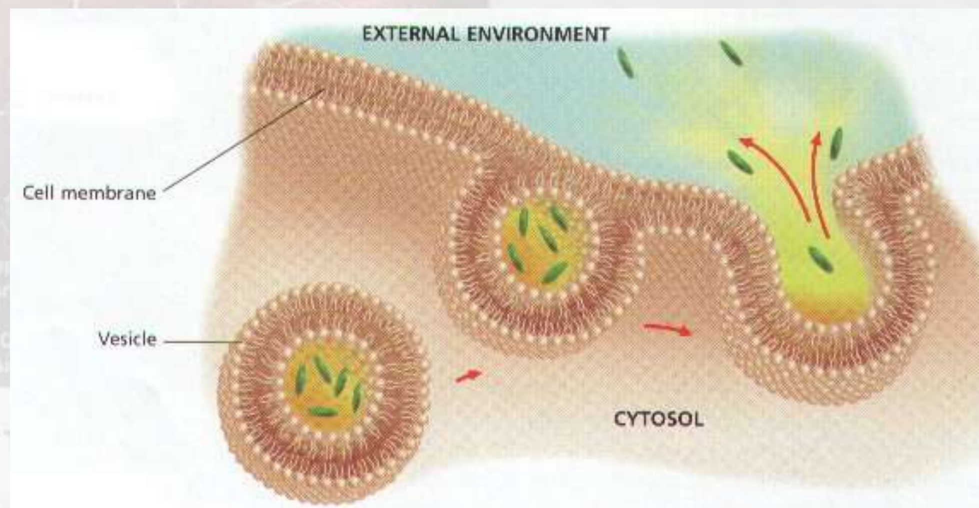
- Under appropriate stimuli, secretory vesicles or vacuoles moves and fuses with plasma membrane and contents of vesicle are removed – Exocytosis or reverse pinocytosis. E.g. Insulin release, enzymes from stomach.
- Internalization by opposite process – Requires energy – Called endocytosis.
- May be pinocytosis or phagocytosis.

Exocytosis, Endocytosis

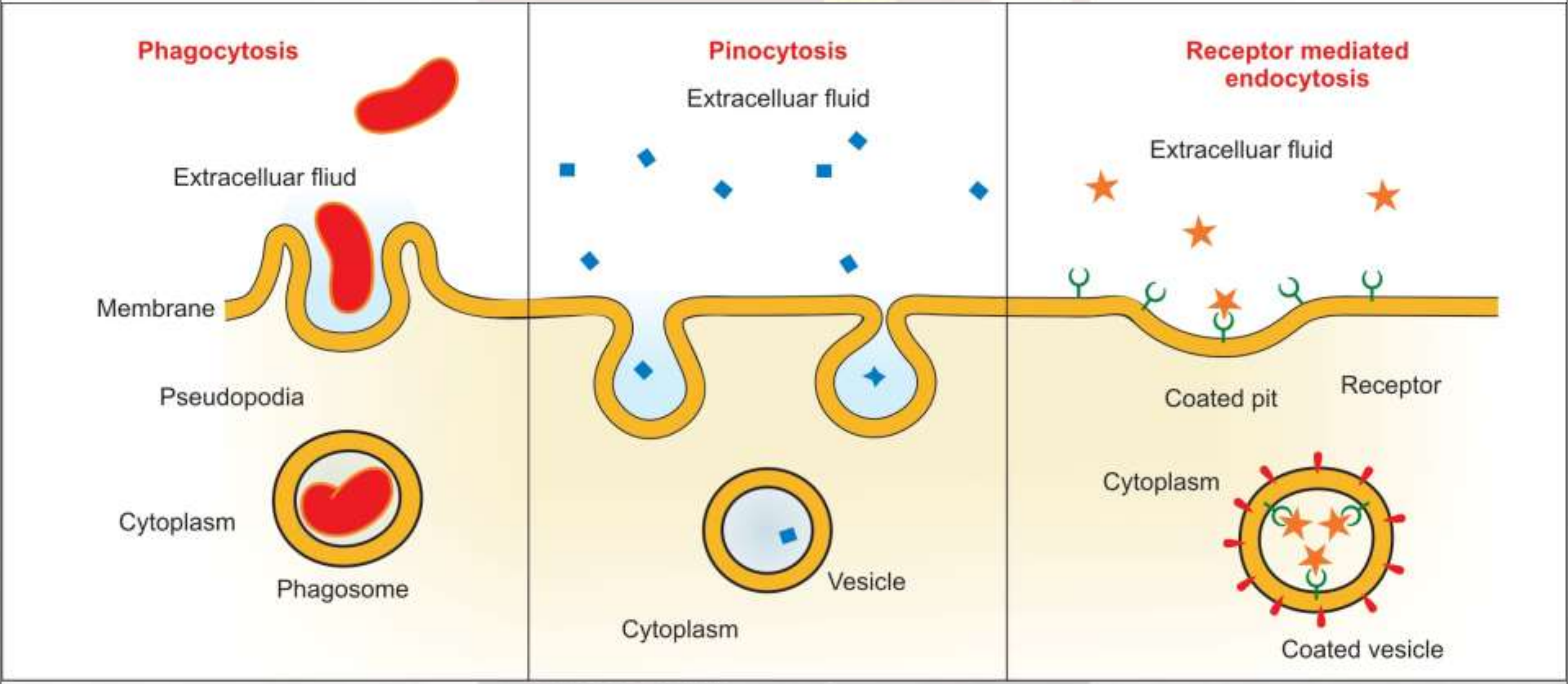


Endocytosis

Exocytosis



Different Types of Endocytosis



Phagocytosis, Pinocytosis

- Pinocytosis – Cell drinking.
  - Adsorptive or selective pinocytosis – Receptor mediated.
  - Clathrin mediated process.
  - E.g. LDL receptor, uptake of viruses
- Phagocytosis – Uptake of bacteria by macrophages and granulocytes.
  - Form phagosomes which fuse with lysosomes to form phagolysosomes.
  - Leads to ‘repiratory burst’.

Some important ABC proteins

ABC Protein	Functional role	Related disease
ABC A1	Transport of cholesterol from cells to HDL	Deficiency causes Tangier disease
ABC B4	p-glycoprotein extruding drugs from cells	Multidrug resistance
ABC C2 (MRP2)	Multidrug resistance associated protein-2	Dubin Johnson syndrome
ATP 7A	Copper transporting ATPase	Menke’s kinky hair syndrome
ATP 7B	Copper transporting ATPase	Wilson’s disease
CFTR	Chloride channel protein	Cystic fibrosis

Exosomes

Exosomes are cell-derived vesicles found in biological fluids from multiple cell types including stem cells, hematopoietic, epithelial, neural and cancer cells. Exosomes are 30-100 nm in size. They may be directly released from the plasma membrane and can be retrieved in many body fluids, such as blood, urine, saliva and cerebrospinal fluid and used as liquid biopsy specimens. Exosomes play key roles in coagulation, intercellular signaling, waste management and other processes. Exosomes are increasingly used as drug carriers. They carry molecules via vesicle trafficking and influence the immune system. They are potential biomarkers for cancer and neurodegenerative disorders. Flow cytometry and electron microscopy can be used for the detection of exosomes and can be purified by ultracentrifugation.