Module 3 lecture 1

Transport across cell membrane

All cells are generally separated from their surrounding environment by plasma membrane. In addition, the eukaryotic cells are compartmentalized by intracellular membranes that form the boundaries and internal structures of various organelles.Thesebiological membranes are semi-permeable in nature that is their permeability properties ensure that the specific molecules and ions readily enter the cell and the waste products leave the cell. These movements of solutes into the cell are mediated through the action of specific transport proteinsthat arepresent on the cell membrane. Such proteins are therefore required for movements of ions, such as Na^+, K^+, $Ca2^+$, and Cl^-, as well as metabolites such as pyruvate, amino acids, sugars, and nucleotides, and even water. Transport proteins are also responsible for biological electrochemical phenomena such as neurotransmission.

Cell membranearchitecture in transport across cell membrane:

The cell membrane plays an important role in transport of molecules. Because it acts as a semi-permeable barrier, allowing specific molecules to cross while fencing the majority of organically produced chemicals inside the cell. Electron microscopic examinations of cell membranes reveal the development of the lipid bilayer model (fluid-mosaic model). The model consists ofphospholipid, which has a polar (hydrophilic) head and two non-polar (hydrophobic) tails. These phospholipids are aligned tail to tail so the non-polar areas form a hydrophobic region between the hydrophilic heads on the inner and outer surfaces of the membrane.

Permeability of molecules across phospholipid bilayer:

Most of the molecule will diffuse across a protein-free lipid bilayer down its concentration gradient, if provided enough time. The diffusion rate is the function of the size of the molecule and its relative solubility in oil. In general, the smaller the molecule and the more soluble in oil (the more hydrophobic or non-polar), the more rapidly it will diffuse across a cell membrane. Small non-polar molecules, such as O_2 and CO_2, readily

dissolve in cell membrane and therefore diffuse rapidly across them whereas small uncharged polarmolecules, such as water or urea, also diffuse across a bilayer, but much more slowly but ethanol diffuses readily. Conclusively it can be said that lipid bilayers are highly impermeable to charged molecules (ions) by considering its size also because the charge and high degree of hydration of such molecules prevents them from entering the hydrocarbon phase of the bilayer. Thus, these bilayers are 10^9 times more permeable to water than to even such small ions as Na^+ or K^+(**M. Lodish et al., 2003**).

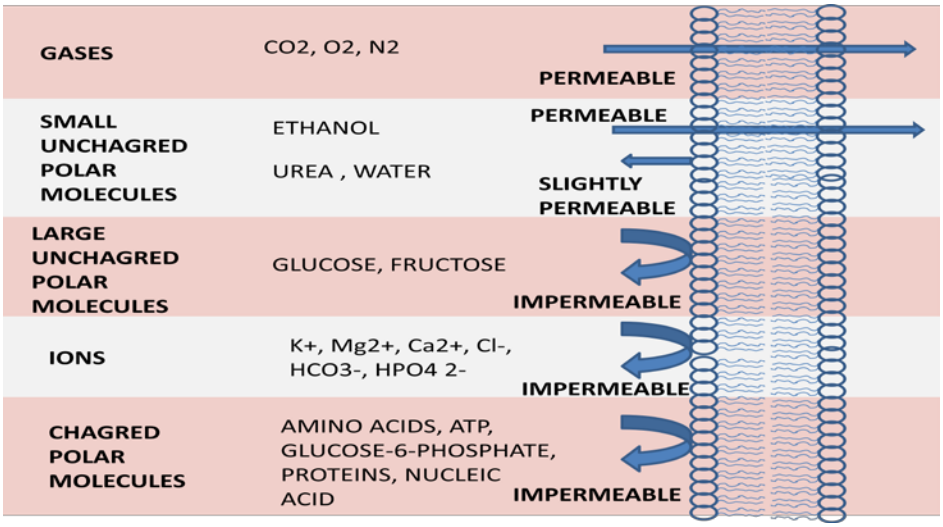

Figure 1:Relative permeability of a pure phospholipid bilayer to various molecules. A bilayer is permeable to small hydrophobic molecules and small uncharged polar molecules, slightly permeable to water and urea, and essentially impermeable to ions and to large polar molecules.

Thermodynamics of transport :

The diffusion of a substance A, across the two sides of a membrane thermodynamically resembles a chemical equilibration.

A(*out*) → A(*in*)

In the following sections, the free energy of a soluteA, varies with its concentration:

$$\overline{G}_A - \overline{G}_A^{o\prime} = RT \ln[A]$$

$$\bar{G}_A = \bar{G}_A^{o,} = RTin\,(A)$$

where

$G_A$is the chemical potential(partial molar free energy) of A (the bar indicates quantity per mole)

$G°`_A$ is the chemical potential of its standard state.

Thus, a difference arises in the concentrations of the substance on two sides of a membrane and generates a chemical potential difference:

$$\Delta\bar{G}_A = \bar{G}_A(in) - \bar{G}_A(out) = RTin\left(\frac{[A]_{in}}{[A]_{out}}\right)$$

If the concentration of A outside the membrane is greater than that inside, ΔG_A for the transfer of A from outside to inside will be negative and the spontaneous net flow of A will be inward. Conversely, if [A] is greater inside than outside, ΔG_A is positive and an inward net flow of A can occur only if an exergonic process, such as ATP hydrolysis, is coupled to it to make the overall free energy change.

The transmembrane movement of ions also depends in charge differences across the membrane, thereby generating an electrical potential difference which is given by:

ΔA=A(in) – A(out),

whereΔAis termed the membrane potential.Consequently, if A is ionic, must be amended to include the electricalwork required to transfer a mole of A across the membrane from outside to inside:

$$\Delta\bar{G}_A = RTin\left(\frac{[A]_{in}}{[A]_{out}}\right) + Z_A\,°\mathrm{F}\,\Delta A$$

$$\Delta\overline{G_A} = RT\ln\left(\frac{[A]_{in}}{[A]_{out}}\right) + Z_A\mathcal{F}\Delta\Psi$$

where

Z_A is the ionic charge of A

F, the Faraday constant, is the charge of a mole of electrons (96,485 C /mol; C is the symbol for coulomb)

G_A is now termed the electrochemical potentialof A.

The membrane potentials of living cells are commonly as high as 100 mV (note that 1 V = 1 J /C).

Types of transport process:

Two types of transport processoccuracross the membrane.

1. Non-mediated transport
2. Mediated transport

Non-mediated transport occurs through the simple diffusion process andthe driving force for the transport of a substance through a medium depends on its chemical potential gradient. Whereasmediated transportrequires specific carrier proteins. Thus, the substance diffuses in the direction that eliminates its concentration gradient; at a rate proportional to the magnitude of this gradient and also depends on its solubility in the membrane's non-polar core. Mediated transport is classified into two categories depending on the thermodynamics of the system:

1. Passive-mediated transport, or **facilitated diffusion:**In this typeof process a specific molecule flows from high concentration to low concentration.

2. Active transport: In this typeof process a specific molecule is transported from low concentration to high concentration, that is, against its concentration gradient. Such an endergonic process must be coupled to a sufficiently exergonic process to make it favorable (ΔG <0).

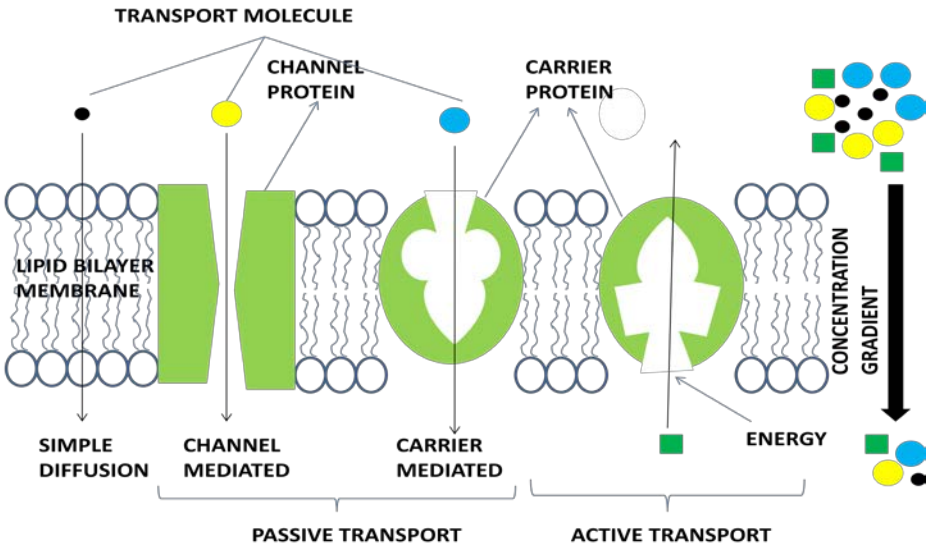

Figure 2: Mediated transport. (A) Passive transport and (B) Active transport

Passive mediated transport:

Substances that are too large or polar diffuse across the lipid bilayer on their own through membrane proteins called carriers, permeases, channels and transporters. Unlike active transport, this process does not involve chemical energy. So the passive mediated transport is totally dependent upon the permeability nature of cell membrane, which in turn, is function of organization and characteristics of membrane lipids and proteins.

Types of passive transport:

1. **Diffusion:**

The process of the net movement of solutes from a region of high concentration to a region of low concentration is known as diffusion. The differences of concentration between the two regions are termed as concentration gradient and the diffusion continues till the gradient has been vanished. Diffusion occurs down the concentration gradient.

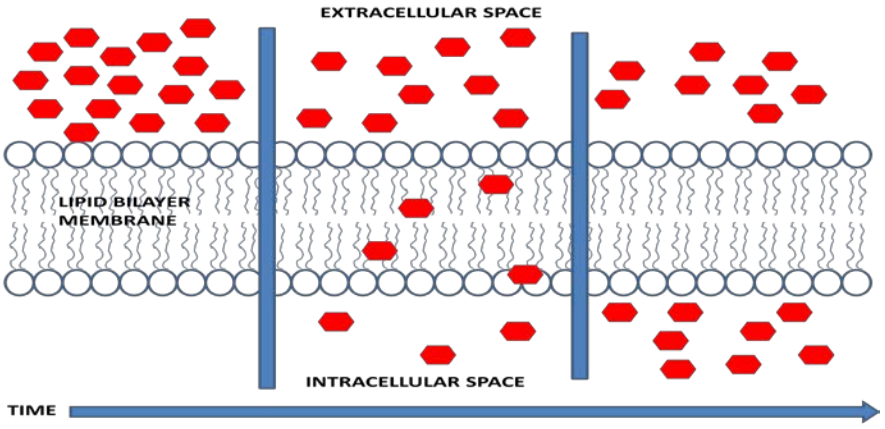

Figure 3: Diffusion.Extracellular space contains high concentration of solutes than intracellular space and hence the solutes move from extracellular space to intracellular space till there is no concentration gradient between the spaces.

2. Facilitated diffusion :

The process of the movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane is known as facilitated diffusion or called carrier-mediated diffusion. Many large molecules, such as glucose, are insoluble in lipids and too large to fit into the porins, therefore, it will bind with its specific carrier proteins, and the complex will then be bonded to a receptor site and moved through the cellular membrane.

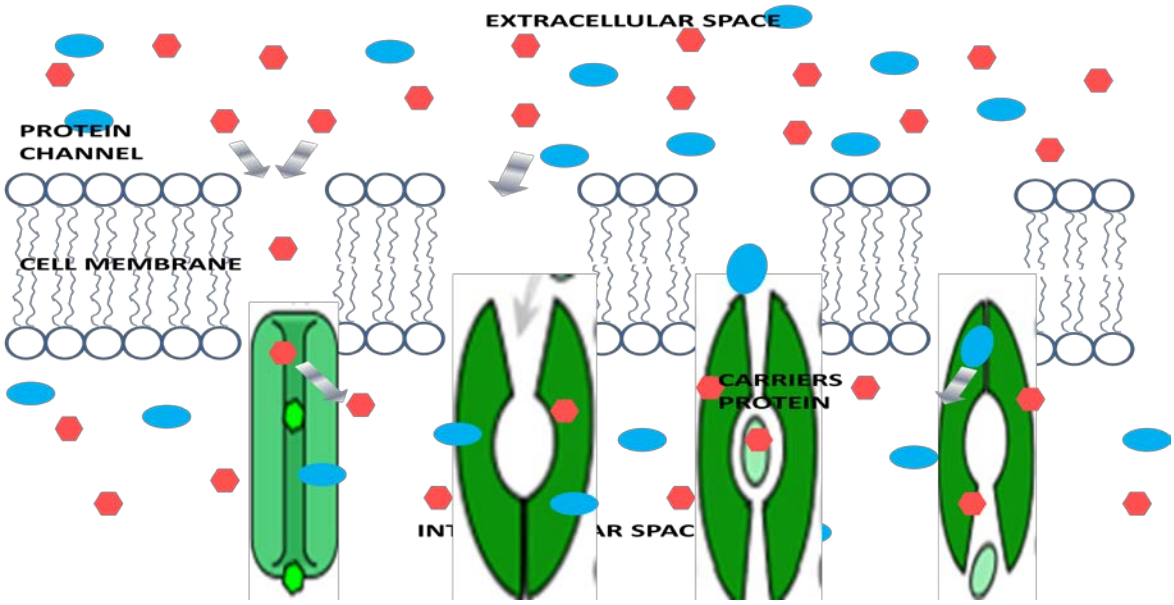

Figure 4: Facilitated transport.Movement of the solutes from extracellular space to intracellular space via carrier proteins and down its concentration gradient.

3. Filtration:

Filtration is the process of the movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the system. Depending on the size of the membrane pores, only solutes of a certain size may pass through it. The membrane pores of the Bowman's capsule in the kidneys are very small, and only albumins (smallest of the proteins)can filter through. On the other hand, the membrane pores of liver cells are extremely large, to allow a variety of solutes to pass through and be metabolized.

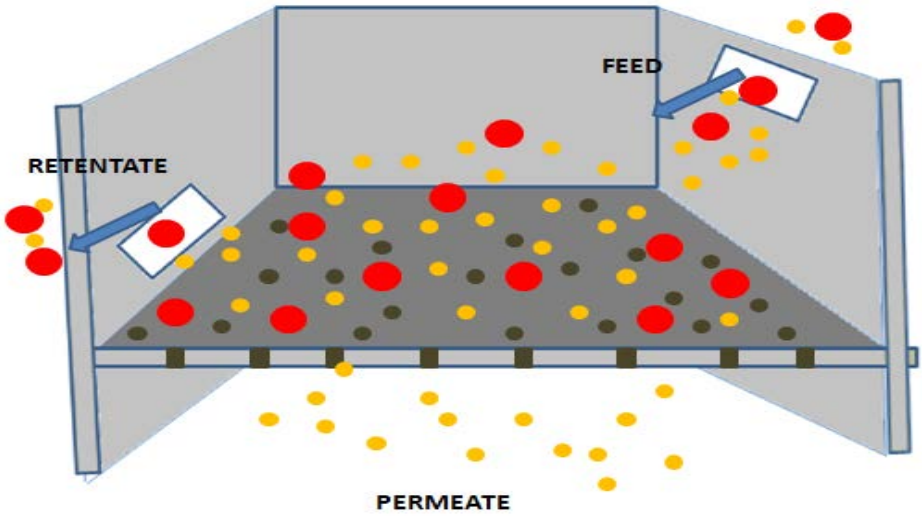

Figure 5: Filtration

4. Osmosis:

Osmosis is the type of diffusion of water molecules across a semi- permeable membrane, from a solution of high water potential to a region of low water potential. A cell with a less negative water potential will draw in water but this depends on other factors as well such as solute potential (pressure in the cell e.g. solute molecules) and pressure potential (external pressure e.g. cell wall).

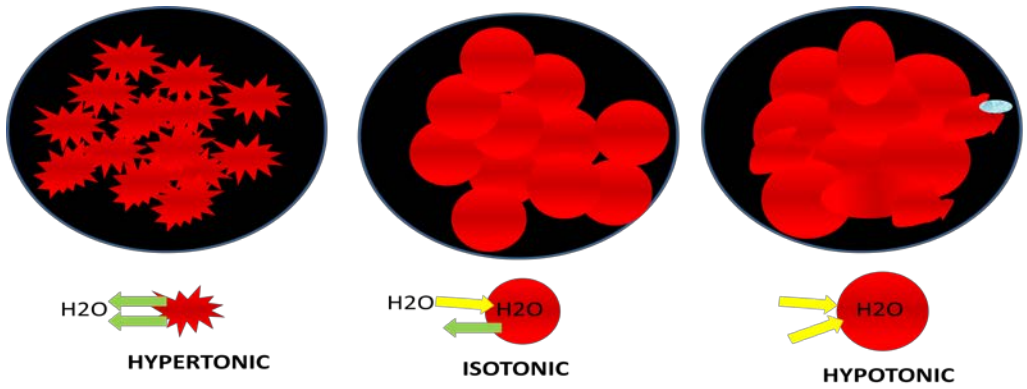

Figure 6: Osmosis.(A) In hypertonic solution, there are more solute molecules outside the cell, which causes the water to be sucked in that direction which leads to the shrinkage of cells. (B) In isotonic solution, there is equal concentration of solute on both sides, henceforth the water with move back in forth. (C) In hypotonic solution, there are less solute molecules outside the cell, since salt sucks and water will move inside the cell. The cell will gain water and grow larger, and finally burst.

Active transport:

Active transport is the movement of a substance against its concentration gradient (i.e. from low to high concentration). It is an endergonic process that, in most cases, is coupled to the hydrolysis of ATP.

Types of active transport:

1. **Primary active transport:**Primary active transport, also called direct active transport, directly uses energy to transport molecules across a membrane.
   Example:Sodium-potassium pump, which helps to maintain the cell potential.

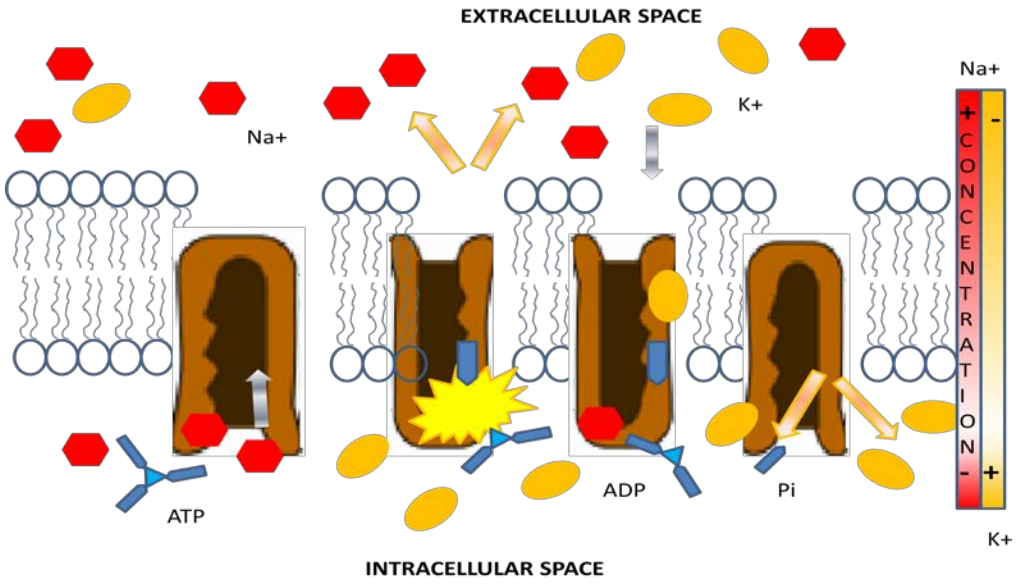

Figure 7: Primary active transport.The action of the sodium-potassium pump is an example of primary active transport.

2. **Secondary active transport:**Secondary active transport or co-transport, also uses energy to transport molecules across a membrane; however, in contrast to primary active transport, there is no direct coupling of ATP; instead, the electrochemical potential difference created by pumping ions out of the cell is instrumental.

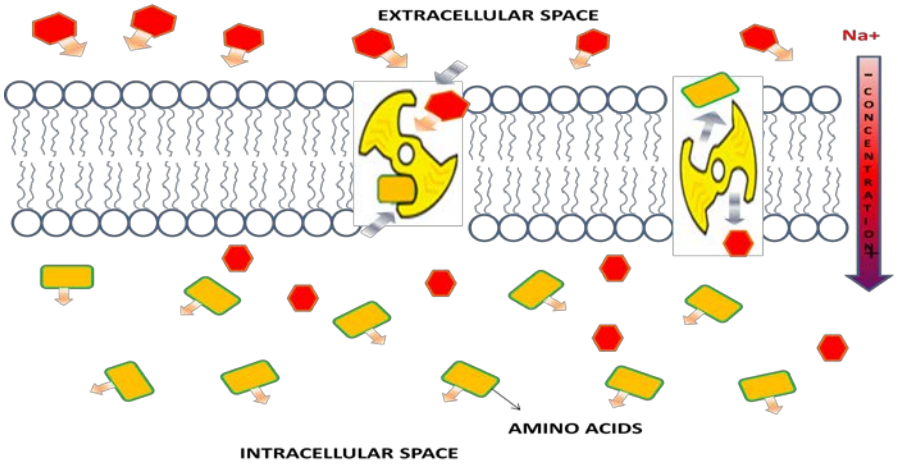

Figure 8: Secondary active transport

The two main forms of active transport are antiport and symport.

(a) Antiport:

In antiport two species of ion or solutes are pumped in opposite directions across a membrane. One of these species is allowed to flow from high to low concentration which yields the entropic energy to drive the transport of the other solute from a low concentration region to a high one.Example: the sodium-calcium exchanger or antiporter, which allows three sodium ions into the cell to transport one calcium out.

(b) Symport:

Symport uses the downhill movement of one solute species from high to low concentration to move another molecule uphill from low concentration to high concentration (against its electrochemical gradient).

Example: glucose symporterSGLT1, which co-transports one glucose (or galactose) molecule into the cell for every two sodium ions it imports into the cell.

Examples:

(A) (Na^+–K^+)–ATPase

(Na^+ - K^+)–ATPaseactive transport system is commonly found in the plasma membranes of higher eukaryotes, which was first characterized by Jens Skou. This transmembrane protein consists of two types of subunits: a 110-kD non-glycosylated α- subunit that contains the enzyme's catalytic activity and ion-binding sites, and a 55-kD glycoprotein β-subunit of unknown function. Sequence analysis suggests that the α- subunit has eight transmembrane α-helical segments and two large cytoplasmic domains. The β- subunit has a single transmembrane helix and a large extracellular domain. The protein may function as an (αβ)2 tetramer *in vivo*.

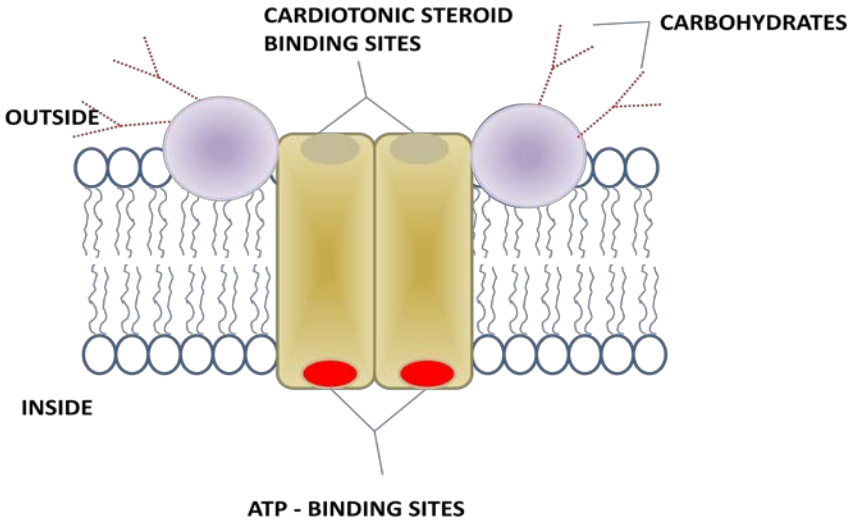

Figure 9: (Na^+ - K^+) – ATPase.This diagram shows the transporter's dimeric structure and its orientation in the plasma membrane. Cardiotonic steroids bind to the external surface of the transporter, thereby inhibiting transport.

The (Na^+ – K^+)–ATPase is also called as the (Na^+ – K^+) pumpbecause it pumps 3 Na^+ out of and 2 K^+ into the cell in presence of hydrolysis of intracellular ATP. The overall stoichiometry of the reaction is:

$3\ Na^+(in) + 2\ K^+(out) + ATP + H_2O \rightarrow 3\ Na^+(out) + 2\ K^+(in) + ADP + Pi$

(B) Ion Gradient–Driven Active Transport

For example, cells of the intestinal epithelium take up dietary glucose by Na^+ -dependentsymport. This process is an example of secondary active transport because Na^+ gradient in these cells is maintained by the (Na^+–K^+)–ATPase.The Na^+ –glucose transport system concentrates glucose inside the cell. Glucose is then transported into the capillaries through a passive-mediated glucose uniport (which resembles GLUT1).

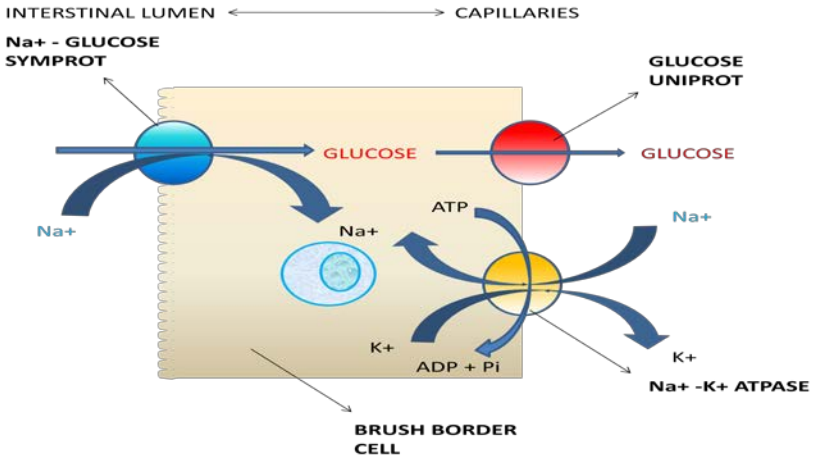

Figure 10: Glucose transport across Interstinal epithelium.The brushlike villi lining the small interstine greatly increases the surface area (a), thereby facilating the absorption of the nutrients. The brush border cells from which the villi are formed (b) concentrate glucose from the interstinal lumen in symport to Na^+ (c), a process that is driven by (Na^+ - K^+) – ATPase, which is located on the capillary side of the cell and functions to maintain a low internal [Na^+]. The glucose is exported to the bloodstream via a passive-mediated uniprot system similar to GLUT1.

Differentiating mediated and non-mediated transport:

Glucose and many other compounds can enter cells by a non-mediated pathway; that is, they slowly diffuse into cells at a rate proportional to their membrane solubility and their concentrations on either side of the membrane. The flux(rate of transport per unit area) of asubstance across the membrane increases with the magnitude of its concentration gradient. If glucose moves across a membrane by means of a transport protein, its flux is no longer linear.

This is one of four characteristics that distinguish mediated from non-mediated transport:

1. **Speed and specificity**-The solubilities of the chemically similar sugars D-glucose and D-mannitol in a synthetic lipid bilayer are similar. However, the rate at which glucose moves through the erythrocyte membrane is four orders of magnitude faster than that of D-mannitol. The erythrocyte membrane therefore contains a system that transports glucose and that can distinguish D-glucose from D-mannitol.
2. **Saturation**-The rate of glucose transport into an erythrocyte does not increase infinitely as the external glucose concentration increases. Such an observation is evidence that a specific number of sites on the membrane are involved in the transport of glucose; which becomes saturatedat high [glucose] and the plot of glucose flux versus [glucose] is hyperbolic. The non-mediated glucose flux increases linearly with [glucose].

3.**Competition**-The curve is shifted to the right in the presence of a substance that competes with glucose for binding to the transporter; for example, 6-*O*benzyl-D-galactose.Competition is not a feature of non-mediated transport, since no transport protein is involved.

4.**Inactivation**-Reagents that chemically modify proteins and hence may affect their functions may inhibit the rapid, saturatable flux of glucose into the erythrocyte.

Interesting facts:

- The binding of the neurotransmitter acetylcholine at certain synapses opens channels that admit Na+ and initiate a nerve impulse or muscle contraction.
- Sound waves bending the cilia-like projections on the hair cells of the inner ear open up ion channels leading to the creation of nerve impulses that the brain interprets as sound.
- Mechanical deformation of the cells of stretch receptors opens ion channels leading to the creation of nerve impulses.
- The crucial roles of the Na+/K+ ATPase are reflected in the fact that almost one-third of all the energy generated by the mitochondria in animal cells is used just to run this pump.

- ABC transporters must have evolved early in the history of life. The ATP-binding domains in archaea, eubacteria, and eukaryotes all share a homologous structure, the ATP-binding "cassette".

Questions:

1. **Carrier molecules that bring materials into cells are**
   a. Lipids
   b. Proteins
   c. Glycogen
   d. Phospholipid
2. **Arrange the following compounds in order of increasing membrane permeability: N_2, water, glucose and RNA.**
   a. RNA>glucose>water>N_2
   b. N_2>water>glucose>RNA
   c. Water>N_2>glucose>RNA
   d. N_2>water>RNA>glucose
3. **The rate of diffusion across the cell membrane is affected by the**
   a. temperature and pinocytosis.
   b. temperature and size of the molecule.
   c. membrane structure and phagocytosis.
   d. shape of glycolipids and glycoproteins.
4. **How many of the following factors would affect the permeability of the cell membrane? • Size of molecules • Lipid solubility of molecules •Presence of transport channels • Presence of ATP inside the cell.**
   a. One.
   b. Two.
   c. Three.
   d. Four.

5. **Which of the following aids the movement of glucose across a cell membrane?**
    a. Protein.
    b. Phosphate.
    c. Glycolipid.
    d. Cholesterol.
6. **In the parietal cells of the stomach, the uptake of chloride ions is coupled to the transport of bicarbonate ions out of the cell. This type of active transport system is called,**
    a. Uniprot
    b. Symprot
    c. Antiprot
7. **Which of the following conditions is required for diffusion to occur?**
    a. ATP energy.
    b. A living cell.
    c. A concentration difference.
    d. A selectively-permeable membrane.
8. **Frog eggs placed in an isotonic solution will**
    a. burst.
    b. shrink.
    c. remain the same.
    d. increase in volume.
9. **When put in a hypotonic environment, an animal cell will**
    a. swell.
    b. shrink.
    c. secrete enzymes.
    d. remain unchanged.
10. **Which of the following conditions would cause red blood cells to burst?**
    a. pH of 7.5.
    b. Temperature of 3°C.
    c. Being placed in distilled water.
    d. Being placed in an 11% salt solution.

11. In an experiment, frog's eggs were placed in a salt solution. After several hours their mass increased significantly. We can therefore conclude that, compared to the frog's eggs, the solution was

a. isotonic.
b. saturated.
c. hypotonic.
d. hypertonic.

12. Which of the following moves material against a concentration gradient?

a. osmosis
b. diffusion
c. active transport
d. facilitated transport

13. Which of the following processes moves molecules using cellular energy?

a. Osmosis.
b. Diffusion.
c. Pinocytosis.
d. Facilitated transport.

14. Which of the following processes would be directly affected by a lack of cellular ATP?

a. Osmosis.
b. Diffusion.
c. Active transport.
d. Facilitated transport.

15. Which of the following will be affected directly if the mitochondria in a cell are not functioning properly?

a. Absorption of alcohol by the cell.
b. The movement of water into and out of the cell.
c. The movement of oxygen across the cell membrane.
d. The movement of sugar from a low to a high concentration.

16. The cell process which uses ATP to bring substances into the cell is

a. Osmosis.

b. Diffusion.

c. Active transport.

d. Facilitated transport.

17. A bacterium is living in a pond where the concentration of sodium ions is 0.005mM. This ion is found in the bacterial cytoplasm at a concentration 0.10 mM. Therefore the sodium ion is probably entering by:

a. Simple diffusion

b. Facilitated diffusion

c. Passive transport

d. Active transport

18. What are the two factors that are responsible for diffusion rate?

19. What are the membrane potentials of living cells?

20. How the opening and closing of ion channels occur in a cell?

21. Explain glucose transporter or GLUT1 with a diagram.

22. What are the different types of mediated transport depending on the thermodynamics of the system?

23. How the mediated transport can be differentiated from non-mediated transport. Explain with a graph.

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Module 3 Lecture 2
Membrane transport facilitators

Membrane transport is assisted by various facilitators to ease their job. We will study a few of them in detail.

Permeases

Permeases are a class of membrane transport proteins which facilitate the diffusion of a specific molecule by passive mediated transport. These are divided into following types:

1. Lactose permease: It is a transmembrane protein that consists of N- and C- terminal domains, each consisting of six membrane-spanning alpha helices in a symmetrical fashion. These two domains are well separated and are joined by a single stretch of polypeptide. There are six side chains amino acids that play an important role in the active transport of lactose through the protein. Some of the examples are: Glutamic Acid 126, Arginine 144, and Glutamic Acid 269 plays role in substrate binding activities where as Arginine 302, Histidine 322, and Glutamic Acid 325 plays a significant role in proton translocation throughout the transport process. These side chains, make up the active site of the protein and found within the large internal hydrophilic cavity of the lactose permease where the substrate is received for transport and it is the location from which it is sent into the cell.

It is an active co-transport that facilitates the passage of lactose across the phospholipid bi-layer of the cell membrane by using the inwardly directed H^+ electrochemical gradient as its driving force. The proton gradient is metabolically generated through oxidative metabolism. The electrochemical potential gradient created by both these systems is used mainly to drive the synthesis of ATP. As a result, the lactose is accompanied from the periplasam to the cytoplasm of the cell by an H^+ proton.

Lactose permease has two major conformational states:

1. E-1, which has a low-affinity lactose-binding site facing the interior of the cell.

2. E-2, which has a high-affinity lactose-binding site facing the exterior of the cell.

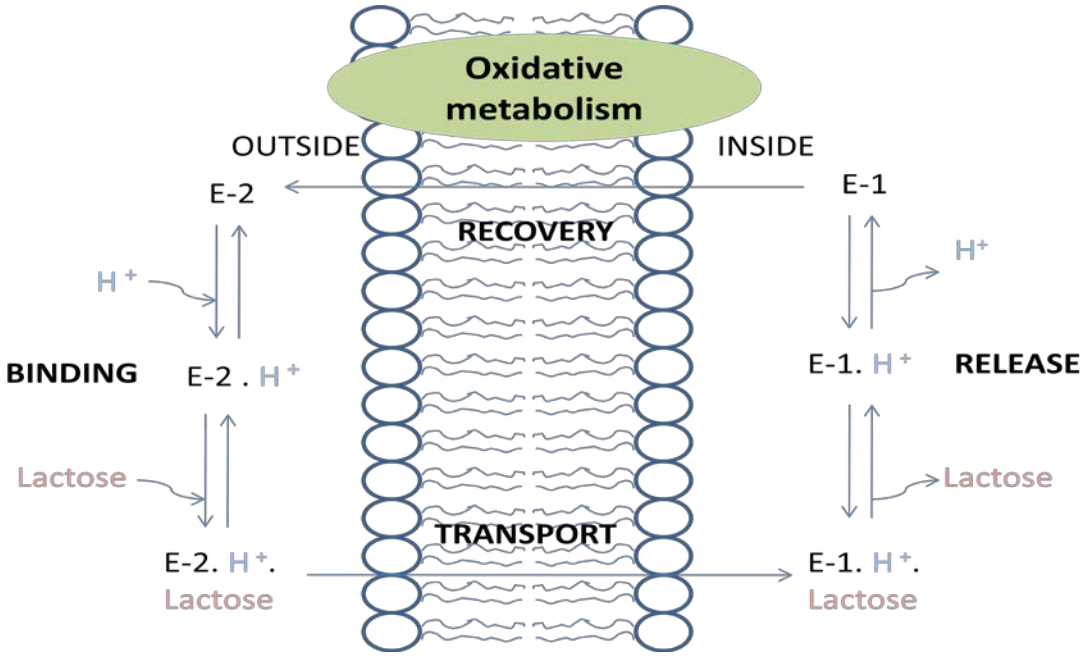


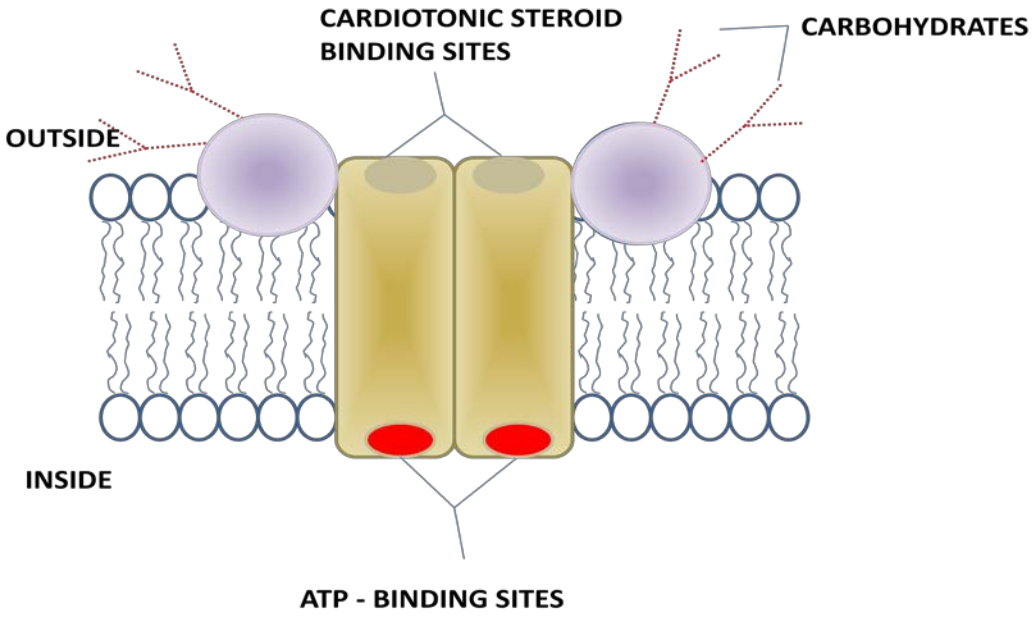
Figure 1: Schematic diagram for the cotransport of H^+ and lactose by lactose permease in *E.Coli*. H^+ binds first to E-2 outside the cell, followed by lactose. They are sequentially released from E-1 inside the cell. E-2 must bind to lactose and H^+ in order to change the conformation to E-1, thereby cotransporting these substances in the cell. E-1 changes the conformation to E-2 when neither lactose nor H^+ is bound, thus completing the transport cycle.

2. β-galactoside permease is a membrane-bound transport protein that facilitates the uptake of β-galactosides across the cell. The common example is melibiose carrier protein from *Klebsiella pneumonia*, which is capable of using hydrogen and lithium cations as coupling cations for cotransport, depending on the particular sugar transported (H^+-melibiose, Li^+-lactose).

3. Amino acid permeases are integral membrane proteins involved in the transport of amino acids into the cell. One of the examples of amino acid permease is histidine permease which is a bacterial ABC protein in *E.coli* and located in the periplasmic space of cell. Histidine binding protein binds histidine tightly and directs it to T sub-units of permease, through which hisitidine crosses the plasma membrane along with ATP hydrolysis.

Na^+/K^+ ATPase :

In mammalian cells, the Na^+ and K^+ gradients are the two major components of the electrochemical gradient across the plasma membrane. The cells maintain a lower intracellular Na^+ concentration and higher intracellular K^+ concentration with relative to extracellular space. Hence, for the generation and maintenance of the electrochemical gradients for Na^+ and K^+, it requires Na^+/K^+ ATPase, which is an ion pump that couples ATP hydrolysis to cation transport. It also helps to set the negative resting membrane potential, which regulates the osmotic pressure to avoid cell lysis. The Na^+/K^+ ATPase belong to P-class ATPase which is commonly found in the plasma membranes of higher eukaryotes. This transmembrane protein consists of two types of subunits: a 110-kD non-glycosylated α- subunit that contains the enzyme's catalytic activity and binding sites for ATP, Na^+ and K^+ ions, and a 55-kD glycoprotein β-subunit of unknown function. The smaller β-subunit has one transmembrane domain that stabilizes the α-subunit and is important in membrane insertion. The α- subunit has eight transmembrane α-helical segments and two large cytoplasmic domains and the β- subunit has a single transmembrane helix and a large extracellular domain. The protein may function as an (αβ)2 tetramer *in vivo*.



Figur 2: Na^+/K^+ ATPase. The diagram shows the transporter's putative dimeric structure and its orientation in the plasma membrane. Cardiotonic steroids bind to the external surface of the transporter, thereby inhibiting transport.

The Na^+/K^+ ATPase is also called as the Na^+/K^+ pump because it pumps 3 Na^+ out of and 2 K^+ in both direction across the membrane in presence of hydrolysis of ATP. The overall reaction is:

3 Na^+(*in*) + 2 K^+(*out*) + ATP + H2O →3 Na^+(*out*) + 2 K^+(*in*) + ADP + P*i*

The important feature to the Na^+/K^+ ATPase is the phosphorylation of a specific Asp residue of the transport protein which phosphorylates only in the presence of Na^+, whereas the resulting aspartyl phosphate residue is subject to hydrolysis only in the presence of K^+. Hence it has two conformations named E1 and E2. The protein appears to operate in the following (explained in figure 4):

1. The protein in the *E*1 state has three high-affinities Na^+ binding sites and two low-affinity K^+ binding sites accessible to the cytosolic surface of the protein. Hence E1 binds three Na^+ ions inside the cell and then binds ATP to yield an *E*1 .ATP.3 Na^+ complex.
2. ATP hydrolysis produces ADP and a "high-energy" aspartyl phosphate intermediate *E*1-P.3 Na^+.
3. This "high-energy" intermediate relaxes to its "low-energy" conformation, *E*1~P.3 Na^+, and releases its bound Na^+ outside the cell.
4. *E*2-P binds two K^+ ions from outside the cell to form an *E*2-P.2 K^+ complex.
5. The phosphate group is hydrolyzed, yielding *E*2 .2 K^+.
6. *E*2 .2 K^+ changes conformation, releases its two K^+ ions inside the cell, and replaces them with three Na^+ ions, thereby completing the transport cycle.

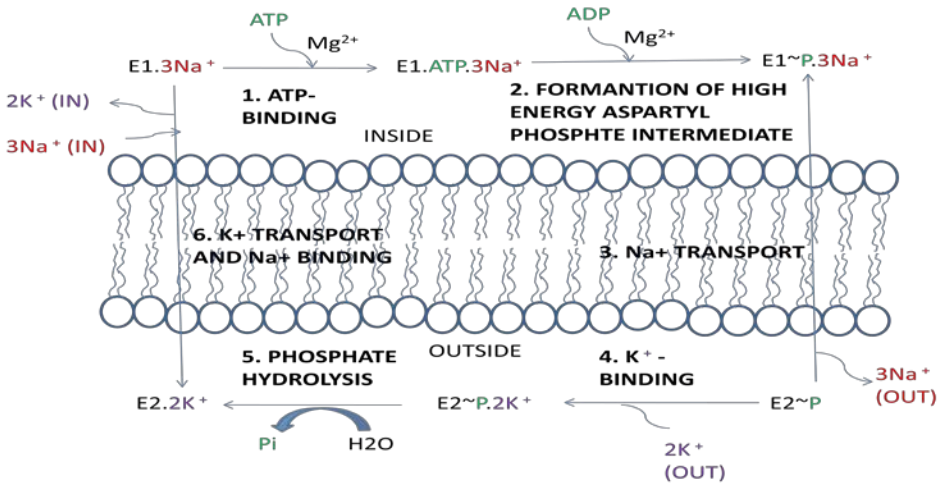


Figure 3: Scheme for the transport of Na^+ and K^+ by the Na^+/K^+ ATPase.

These are mostly target of a large number of toxins and important drug target. Some of the examples are: the naturally occurring steroids called cardiac glycoside such as ouabain and digitalis, inhibit ion transport by Na^+/K^+ ATPase by binding reversibly to the extracellular side of pump which in turn inhibit ATP hydrolysis and ion transport. Other toxins like palytoxin from marine corals are also specific inhibitor. They block the ATPase in an open state, allowing ions to flow down their concentration gradient, which destroys electrochemical gradient.

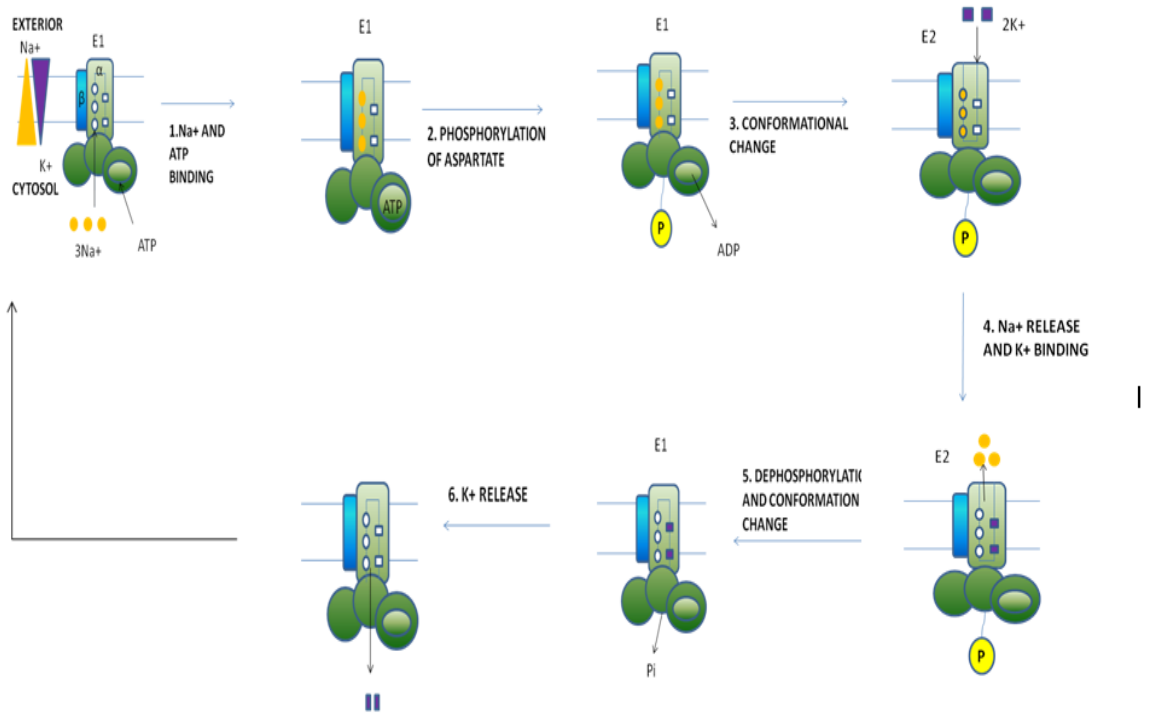


Figure 4: Operational model of the Na^+/K^+ ATPase in the plasma membrane. Only one of the two catalytic α subunits of this P-class pump is depicted. It is not known whether just one or both subunits in a single ATPase molecule transport ions. Ion pumping by the Na^+/K^+ ATPase involves phosphorylation, dephosphorylation, and conformational change. In this case, hydrolysis of the E2–P intermediate powers the E2 → E1 conformational change and concomitant transport of two ions (K^+) inward. Na^+ ions are indicated by red circles; K^+ ions, by purple squares; high-energy acyl phosphate bond, by ~P; low-energy phosphoester bond, by –P.

Ca^{2+} ATPase

Eukaryotic cells maintain a low concentration of free Ca^{2+} in the cytosol (10^{-7} M) whereas the extracellular concentration is very high on the opposite face (10^{-3} M). Henceforth, a small influx of Ca^{2+} significantly increases the concentration of free Ca^{2+} in the cytosol and the flow of Ca^{2+} down its steep concentration gradient in response to the extracellular signals is one of the means of transmitting these signals rapidly across the plasma membrane. Hence cells maintain a steep Ca^{2+} gradient across the plasma membrane. The $Ca2^{+}$ ATPases are commonly found in muscle cells and neurons. The skeletal muscle have specialized structure of large intracellular Ca^{2+} stores called sarcoendoplasmic recticulum which controls Ca^{2+} uptake and release throughout the cell volume. These are mainly responsible for Ca^{2+} extrusion from cytosol in muscle cells which is required to stop muscle contraction and to initiate relaxation.

Ca^{2+} transporters are the common example of P-type transport ATPase. It is also known as Ca^{2+} pump or Ca^{2+} ATPase or SERCA pump (Sacroendoplasmic recticulum Ca^{2+} ATPase). These transporters actively pump Ca^{2+} out of the cell and helps in maintaining the gradient. The structure of Ca^{2+} pump has an asymmetrical arrangement of transmembrane and cytosolic domains that undergo movements during Ca^{2+} transport. It contains 10 tranmembrane α-helices and two cytoplasmic loops between the transmembrane α-helices. The transmembrane α-helices form Ca^{2+} binding site which binds two Ca^{2+} ions from cytosol. And the two cytoplasmic loops form three separate domains: nucleotide binding domains that binds ATP, actuator domain that contains catalytic phoshorylation site and P domain which is important for transmission of conformational changes between cytosolic and transmembrane domains. In unphosphorylated state, the two helices are disturbed and form a cavity for binding of two Ca^{2+} ions from the cytosolic side of the membrane. ATP also binds to a binding site on the same side of the membrane and the subsequent transfer of the terminal phosphate group of ATP to an aspartic acid of an adjacent domain lead to a drastic rearrangement of the transmembrane helices. This rearrangement disturbs the Ca^{2+} binding site and releases Ca^{2+} ions on the other side of the membrane that is into the lumen of SR. With respect to figure 5 and 6, the mechanism of the Ca^{2+} ATPase in the SR membrane can be understood clearly through following steps:

1. The protein in E1 conformation has two high affinity binding sites for Ca^{2+} ions accessible from the cytosolic side and ATP binds to a side on cytosolic surface.
2. In the presence of Mg^{2+}, the bound form of ATP is hydrolyzed to ADP and phosphate. Later the liberated phosphate is transferred to a specific aspartate residue in the protein, forming the high-energy acyl phosphate bond denoted by E1 ~ P.
3. Then the protein undergoes a conformational change and generates E2, which has two low-affinity Ca^{2+} binding sites accessible to the SR lumen.
4. The free energy of E1 ~ P is greater than E2-P, and this reduction in free energy leads to the E1 → E2 conformational change. Simultaneously, the Ca^{2+} ions also dissociate from the low-affinity sites to enter the SR lumen, following which the aspartyl-phosphate bond is hydrolyzed.
5. Dephosphorylation then again leads to the E2 → E1 conformational change, and E1 is ready to transport two more Ca^{2+} ions.

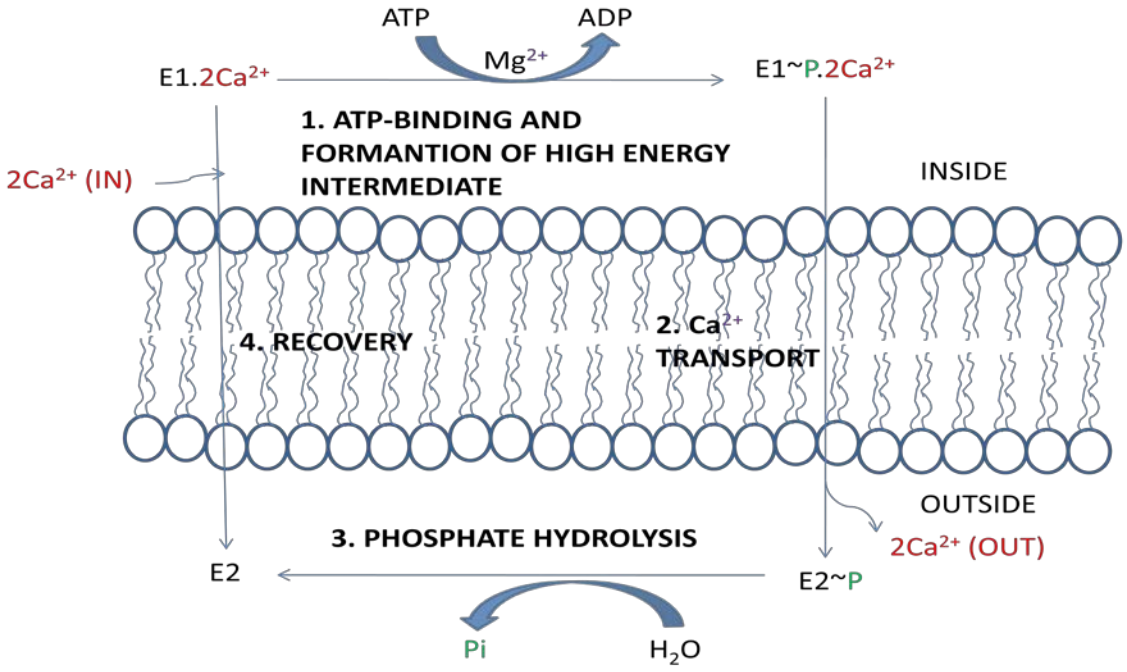


Figure 5: Scheme for the active transport of Ca^{2+} by the Ca^{2+} ATPase. Here (*in*) refers to the cytosol and (*out*) refers to the outside of the cell for plasma membrane Ca^{2+} ATPase or the lumen of the endoplasmic reticulum (or sarcoplasmic reticulum) for the Ca^{2+} ATPase of that membrane.

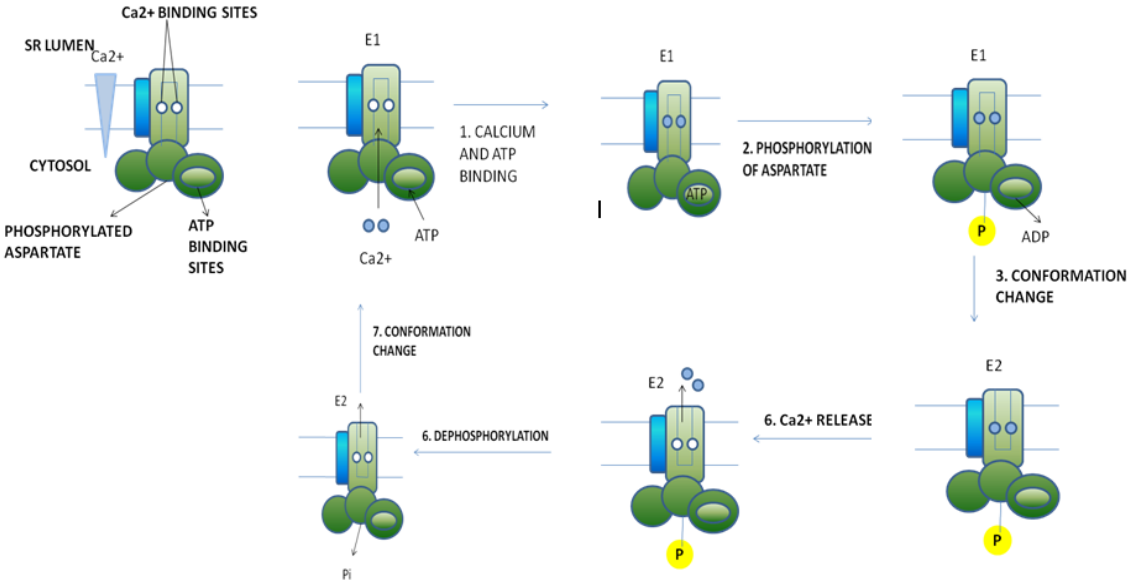


Figure 6: Operational model of the Ca^{2+} ATPase in the SR membrane of skeletal muscle cells. Only one of the two catalytic α subunits of this P-class pump is depicted. E1 and E2 are alternative conformations of the protein in which the Ca^{2+} binding sites are accessible to the cytosolic and exoplasmic faces, respectively. An ordered sequence of steps (1 – 6), as diagrammed here, is essential for coupling ATP hydrolysis and the transport of Ca^{2+} ions across the membrane. In the figure, ~P indicates a high-energy acyl phosphate bond; –P indicates a low-energy phosphoester bond.

Interesting facts:

- The X-ray crystal structure of lactose permease was first solved in 2003 by J. Abramson et al.
- Ouabain is a cardiac glycoside toxin. Potent inhibitors that bind to potassium binding sites. In the presence of Ouabain, Na^+/K^+ ATPase cannot return to its resting state.
- One major type of gradient linked active permeases is the sodium-glucose symport carrier.

Questions:

1. **Which of the following uses energy to transport molecules or ions against their concentration gradient?**

a. Voltage-gated Na+ channel
b. Acetylcholine receptor
c. Glucose transporter
d. ATP-ADP transporter
e. Na+/K+-ATPase

2. **A membrane-spanning transporter protein that is also characterized as a "symporter" would be involved in which one of the following transport processes?**

a. Simple transport (e.g., lactose via Lac permease)
b. Simultaneous transport of one type of molecule into the cell and a different molecule out of the cell (e.g., Na+ "pump" to move Na+ out of the cell)
c. Transport of potassium ions into the cell without any other ion or molecule being transported in any direction
d. Unidirectional transport into the cell of only one type of molecule (found in very low concentration in the periplasm) using the ATP-driven ABC translocation system

3. **The sodium-potassium pump passes**

a. more Na+ out than K+ in
b. K+ out and Na+ in on a one-for-one basis
c. Na+ out and K+ in on a one-for-one basis
d. K+ and Na+ in the same direction

4. **The sodium-potassium pump moves sodium and potassium ions against the concentration gradient.**

a. True
b. False

5. **The Na^+-K^+ pump consumes a third of the total ATP supply of a typical animal cell and is responsible for maintaining the high concentration of K^+ inside cells, for controlling cell volume, and for driving the uptake of sugars and amino acids in the intestine and kidneys.**
   a. True
   b. False

6. **The energy needed to power the sodium-potassium pump is provided by the**

a. Binding of ATP to the pump
b. Transport of ATP by the pump.
c. Splitting of ATP.
d. Formation of ATP.

7. **Which of the following moves Ca^{2+} back into the tubules of the SR after a contraction?**

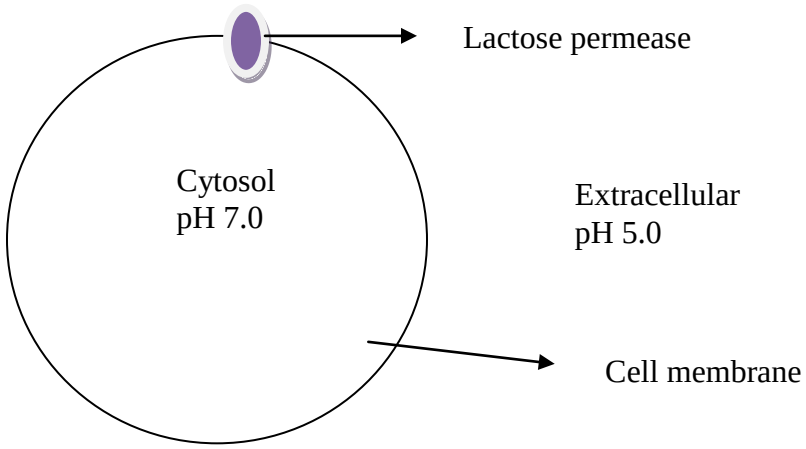
a. The ATP-dependent H^+ pump
b. The ATP-dependent myosin pump
c. Simple diffusion
d. The ATP-dependent Na^+/K^+ pump
e. The ATP-dependent Na^+/K^+ pump
f. The ATP-dependent calcium pump

8. **SERCS pumps actively transport calcium:**

a. From ER to cytosol
b. From cytosol to ER
c. From extracellular space to the cytosol
d. From the cytosol to the extracellular space
e. From the mitochondria to the cytosol

9. **In each cycle, the Na^+-K^+ pump transfers ____K^+ ions in the cell and ____ Na^+ out of the cell.**

10. **Bacterial lactose permease is a symporter of lactose and H+. When the lactose concentrations in the cytosol and in the extracellular space are identical but the pH's in the two locations are different as indicated below, which direction would lactose be transported? Explain briefly why you think that way.**



11. **Why the sodium-potassium transport mechanism is called a pump?**
12. **Explain Na^+/K^+ pump with a schematic diagram.**
13. **Explain Ca^{2+} pump with a schematic diagram**
14. **What are the types of permeases?**

References

1. Donald Voet, Judith G. Voet, Charlotte W. Pratt (2008); Fundamentals of Biochemistry: Life at the Molecular Level: Chapter 10 Membrane transport, 3rd Edition
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Module 3 Lecture 3

Lysosome and vacuolar membrane

In earlier lecture we have studied about plasma membrane. However some cell organelles have depending on the function which they perform have modified membranes. We will study the membrane of a lysosome and vacuoles in detail in this lecture.

Lysosomes:

Lysosomes are central, acidic and membrane bound organelles that contain hydrolase enzyme for the breakdown of all types of biological polymers- proteins, nucleic acids, carbohydrates and lipids. They are mostly found in animal cells, while in yeast and plants, it acts as lytic vacuoles. It is enclosed by membrane known as lysosomal membrane that maintains the digestive enzyme at pH 4.5. Figure 1 shows the structure of lysosome.

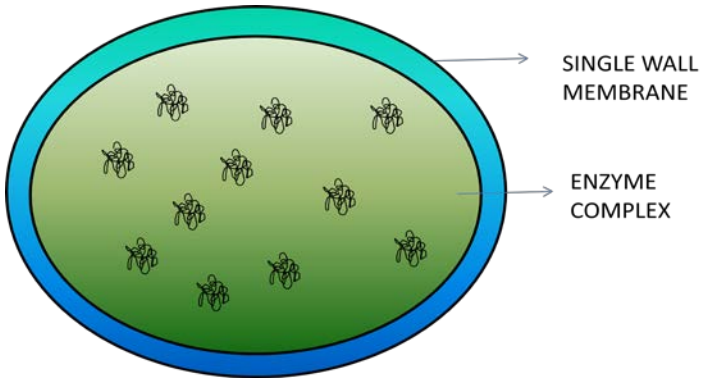


Figure 1: Lysosome

Functions of lysosomes:

- Maintains pH by pumping protons from cytosol across the membrane via proton pumps and chloride ion channels.
- Protects the cytosol and rest of the cells from degradative enzymes within the lysosome.
- Acts as digestive system of the cell, serving both to degrade material taken up from the outside of the cell and to digest obsolete components of cell itself.

- Sequestration of lysosomal enzymes.
- Mediation of fusion events between lysosomes and other organelles.
- Transport of degradation products to the cytoplasm

Lysosomal Membrane: To perform its function with efficacy the lysosomal membrane needs some additional features in its membrane. It is slightly thicker than that of the plasma membrane. It contains substantial amounts of carbohydrate component, particularly sialic acid. In fact, most lysosomal membrane proteins are highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration. Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue. The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H+ inside the organelle. Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

Lysosomal membrane composition:

The V-class H^+ ATPase pump is generally present in lysosomal membrane. This class of ATPase pump only transports H^+ ions. Its main function is to acidify the lumen of the organelles. The proton gradient between the lysosomal lumen (pH ≈4.5–5.0) and the cytosol (pH ≈7.0) depends on ATP production by the cell.

These V-class proton pumps contain two domains: a cytosolic hydrophilic domain (V_1) and a transmembrane domain (V_0) with multiple subunits in each domain. Binding and hydrolysis of ATP by the B subunits in V_1 provides the energy for pumping of H^+ ions

through the proton-conducting channel formed by the c and a subunits in V_0. These V-class proton pumps are not phosphorylated and dephosphorylated during proton transport. Figure 2 depicts a V-class proton pump.

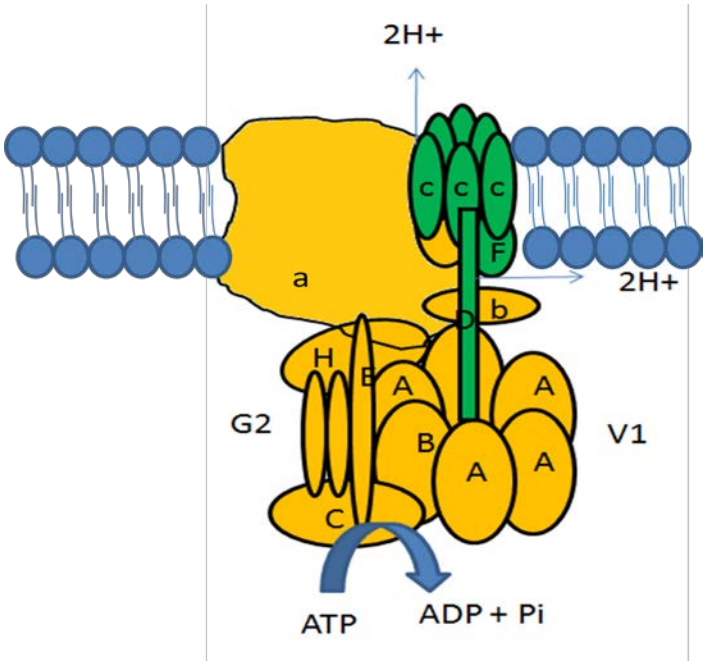
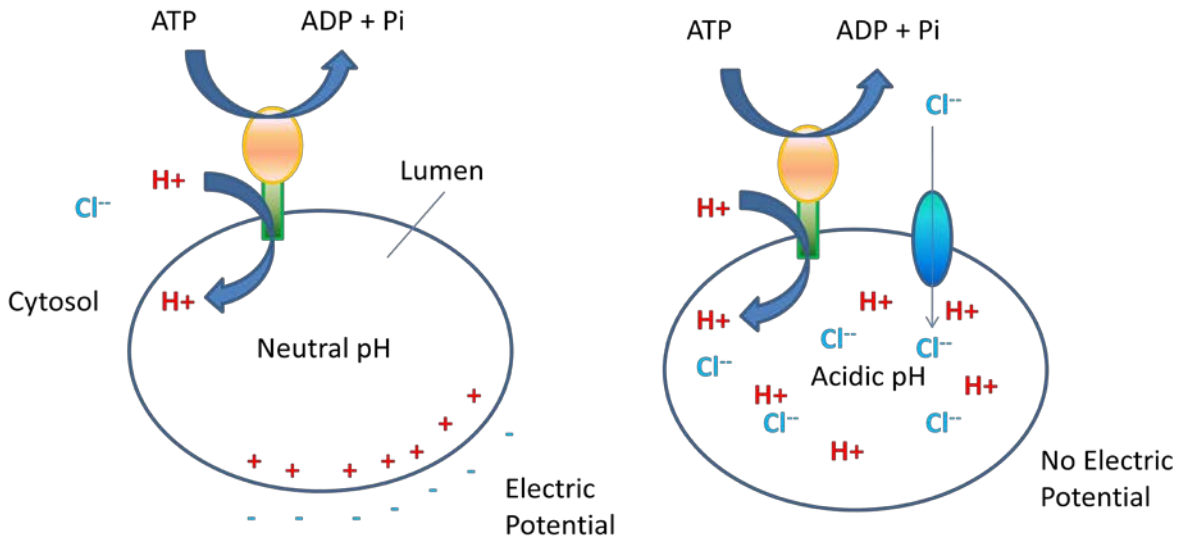


Figure 2: V-class proton pump

These protons cannot acidify by themselves because a net movement of electric charge occurs. Only a few protons build up positive H^+ ions on exoplasmic face (inside) and for each H^+ pumped across, a negative ion will be left behind on cytosolic face, building negative charged ions. These oppositely charged ions attract each other on opposite faces of the membrane, generating a charge separation, or electric potential, across the membrane. If more protons are pumped, the excess positive ions on exoplasmic face repels other H^+ ions and prevents pumping of extra proton long before a significant transmembrane H^+ concentration gradient had been established .



(a) **(b)**

Figure 3: Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change occurs in the intraluminal pH. (b) If the organelle membrane also contains Cl^- channels, anions passively follow the pumped protons, resulting in an accumulation of H^+ ions (low luminal pH) but no electric potential across the membrane.

Lysosomal membrane proteins:

Lysosomes are formed by the fusion of transport vesicles budded from Golgi network with endosomes, which contain molecules taken up at the cell surface. And its membrane proteins are usually highly glycosylated proteins decorating the luminal surface of lysosomal membranes. They are most often known as lysosomal associated membrane proteins (LAMP). LAMP-1, LAMP-2 and LIMP-2 are the most abundant components of this membrane. And mainly involved in transport of newly synthesized hydrolases to the lysosome (lysosomal integral membrane protein 2 (LIMP2)) and across the lysosomal membrane (the V-type H^+-ATPase complex and chloride channel protein 7 (CLC7).

Vacuolar membrane:

Vacuoles are the membrane bound sac within the cytoplasm which are filled with water containing organic and inorganic molecules including enzymes and mostly present in plants, fungi and some animals. This vacuole slowly develops as the cell matures by fusion of smaller vacuoles (vesicles) derived from the endoplasmic reticulum and Golgi apparatus.

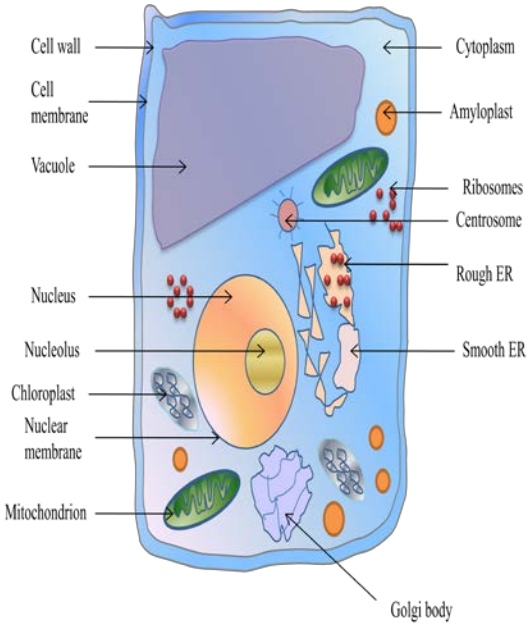


Figure 4: Plant cell structure

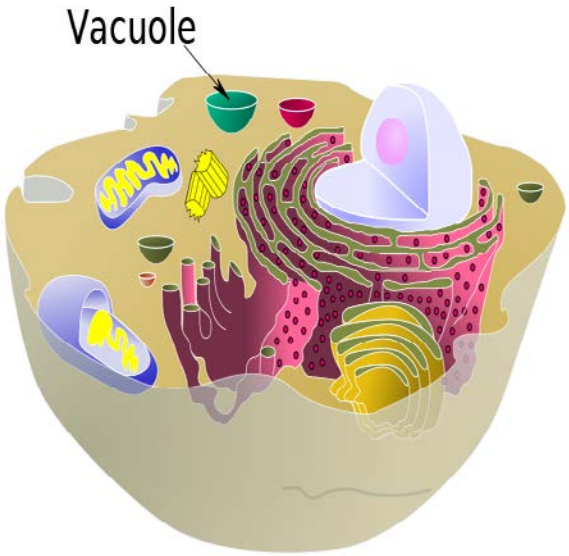


Figure 5: Animal cell structure

Function of vacuoles:

- Acts as storage organelles and contains water and small molecules. Stores salts, minerals, nutrients, proteins, pigments, helps in plant growth, and plays an important structural role for the plant.
- Maintains internal hydrostatic pressure or turgor pressure within the cell
- Maintains an acidic internal pH
- Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole. Also maintains turgor pressure against the cell wall. Because of osmosis, water diffuses into the vacuole, and exerting pressure on the cell wall. And water loss leads to shrinkage of the cell. Hence turgor pressure needs to be maintained. Turgor pressure also dictates the rigidity of the cell and is associated with the difference between the osmotic pressure inside and outside of the cell.
- In seeds, stored proteins needed for germination are kept in protein bodies, which are modified vacuole.
- Regulating the movements of ions around the cell.
- Transports proton from cytosol to vacuole and hence stabilizes cytoplasmic pH making the vacuolar interior most acidic by creating a proton motive force which in turn used for the transport of nutrients into and out of the cell and allows degradative enzymes to act.
- Vacuoles also often store the pigments that give certain flowers their colors, which aid them in the attraction of bees and other pollinators, but also can release molecules that are poisonous, odoriferous, or unpalatable to various insects and animals, thus discouraging them from consuming the plant.

Plant vacuoles:

Most of the plant cell contains large, single central vacuoles and can occupy at least 30% to 80% of the cell. Generally vacuole is surrounded by membrane known as tonoplast, or vacuolar membrane. It separates the vacuolar contents from cell's cytoplasm and an important and highly integrated component of the plant internal membrane network (endomembrane) system. The vacuole solution (also known as cell sap) differs markedly from that of the surrounding cytoplasm.

Vacuolar membrane:

The V-class H+ ATPase pump is present in vacuolar membrane. More details of V-class H+ ATPase pump is described earlier (Figure 2 and Figure 3).

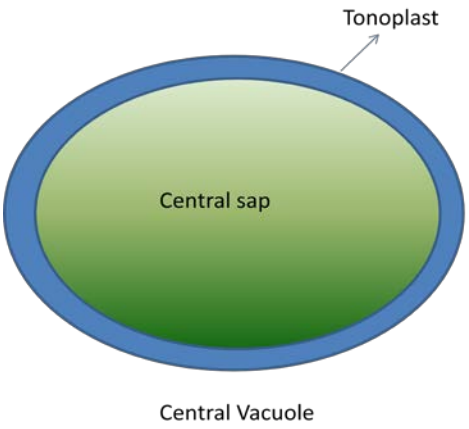


Figure 6: Plant cell vacuole

Other transport proteins present in vacuolar membrane:

1. Proton Pump:

Proton pumps play a central role in the function of the tonoplast by generating a transmembrane H+ electrochemical gradient which can be utilized to drive the transport of solutes. The tonoplast contains different proton pumps, an ATPase and a PPase. V-ATPases (vacuolar-type) are present on different membranes of eukaryotic cells and is constituted of 13 subunits whereas tonoplast PPase is also an integral entity of the tonoplast and consists of one 80 kDa protein.

2. Aquaporins:

Major intrinsic membrane proteins (MIPs), are very small hydrophobic proteins abundantly present in membranes. But these MIPs form water channels. Later α-TIP (tonoplast intrinsic protein) which is a member of MIPs was described and found abundantly. Another major membrane protein of the central vacuole is the γ-TIP (observed in radish). Both TIPs have been shown to act as water channels. α-TIP is associated with the storage vacuole while the γ-TIP is localized on the lytic vacuole. Interestingly, α-TIP has to be phosphorylated in order to exhibit water channel activity.

3. ABC transpoters:

Another class of transporters are ABC type transporters, which are directly energized by MgATP and do not depend on the electrochemical force. Their substrates are organic anions formed by conjugation, e.g. to glutathione.

Examples of solute transport across vacuolar membrane in plant cells:

Transport of products of primary metabolites:

The various types of Primary metabolites could be:

1. Carbohydrates: Sucrose uptake occurs by facilitated diffusion in leaf vacuoles. Later it was also observed that active transport of sucrose takes place for vacuoles isolated from sugar cane cell cultures, which accumulates sucrose at concentrations comparable to those in the stalk tissue and tomato fruit vacuoles. Furthermore, it was also found that sucrose transport was stimulated by MgATP and to occur via a sucrose/H+ antiport in red beet. Larger carbohydrates such as stachyose, which is present in large quantities in *Stachys sieboldi*, may also be accumulated in the vacuole by proton antiport mechanisms. Many sugar alcohols also found in plants accumulate within the vacuoles. Transport of sorbitol across the tonoplast appears to be ATP-dependent in case of immature apple fruit tissue. Transport experiments suggest that mannitol crosses the tonoplast by facilitated diffusion.

2. Amino acid:

The first amino acid transport system was observed in barley plants. These are carriers or channles which are modulated by free ATP (but not by MgATP) which induces inward as well as outward fluxes of all amino acids tested.

3. Organic acids:

With context to organic acids, malate transport across the vacuolar membrane has been studied most intensively. This is due to the central role of malate in plant metabolism. The uptake of maltate is mainly governed by the electrical component of the electrochemical potential generated by the proton pumps. This channel also mediates uptake of succinate, fumarate, and oxaloacetate. The malate channel is not affected by cytosolic Ca2+ or ATP and it is a 32 kDa subunit protein. Citrate crosses the tonoplast using the same transporter as malate.

4. Inorganic anions:

The H+ pumps generate a positive potential inside the vacuole, which is the driving force for anion movements. Anion-dependent dissipation of a proton-pump generated by anions revealed that NO_3^- permeates more rapidly than Cl^- and $SO4^-$ whereas HPO_4^{2-} crossed the tonoplast considerably slowly.

Chloride:

An ATP-dependent Cl^- uptake was studied in barley mesophyll vacuoles. Later a vacuolar Cl^- channel (VCL) was identified in *Vicia faba* guard cells which is activated by a calcium dependent protein kinase (CDPK) in the presence of ATP and $Ca2^+$ and, to a weaker extend (22%), by protein kinase A. The VCL channel was activated at physiological potentials enabling Cl^- uptake into vacuoles.

Nitrate:

Amongst the anions it exhibits the highest permeability through the vacuolar membrane. It was concluded in one of the experiment that a membrane potential driven nitrate transporter, a NO_3^-/H^+ antiporter is present on the tonoplast.

Sulphate:

Using tonoplast vesicles, it has been shown that $SO4^{2-}$ and HPO_4^{2-} anions cross the tonoplast slowly as compared to NO_3^- or Cl^-. It has been found that SO_4^{2-} uptake is stimulated by Mg^+-ATP.

Phosphate:

Pi starvation leads to an efflux of Pi from the vacuole. It has been shown that Pi concentrations in the cytosol are maintained at a constant level in *Acer pseudoplatanus* cells using ^{31}P NMR.

5. Inorganic cation:

The membrane potential of the cytosol with respect to the vacuole is negative (20–40 mV). This implies that cations are excluded from the vacuole unless transport is coupled to an energy-dependent uptake mechanism.

Potassium:

Several channels exhibiting potassium permeability have been described. The first channel demonstrated for vacuolar membrane was called SV (slow activating vacuolar) channel. This channel is a slow activated channel and is associated with Ca^{2+} and calmodulin-induced K^+ and Ca^{2+} fluxes. These channels have been reported for the permeability of Na^+ if Ca^{2+} concentrations are increased by a signal. Secondly, FV (fast vacuolar) channel activates instantaneously in response to voltage changes. These channels may allow the release of K^+ at low Ca^{2+} concentrations. Thirdly, the vacuolar K^+ (VK) channel is activated instantaneously but it can be distinguished from the FV channel. It is voltage independent and fully activated at low cytosolic pH.

A K^+/H^+ antiport mechanism has been also reported for tonoplast enriched fractions from zucchini, *Brassica napus* hypocotyls, and *Atriplex*.

Sodium:

Na^+ accumulation is accompanied by vacuolar alkalinization in barley roots. This was established by using NMR spectroscopy.

Calcium:

Calcium plays a central role in signal transduction and higher concentrations are observed in apoplast and within the vacuole. An energized, highly specific calcium uptake by the vacuole is, therefore, a prerequisite for maintaining a low cytosolic calcium concentration. P-type Ca-ATPases have been identified at the plasma membrane, the ER, and the vacuolar membrane. A Ca^{2+} pump called a Ca^{2+}/H^+ antiporter has been demonstrated in vacuolar membrane fractions. This antiporter exhibits a far lower affinity

than the Ca^{2+}-ATPase. Also, a vacuolar voltage gated Ca^{2+} channel (VVCa) has been reported which is activated on membrane hyperpolarization.

Magnesium:

The presence of a Mg^{2+}/H^{+} antiporter has been described for the vacuole-like lutoids of *Hevea brasiliensis* and tonoplast vesicles isolated from maize roots.

Heavy metals:

Plants need some heavy metals such as Cu^{2+} or Zn^{2+} as micronutrients. Therefore, they need to be transported and a large portion of the heavy metals absorbed by the cell is usually concentrated within the vacuole. A vacuolar Cd^{2+}/H^{+} antiport activity has been demonstrated. However, it is known that plants form chelates with heavy metals by synthesizing phytochelatins (PCs), and these PCs can be transported into vacuoles of *Schizosaccharomyces* as apoPC or as PC-Cd complexes by ABC transporters. Vacuoles of higher plants are also known to transport phytochelatins.

Transport of products of the secondary metabolites: Involvement of secondary energized transporters and directly energized, ABC-type transporters

Plants synthesize an huge number of secondary metabolites and many of these have been found to be exclusively localized in the vacuole. The electrochemical gradient established by the two vacuolar proton pumps is used by the secondary energized transporters as a source of energy. It was demonstrated that the ΔpH was essential for the uptake of a number of phenolics, such as esculin, *o*-coumaric acid glucoside, apigenin- 7-(6-*O*-malonyl) glucoside, and anthocyanins from carrot. Recently it became evident that in addition to transporters depending on the proton motive force, directly energized transporters are also present on the vacuolar membrane. The first demonstration for a directly activated transport of solutes into the vacuole was provided for glutathione conjugates. Flavonoid glucuronides, a secondary plant compounds in rye vacuoles are transported by directly energized transport processes. Furthermore, studies with lucifer yellow, a sulfonated compound also indicates that sulfonated and sulfated secondary compounds cross the tonoplast by direct energization.

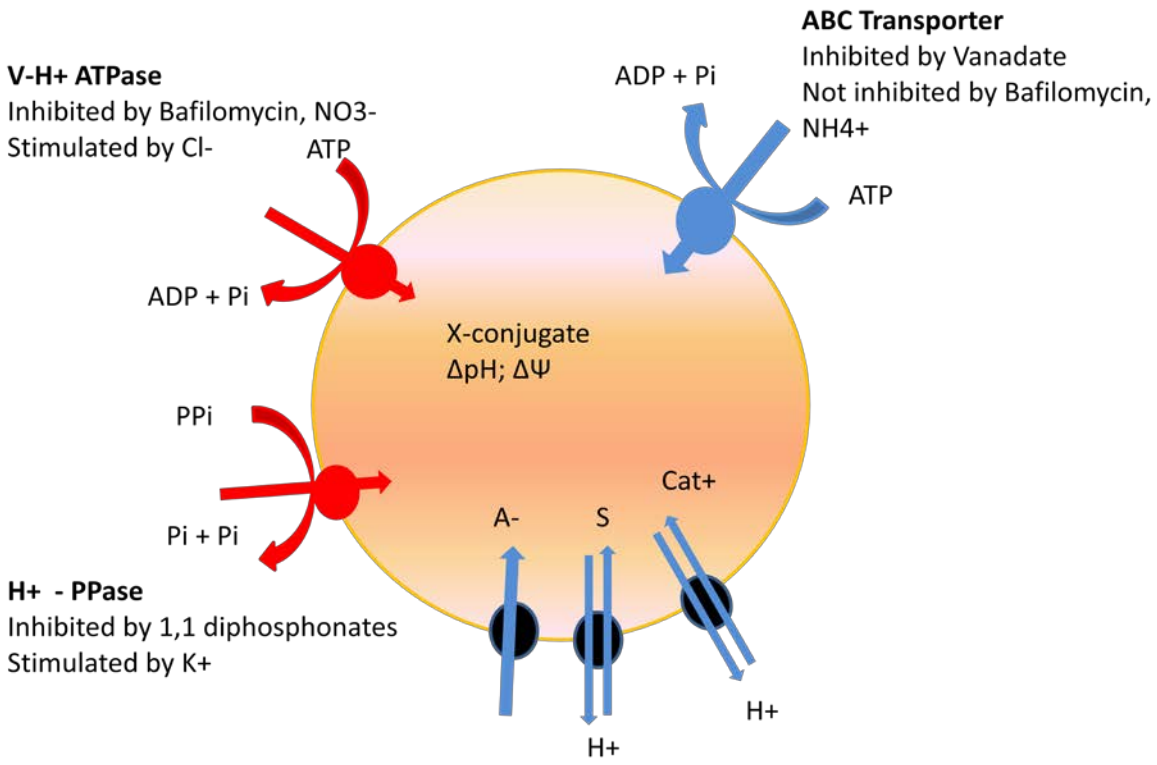


Figure 7: Proton pumps establishing a electrochemical gradient (red), secondary energized uptake mechanisms (green), and directly energized, ABC-type transporters (blue) of the plant vacuole. S, neutral solute; A–, anion; cat+, cation; X-conjugate, conjugate of a compound X (secondary metabolite or xenobiotic) with a hydrophilic compound such as glucose, glutathione, an amino acid, malonate, or sulphate.

Interesting facts:

- During endocytosis, these intra-lysosomal membranes are formed and prepared for digestion by a lipid-sorting process during which their cholesterol content decreases and the concentration of the negatively charged bis(monoacylglycero)phosphate increases.
- Lysosomal enzyme disorders contribute to several human diseases, either due to genetic defects in its enzyme expression or the escape of lysosomal enzymes (lysozymes) into extralysosomal medium.
- Permeabilization of lysosome, has been shown to initiate a cell death pathway or apoptosis.

Questions

1. Which pump is present in lysosomal membrane?
    a. P-class pump
    b. ABC transporter
    c. V-class pump
    d. F-class pump
2. The pH of the lysosomal compartment is
    a. 4
    b. 4.6
    c. 5
    d. 5.6
3. Which of the following correctly matches an organelle with its function?
    a. mitochondrion….photosynthesis
    b. Nucleus….cellular respiration
    c. Ribosome….manufacture of lipids
    d. Lysosome….movement
    e. Central vacuole….storage
4. Lysosomes are reservoirs of
    a. Hydrolytic enzymes
    b. Fat
    c. Secretory glycoproteins
    d. RNA
5. A function of lysosomes is
    a. Syntheisis
    b. Hydrolysis
    c. Replication
    d. Respiration

6. For digestion to occur in a vacuole, the vacuole must first fuse with
    a. Nucleus
    b. Ribosome
    c. Lysosome
    d. Golgi bodies
7. Lysosomes can be expected to be present in large numbers in cells which
    a. Have cilia.
    b. Produce centrioles.
    c. Are actively dividing.
    d. Carry out phagocytosis.
8. For digestion to occur in a vacuole, the vacuole must first fuse with
    a. Nucleus
    b. Ribosome
    c. Lysosome
    d. Golgi body
9. The proton gradient between the lysosomal lumen (pH ≈4.5–5.0) and the cytosol (pH ≈7.0) depends on ATP production by the cell.
    a. True
    b. False
10. What is the function of permanent vacuole?
    a. Supports and protects the cell
    b. Controls what enters and leaves the cell
    c. Controls the cell
    d. Stores water and mineral ions
    e. Stores water and mineral ions
11. Vacuole is surrounded by membrane called

a. Tonoplast
b. Chloroplast
c. Plasma membrane

18. What are the most abundant components of lysosomal membrane?
19. Write the composition and functions of vacuolar membrane.

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Module 3 Lecture 4
ATP dependent proton pumps

Proton pump

The proton pump is a transmembrane protein that is capable of transport of protons across the cell membrane, mitochondria and other cell organelle.

ATP dependent proton pumps

ATP dependent proton pumps or transport ATPase are the pumps that transport H^+ ions against their concentration gradients. These pumps are transmembrane proteins with one or more binding sites for ATP located on the cytosolic face of the membrane and these proteins are called ATPases. They normally do not hydrolyze ATP into ADP and Pi unless H^+ ions are simultaneously transported. Because of this tight coupling between ATP hydrolysis and transport, the energy stored in the phosphoanhydride bond is not dissipated but rather used to move ions or other molecules uphill against an electrochemical gradient.

ATP dependent proton pumps can be categorized into different classes. Generally, ATP dependent proton pumps are divided into 4 classes:

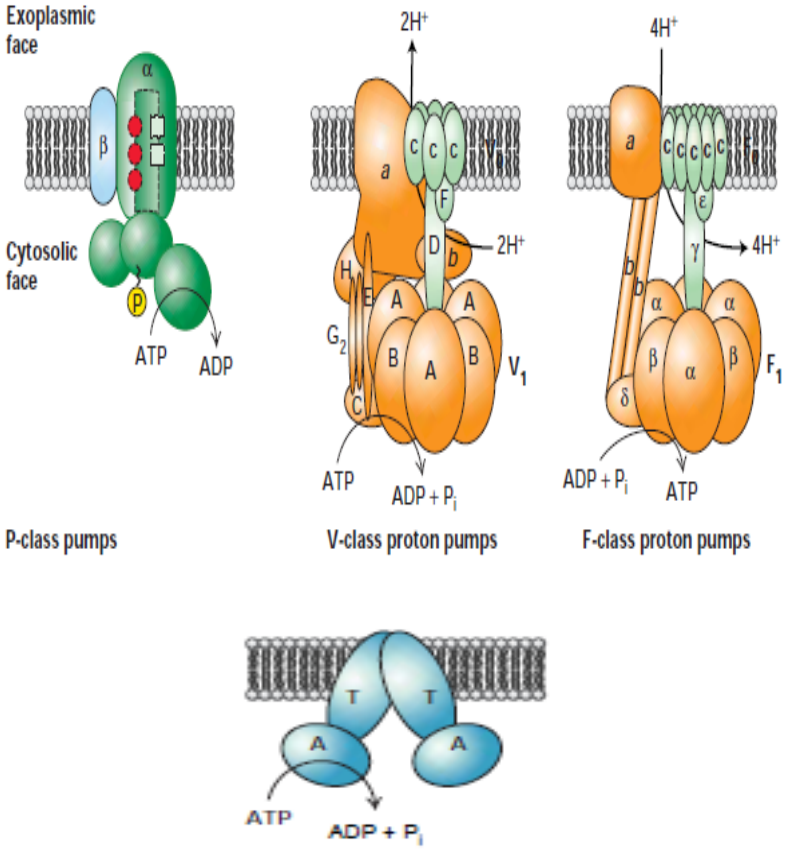

Figure 1: Different types of ATP dependent proton pumps

1. **P-class ion pumps:**

These are multipass transmembrane proteins having two identical catalytic α-subunits that contain an ATP binding site. Some have two smaller β-subunits that usually have regulatory functions. During the transport process or pumping cycle at least one of the α-subunit must be phosphorylated and the H^+ ions are thought to move through the phosphorylated subunit. This class includes many ion pumps that are responsible for setting up and maintaining gradients of Na^+, K^+, H^+ and $Ca2^+$ across the cell membrane.

a) The common P-type pump is mostly found in parietal cells of the mammalian stomach which transport protons (H^+ ions) out of cell and K^+ ions into the cell and is mainly responsible for the acidification of the stomach contents. The pump is known as H^+/K^+ ATPase. It is a heterodimeric protein. The H^+/K^+ ATPase transports one H+ from the cytoplasm of the parietal cell in exchange for one K^+ retrieved from the gastric lumen. As an ion pump the H^+/K^+ ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transferred from ATP to the H^+/K^+ ATPase during the transport cycle.

b) Another example of P-type pump is Na^+/K^+ ATPase in the plasma membrane, which generates low cytosolic Na^+ and high cytosolic K^+ concentration which is typical of animal cells (discussed in earlier lecture).

c) Certain Ca^{2+} ATPase pump Ca^{2+} ions out of the cytosol into the external medium while others pump Ca2+ from the cytosol into the endoplasmic recticulum or into the specialized sarcoplasmic reticulum, which is more common in muscle cells (discussed in earlier lecture).

2. **F-class ion pumps:**

The F class ion pumps contain different transmembrane and cytosolic subunits. They are known for only transport of protons, in a process that does not involve phosphoprotein intermediate. They generally behave as reverse proton pump by synthesizing ATP from ADP and Pi by movement of protons from the exoplasmic to the cytosolic face of the membrane down the proton electrochemical gradient. Therefore, these pumps are also known as ATP synthases or F_0F_1 complex. F-class ion pump is most common in bacteria, yeast and animal mitochondria and also in chloroplast.

The F_0F_1 complex is a multi-protein having two components F_0 and F_1. Both are multimeric proteins. The F_0 component contains three integral membrane proteins named a, b and c. The a and two b subunits are linked tightly but not to the donut- shaped ring of c subunits. And the F_1 component is water soluble complex of five distinct polypeptides with the composition $\alpha 3\beta 3\gamma\delta\varepsilon$. The lower part of the F_1 γ subunit is a coil which fits into the centre of the c-subunit ring of F_0 and appears rigidly attached to it. The F_1 ε subunit is rigidly attached to γ and also forms rigid contacts with c subunits. The F_1 and subunits associate in alternating order to form a hexamer αβαβαβ. The F_1 δ subunit is permanently linked to one of the F_1 subunits and also to the b subunit of F_0. Thus the F_0 a and b subunits and the δ subunit and (αβ)3 hexamer of the F_1 complex form a rigid structure anchored in the membrane. The rodlike b subunits form a stator that prevents the (αβ)3 hexamer from moving while it rests on the γ subunit.

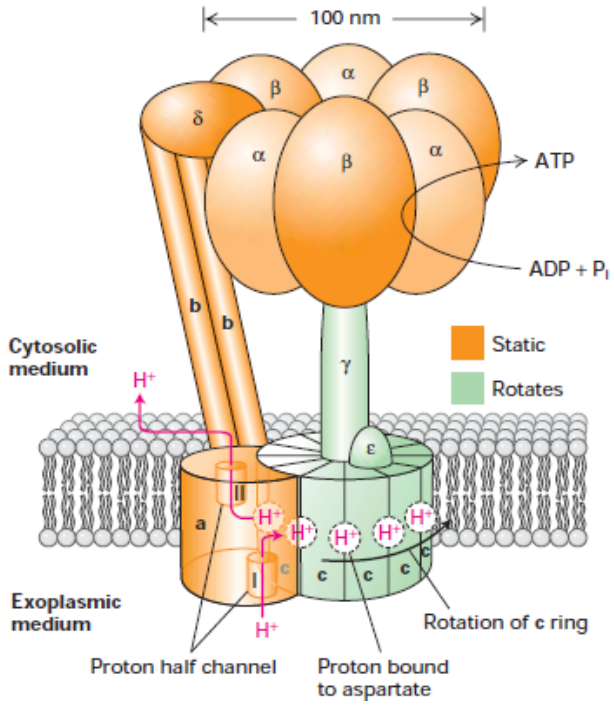

Figure 2: Model of the structure and function of ATP synthase (the F_0F_1 complex) in the bacterial plasma membrane.

The F_0 portion is built of three integral membrane proteins: one copy of a, two copies of b, and on average 10 copies of c arranged in a ring in the plane of the membrane. Two proton half-channels lie at the interface between the a subunit and the c ring. Half-channel I allows protons to move one at a time from the exoplasmic medium and bind to aspartate-61 in the center of a c subunit near the middle of the membrane. Half-channel II (after rotation of the c ring) permits protons to dissociate from the aspartate and move into the cytosolic medium.

3. V-class ion pumps:

It is almost similar to F-class ion pumps in structure and function. But none of their subunits are related to each other. F-class pumps operate in reverse direction to F-class. These pumps generally function to maintain low pH of plant vacuoles and lysosome and other acidic vesicles in animal cells by pumping protons from cytosolic to exoplasmic face (inside) of membrane against the proton electrochemical gradient. The acidification between the lysosomal lumen and cytosol lumen can be maintained by production of ATP by cells.

These V-class proton pumps contain two domains: a cytosolic hydrophilic domain (V_1) and a transmembrane domain (V_0) with multiple subunits in each domain. Binding and hydrolysis of ATP by the B subunits in V_1 provide the energy for pumping of H^+ ions through the proton-conducting channel formed by the c and a subunits in V_0. These V-class proton pumps are not phosphorylated and dephosphorylated during proton transport.

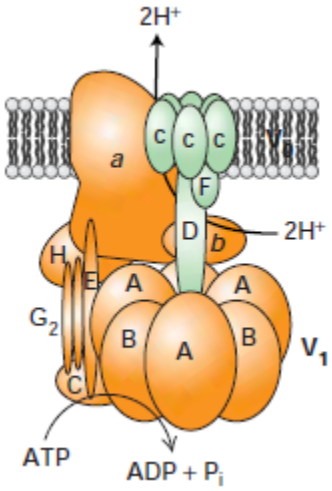

Figure 3: V-class proton pump

These protons cannot acidify by themselves because a net movement of electric charge occurs. Only a few protons build up positive H^+ ions on exoplasmic face (inside) and for each H^+ pumped across, a negative ion will be left behind on cytosolic face, building negatively charged ions. These oppositely charged ions attract each other on opposite faces of the membrane, generating a charge separation, or electric potential, across the membrane. If more protons pumped, the excess positive ions on exoplasmic face repels other H^+ ions and prevents pumping of extra proton long before a significant transmembrane H^+ concentration gradient had been established. If the organelle lumen or

the extracellular space has to be acidified, the net movements of protons must be accompanied either by movement of equal number of anion eg Cl^- in same direction or my movement of different cation in the opposite direction. The first process occurs in lysosomes and plant vacuoles whose membrane contains V-class H^+ ATPase and anion channels for Cl^- movement. And the second process is observed in the lining of the stomach which contains a H^+/K^+ ATPase and pumps one H^+ outward and one K^+ inward.

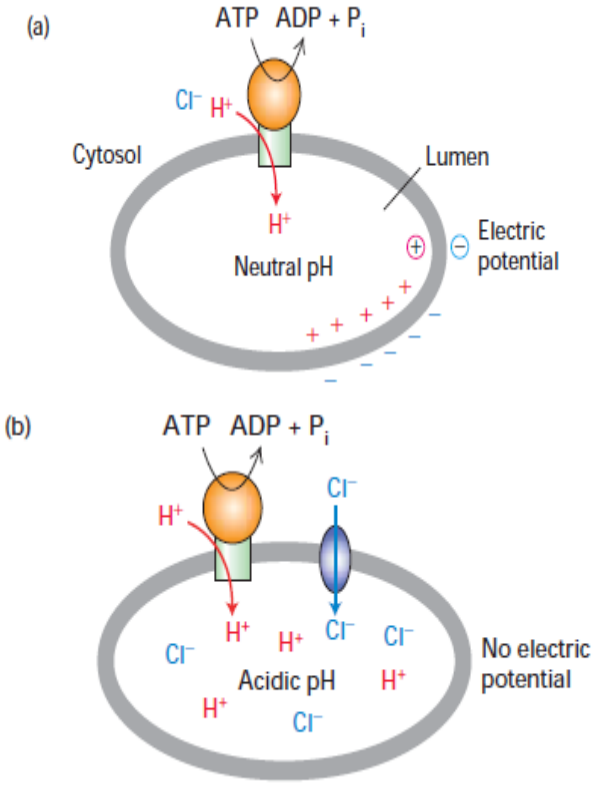

Figure 4: Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change in the intraluminal pH. (b) If the organelle membrane also contains Cl^- channels, anions passively follow the pumped protons, resulting in an accumulation of H^+ ions (low luminal pH) but no electric potential across the membrane.

4. ABC (ATP binding cassettes) superfamily:

The final class of ATP-powered pumps is a large family of multiple membranes. This class includes several hundred different transport proteins found in all organisms ranging from bacteria to mammals. Each ABC protein is specific for single substrate or group of related substrate, which may be ions, sugars, amino acids, phospholipids, cholesterol, peptides, polysaccharides or proteins. All ABC transport protein share a structural organization consisting of four core domains: two transmembarne (T) domains, forming

the passageway through which transported molecules cross the membrane and two cytosolic ATP-binding (A) domains. The core domains are generally present in separate polypeptides which are more common in bacterial cell. In others, the core domains are fused into one or two multidomain polypeptides. ATP binding leads to dimerization of two ATP-binding domains and ATP hydrolysis leads to their dissociation. Theses structural changes in the cytosolic domains are thought to be transmitted to the transmembrane segments, driving cycles of conformational changes that alternately expose substrate-binding sites on one side of the membrane and then on the other. In this way, ABC transporters use ABC binding and hydrolysis to transport small molecules across the bilayer. Some common example of ABC transporters are found in bacterial plasma membranes which contain amino acid, sugar and peptide transporters. These cells use H^+ gradient across the membrane to pump variety of nutrients into the cell. It is also present is mammalian plasma membrane that contains transporters of phospholipids, small lipophillic drugs, cholesterol and other small molecules. One example of eukaryotic ABC transporters is multidrug resistance (MDR) protein which has the ability to pump hydrophobic drugs out of the cytosol. Overexpression of these MDR protein in human cancer cells, make the cells resistant to variety of chemically unrelated cytototoxic drugs.

Interesting facts:

- Valinomycin is a carrier for potassium.
- Lactose permease has been crystallized with thiodigalactoside (TDG), an analog of lactose.
- Adenine nucleotide translocase (ADP/ATP exchanger), which catalyzes 1:1 exchange of ADP for ATP across the inner mitochondrial membrane.
- The reaction mechanism for a P-class ion pump involves transient covalent modification of the enzyme.
- Gramicidin is an example of a channel. It is an unusual peptide, with alternating D and L amino acids. In lipid bilayer membranes, gramicidin dimerizes and folds as a right handed β-helix. The dimer just spans the bilayer.

Questions

1. The functional mechanism of P-class ion pumps is ..... by the ATP.
2. V-class pumps pumps exclusively .....
3. Substance concentration + electric potential = ... which determines the energetically favorable direction of transport a charged molecule across a membrane.
4. Differentiate among Transporters, pumps and channels.
5. Is calcium pump and ATP dependent proton pump are same?
6. Describe ABC (ATP binding cassettes) superfamily.
7. Differentiate between V class proton pump and P-class ion pumps.
8. What are F-class ion pumps? How do they differ from the other classes of ion pumps?
9. What is the main function of a V-class proton pump?
10. Give atleast three examples of ATP-binding cassettes.
11. Give a brief overview of the structural organization of the ABC transport proteins.

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Module 3 Lecture 5
Cotransport: Symport, Antiport

Transporters:

Transporters (also known as carriers) are the membrane proteins that transport a wide variety of ions and molecules across the lipid bilayer membrane.

Cotransporters:

Cotransporters are proteins that transport two different solutes such as glucose and amino acids simultaneously across the cell membrane against a concentration gradient. It mediates coupled reactions in which an energetically unfavorable reaction (uphill movement of molecules) is coupled to an energetically favorable reaction. Unlike ATPase pump, it uses the energy stored in electrochemical gradient. This is called secondary mediated active transport (discussed in earlier lecture). An important feature is that neither molecule can move alone; movement of both molecules together is obligatory, or coupled. One of the common example is the energetically movement of Na^+ ions into the cell across the plasma membrane driven both by its concentration gradient and by the transmembrane voltage gradient, which can be coupled to movement of the transported molecule (glucose) against its concentration gradient.

How cotransportersare differentiated from uniporters?

Both transporters share some common feature with respect tostructural similarities, operation at equivalent rates, and undergo cyclical conformational changes during transport of their substrates. They differ in that uniporters can only accelerate thermodynamically favourable transport down a concentration gradient, whereas cotransporters can harness the energy of a coupled favourable reaction to actively transport molecules against a concentration gradient.

Types of cotransports:

On the basis of movement of solutes, cotranporters can be divided into following categories:

1. **Symport:**When the transported molecule and cotransported ion move in the same direction, the process is called symport.
2. **Antiport:**When the transported molecule and cotransported ion move in the opposite direction, the process is called antiport.

Both the above mentioned cotranporter move one solute against its transmembrane concentration gradient. This movement is powered by coupling to the movement of second solute down its transmembrane concentration gradient.

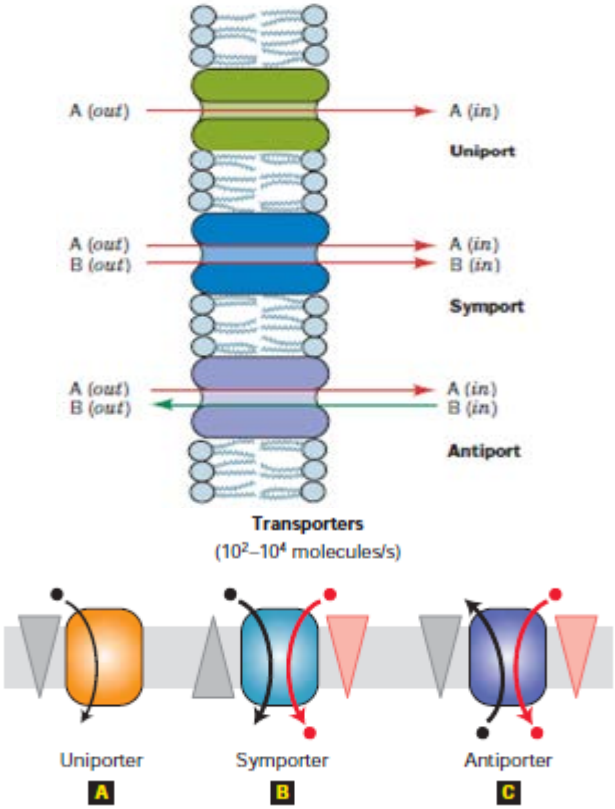

Figure 1: Transporters, which fall into three groups, facilitate movement of specific small molecules or ions.(A) Uniporters transport a single type of molecule down its concentration gradient. Cotransport proteins (symporters (B), and antiporters (C)) catalyze the movement of one molecule againstits concentration gradient (black circles), driven by movement of one or more ions down an electrochemical gradient (red circles). Differences in the mechanisms of transport by these three major classes of proteins account for their varying rates of solute movement.

On the basis of movement of ions, cotransporters can also be categorized into:

1. Cationcotransporter:Example of cation transporter is Na^+/H^+antiporter, which exports H^+ from cells coupled to the energetically favorable import of Na^+.
2. Anion cotransporter:Example of anion transporter is exchange of Cl^- and HCO_3^- across the plasma membrane.

Some common examples of cotranporter are:

1. **Oligosaccharide/H^+ symporter:**They are also known as LacYsymporter. It is most common in bacterial cell which has lactose permeaseLacY and functions as symporter. It uses the free energy released from translocation of H^+ down its electrochemical gradient to drive the accumulation of nutrients such as lactose against its concentration gradient. The H^+ gradient across the cytoplasmic membrane is established by the respiratory chain and by the action of F_1F_0-ATPase, which couples ATP hydrolysis to the export of protons from the cell. For LacY, the stoichiometry of lactose and H^+ translocation is 1:1, with both substances movement in the same direction. Thus, the lactose gradient can drive the uphill translocation of protons and generate an inward or outward H^+ gradient, depending on the direction of the lactose concentration gradient.

   Structure of Lac Y symporter: Structurally theLacYsymporter contains 12 transmembarne helices which are connected by hydrophilic loops and cytoplasmic N- and C- termini. There are two domains of 6 transmembranesegments each, forming a symmetrical structure. The hydrophilic cavity which lies in the centre of lipid bilayer forms the substrate binding site. This substrate binding site is accessible from either the intracellular or extracellular side of the membrane but never to both sides simultaneously. Protonation and binding of lactose in the outward-facing conformation induces a conformation change, resulting in inward-facing conformation. This structural arrangement involves binding of both substrates initially and allows for coupled and then simultaneous transport. Release of lactose and protons into the cell then induces a transition back to the outward-facing conformation. Hence it lowers the energy barrier between inward and outward-facing conformation and facilitates interconversion.

2. **Glycerol-3-phosphate transport (GlpT):**It is an antiporter that accumulates glycerol-3-phosphate into the cell for energy production and phospholipid synthesis. GlpT is an organic phosphate/inorganic phosphate exchange which is driven by Pi gradient. Similar to LacY, it has also symmetrical N- and C- terminal domains, each consisting of 6 transmembrane segments surrounding the substrate translocation pathway. It also works as same mechanism asLacY but glycerol-3-phosphate binds

and phosphate is released in the outward conformation and opposite occurs in the inward conformation.

3. **Na^+ linked symporter:**This symporter imports amino acid and glucose into the animal cells against the concentration gradient. Anexample is GLUT protein which imports glucose from the blood downs its concentration gradient. On the other hand, certain cells such as those lining the small intestine and kidney tubules, import glucose from intestinal lumen or forming urine against a large concentration gradient. Such cells utilize two Na^+/one glucose symporter, a protein that couples to import one glucose to import two Na^+. This symporter contains 14 transmembrane α helices with both its N- and C- termini extending in the cytosol. The N-terminal portion of the protein, including helices 1–9, is required to couple Na^+ binding and influx to the transport of glucose against a concentration gradient.

The following steps occur for transport of Na^+ and glucose:

1. Simultaneous binding of Na^+ and glucose to the conformation with outward-facing binding sites
2. A second conformation generates with inward facing side
3. Dissociation of Na^+ and glucose into the cytosol
4. The protein reverts back to original outward-facing conformation, ready to transport the next substrate

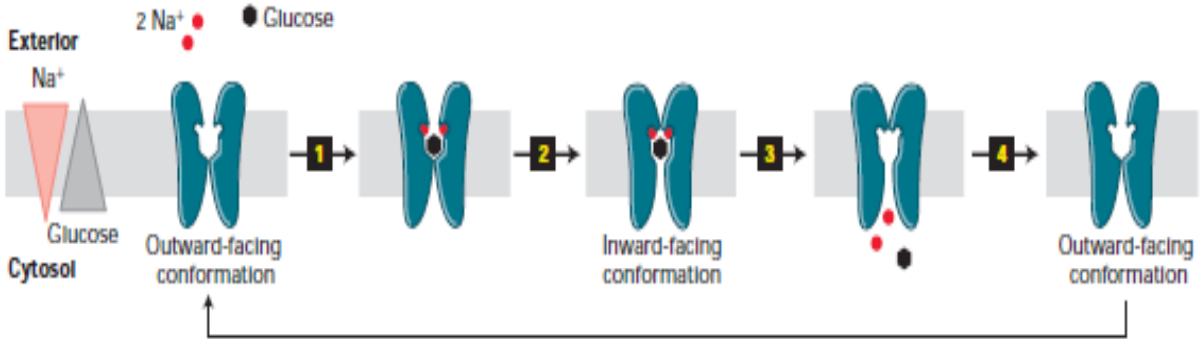

Figure 2: Operational model for the two-Na^+/one glucose symporter.

The overall reaction is:

$2Na^+$ (out) + Glucose (out) ↔ $2Na^+$(in) + Glucose (in)

The free energy change for the symport transport of two Na^+ and one glucose is the sum of the free energy changes generated by the glucose concentration gradient (1 molecule transported), the Na^+ concentration gradient (2 Na^+ ions transported), and the membrane potential(generated by two Na^+tranported):

$$\Delta G = RT\ in\ \frac{[glucose_{in}]}{[glucose_{out}]} + 2RT\ in\ \frac{[Na^+_{in}]}{[Na^+_{out}]} + 2FE$$

When ΔG=0 and the free energy released by movement of Na^+ into cells down its electrochemical gradient has a free energychange Δ*G* of about -3 kcal per mole of Na^+ transported.Thus the Δ*G* for transport of two moles of Na^+inward is about -6 kcal. By substituting in above equation, the ratio of glucose (in)/glucose (out) =30,000. Thus if 2 moles of Na^+ inward then it generates an intracellular concentration of glucose of 30,000 times more than extracellular glucose. Thus if only one Na^+ ion were imported per glucose molecule, then the available energy could generate a glucose concentration gradient (inside- outside) of only about 170-fold. Thus by coupling the transport of two Na^+ ions to the transport of one glucose, the two-Na^+/one-glucose symporter permits cells to accumulate a very high concentration of glucose relative to the external concentration.

4. **Na^+ linked antiporter:**A cotranporter, $3Na^+/Ca2^+$antiporter in cardiac muscle cell maintain a low concentration of Ca^{2+} in cytosol. The reaction for this cation transporter is:

$3Na^+$ (out) + Ca^{2+}(in) ↔ $3Na^+$ (in) + Ca^{2+}(out)

The movement of three Na^+ ions is required to power the export of one Ca^{2+} ion from the cytosol with a [Ca^{2+}] of ≈2 * 10^{-7} M to the extracellular medium with a [Ca^{2+}] of 2 * 10^{-3} M, a gradient of some 10,000-fold form. By lowering cytosolic Ca^{2+}, operation of the Na^+/Ca^{2+}antiporter reduces the strength of heart muscle contraction.

Function of cotransporter:

1. **Regulation of cytosolic pH:**

The anaerobic metabolism of glucose yields lactic acid whereas the aerobic metabolism yields CO_2, which reacts with water to form carbonic acid (H_2CO_3). This weak acid dissociates yielding H^+ ion or proton. If these excess protons werenot removed from cells, then the cytosolic pH would drop and will be unfavourable to cellular fractions. Hencecotransports are required to remove excess of protons. One is Na^+HCO3^-/Cl^- antiport imports one Na^+ down its concentration gradient together with one $HCO3^-$ in exchange for export of one Cl^- against its concentration gradient. The enzyme named carbonic anhydrase catalyzes dissociation of imported HCO_3^- ions into CO_2 and OH^- by the reaction:

$HCO^{3-} \leftrightarrow CO_2 + OH^-$

$OH^- + H^+ \rightarrow H_2O$

Then CO_2 diffuses out of the cell and OH^- ions combine with intracellular protons, forming water. Thus the overall action of this transport is to consume cytosolic H^+ ions, thereby raising cytosolic pH.

Secondly Na^+/H^+antiporter plays an important role in raising cytosolic pH which couples entry of one Na^+into the cell down its concentration gradient to export of one H^+ ion.

Thirdly, anion antiporterthatcatalyzes the one-for-one exchange of HCO_3^- and Cl^- across the plasma membrane. At high pH, this Cl^-/HCO_3^-antiporterexports HCO_3^- in exchange for Cl^-, thus lowering the cytosolic pH. The import of Cl^- down its concentration gradient (Cl^-(medium) >Cl^-(cytosol)) powers the reaction.

The activity of all these antiports depends upon pH.The two antiportersthat operate to increase cytosolic pH are activated when the pH of the cytosol falls. Similarly, a rise in pH above 7.2 stimulates the Cl^-/HCO_3^-antiporter, leading to a more rapid export of HCO_3^- and decrease in the cytosolic pH. In this manner the cytosolic pH of growing cells is maintained very close to pH 7.4.

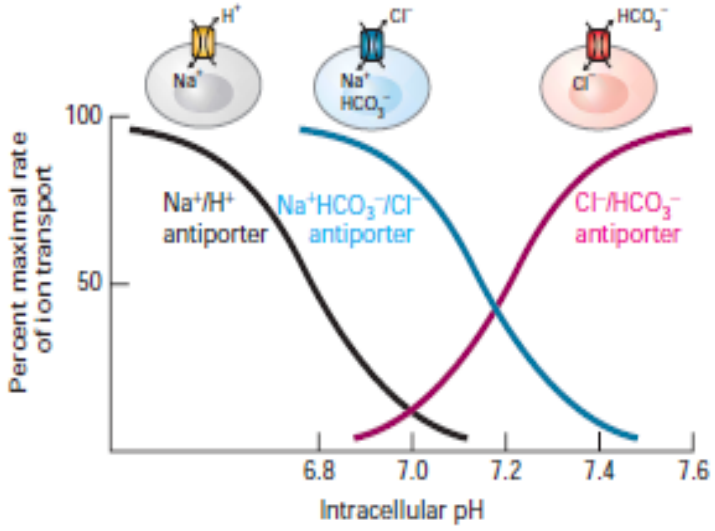

Figure 3: The activity of membrane transport proteins that regulate the cytosolic pH of mammalian cells changes with pH.Direction of ion transport is indicated above the curve for each protein

2. Accumulation of metabolites and ions inplant vacuoles:

The lumen of plant vacuoles is much more acidic(pH 3 to 6) than is the cytosol (pH 7.5). The vacuolarmembrane contains Cl^- and NO_3^- channels that transportthese anions from the cytosol into the vacuole against their concentration gradients and is drivenby the inside-positive potential generated by the H^+ pumps.

One more example is proton/sucrose antiporterin the vacuolar membrane that accumulate sucrose in plant vacuoles. During photosynthesis, sucrose is generated and stored in vacuole. But during night these stored sucrose moves into the cytoplasm and is metabolized to CO_2 and H_2O with generation of ATP from ADP and Pi. The inward movement of sucrose is governed by movement of H^+ which is favoured by its concentration gradient(lumen cytosol) andby the cytosolic-negative potential across the vacuolar membrane.

Uptake of Ca^{2+} and Na^{+} into the vacuolefrom the cytosol against their concentration gradients issimilarly mediated by proton antiporters.

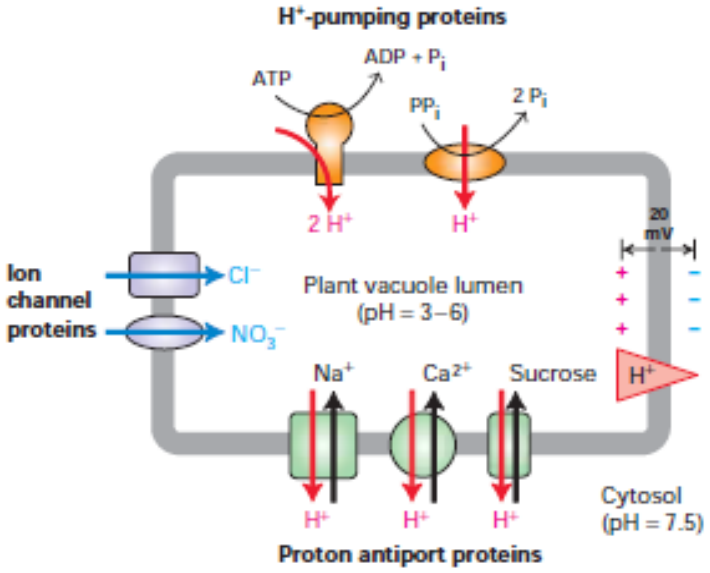

Figure 4: Accumulation of ions and sucrose by the plant vacuole.The vacuolar membrane contains two types of proton pumps (orange): a V-class H^+ ATPase (*left*) and a pyrophosphate-hydrolyzing proton pump (*right*) that differs from all other ion-transport proteins and probably is unique to plants. These pumps generate a low luminal pH as well as an inside positive electric potential across the vacuolar membrane owing to the inward pumping of H^+ ions. The inside-positive potential powers the movement of Cl^- and $NO3^-$ from the cytosol through separate channel proteins (purple). Proton antiporters (green), powered by the H+ gradient, accumulate Na^+, $Ca2^+$, and sucroseinside the vacuole.

Interesting facts:

- Na+/K+/2Cl- symporter in the loop of Henle in the renal tubules of the kidney transports 4 molecules of 3 different types; a sodium ion (Na+), a potassium ion (K+) and two chloride ions (2Cl-).
- In the roots of plants, the H+/K+ symporters are only one member of a group of several symporters/antiporters that specifically allow only one charged hydrogen ion (more commonly known as a proton) and one charged K+ ion. This group of carriers all contribute to modulate the chemiosmotic potential inside the cell.

Questions:

1. An example of cation transporter is ..... and an example of anion transporter is .....
2. The activity of antiports depends upon .....
3. Glycerol-3-phosphate transport is
   a. symport
   b.uniport
   c. antiport
   d. ATP dependent transport.
4. What are transporters and cotransporters?
5. Differentiate between symport and antiport.
6. Describe Na+ linked antiporters.
7. What are uniports?
8. Describe the mechanism of accumulation of metabolites and ions in plant vacuoles.
9. What are the functions of cotransporters?
10. What are LacYsymporters?Describe their structure.

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Module 3 Lecture 6

Transport in prokaryotic cells

Transport in prokaryotic cells: The transport system of a cell depends upon the substrate requirements of the cell, the bioavailability of the substrate and the environmental conditions. It also depends on the metabolic features and physiological state of the organism. Prokaryotic cells have simpler structure and mostly are unicellular. Hence their transport system is different from higher eukaryotes. Here we will study the transport in prokaryotic cells with respect to bacteria.

Membranes in bacteria: Membranes play a major role in transport. The different types of membrane found in bacteria are:

1. **Cytoplasmic membrane, in all bacteria**

The inner membrane of a cell is different from outer membrane of a cell. And the space between these membranes is called periplasam. The membrane is symmetrical, with an equal distribution of lipids (exclusively phospholipids, mainly phosphatidylethanolamine, phosphatidylglycerol and cardiolipin) among the inner and outer surface. Some of the functions associated with cytoplasmic membrane which has role in transport mechanism of cell are:

- Osmotic and permeability barrier
- Presence of transport system for various solutes
- Synthesis of membrane lipids
- Assembly and synthesis of extracytoplasmic proteins
- Coordination of DNA replication and segregation with septum formation and cell division
- Energy generation functions such as electron transport system, establishment of photon motive force and transmembrane ATP-synthesizing ATPase

2. Outer membrane, mostly in gram negative bacteria

The outer membrane is highly asymmetrical, with the inner leaflet, oriented to the periplasam. The outer leaflet, facing the external medium contains lipopolysaccharides (LPS) constituting of three parts: lipid A as anchor, the core oligosaccharide functioning as spacer element and an O-specific polysaccharide consisting of oligosaccharide repeating unit. Proteins are the integral components or associated with OM. Some of the functions associated with OM are:

- Involved in transport mechanism.
- Contribution of membrane integrity
- Serves as anchor for flagellae, fimbriae and pili. Hence important for locomotion, cell-cell interaction, adhesion to surfaces and formation of biofilms.
- LPS are major antigenic determinants, preventing entry of cell-damaging components and serve as receptor for a number of bacteriophages.

3. Cell walls of gram positive bacteria

The cell walls of gram positive bacteria are devoid of outer membrane but possess a thick murein layer. In Gram-positive bacteria, teichoic acids are covalently linked to peptidoglycan. Teichoic acids are polyol phosphate polymers with a strong charge. They are strongly antigenic and absent in Gram-negative bacteria. In some species, teichuronic acids are found as lipoteichonic acids which are composed of glycerol teichoic acid linked to glycolipid. Additional wall components can be polysaccharides, lipids and proteins.

4. Membrane that forms envelope in mycobacteria

Membrane that forms envelope in mycobacteria is characterized by their low permeability, which contributes resistance of the microbes to therapeutic agents. It contains two special features: an outer lipid barrier based on a monomer of mycolic acids and a capsule-like coat of polysaccharide and protein. The cell wall contains a large amount of C_{60}-C_{90} fatty acids, mycolic acids that are covalently linked to arabinogalactan.

Transport process:

Transport process can be divided into four classes on the basis of driving forces and modes of energy coupling (Milton H. Saier et al., 2000):

1. **Passive diffusion:**

The passive diffusion occurs along the concentration gradient and without the use of metabolic energy. Some solutes pass the permeability barrier of a lipid bilayer by passive diffusion. This is valid for small apolar molecules and small slightly polar but uncharged molecules like water and dissolved gases. Some other solutes are also transported via channels or channel type proteins to overcome in a diffusion-controlled movement.

2. **Primary active transport:**

Primary active transport is characterized by coupling translocation of solute directly to a chemical or photochemical reaction. Primary source includes pyrophosphate bond hydrolysis, methyl transfer and decarboxylation. Transport of Na^+ and K^+ by carrier protein, Na^+ - K^+ ATPase, is the most common example of primary active transport.

3. **Secondary active transport:**

In secondary active transport the translocation step across the membrane is coupled to the electrochemical potential of a given solute. The solute chemical potential created by primary active transport systems is the driving force, which allows an uphill transport of another solute, against its own concentration gradient. The uptake can be mediated as uniprot, symport and antiport. A common example of secondary active transport is the symport of Na^+ and glucose. The transmembrane protein Na^+ - glucose transporter, acts as a carrier, allows Na^+ and glucose to enter the cell together. The Na^+ flow down their concentration gradient while the glucose molecules are transported against their concentration gradient into the cell. Later the Na^+ is pumped back out of the cell by the Na^+ -K^+ ATPase.

4. Phosphophenolpyruvate: sugar phosphotransferase system (Pts):

Pts translocation process is exclusive to bacterial species which phosphorylates its carbohydrate substrates during transport.

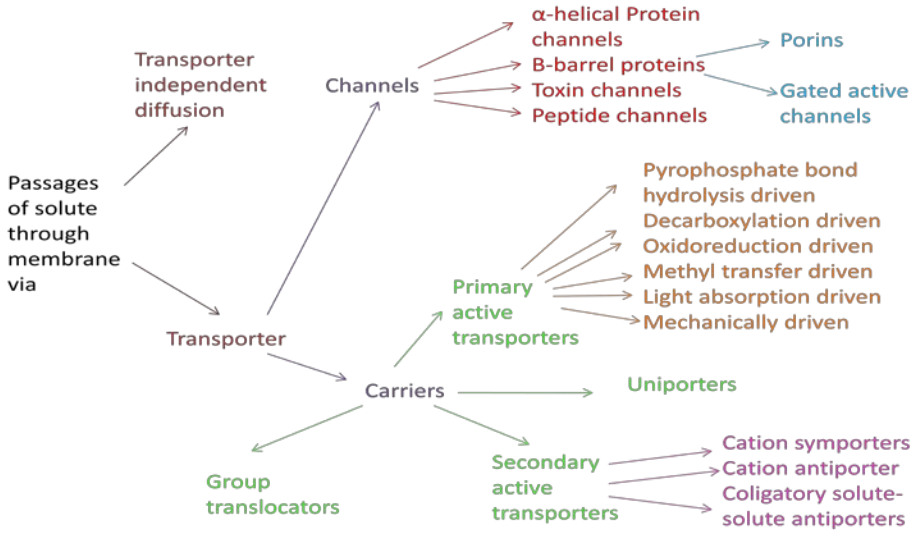

Figure 1: Classification of major types of transport mechanism across biological membranes based on function and phylogeny

The major transport mechanism based on the mode of transport, energy coupling mechanism and substrate specificity and protein phylogenetic grouping that reflects structure, function and its mechanism are:

1. Transport independent diffusion

   Gases (such as O_2 and CO_2); hydrophobic molecules (such as benzene) and small polar but uncharged molecules (such as H_2O and ethanol) are able to diffuse across the plasma membrane.

2. Transport dependent diffusion

This transport takes allows polar and charged molecules such as carbohydrates, amino acids, nucleotides and ions, to cross the plasma membrane.

a. Channels

Some examples are voltage gated channels which open in response to change in electric potentials; others called ligand gated channels open in response to the binding of the ligand.

(i) α-helical protein channel

(ii) β-barrel proteins

(iii)Toxin channels

(iv) Peptide channels

b. Carriers

The common example is the movement of glucose mediated by carrier protein called glucose transporter (GLUT).

(i) Primary active transport: Mechanically driven, Light absorption driven, Methyl transfer driven, Oxidoreduction driven, Decarboxylation driven, Pyrophosphate bond hydrolysis driven

(ii) Uniporters

(iii) Secondary active transport: Cation symporters, Cation antiporters, Solute solute antiporters

(iv) Group translocators

Some examples of transporter in bacteria can be studied with the following examples:

1. Phosphate transport:

Two major phosphate transport systems are involved in bacteria:

a. Low affinity Pit (phosphate inorganic transport) system

b. High affinity Pst (phosphate specific transport) system

Pit consists of a single trans-membrane protein and is constitutively expressed secondary transporter. This system is characterized by uptake of phosphate which is in the form of a neutral metal phosphate complex and is in symport with a proton. This transport of phosphate is achieved by binding and dissociation of the neutral metal phosphate complex and H^+ on the outer and inner surface of the trans-membrane protein carrier protein. Pit is reversible and therefore allows both import and export of divalent ions and phosphate. Also it has a relatively low specificity for both phosphate and arsenate (toxic analogue of phosphate).

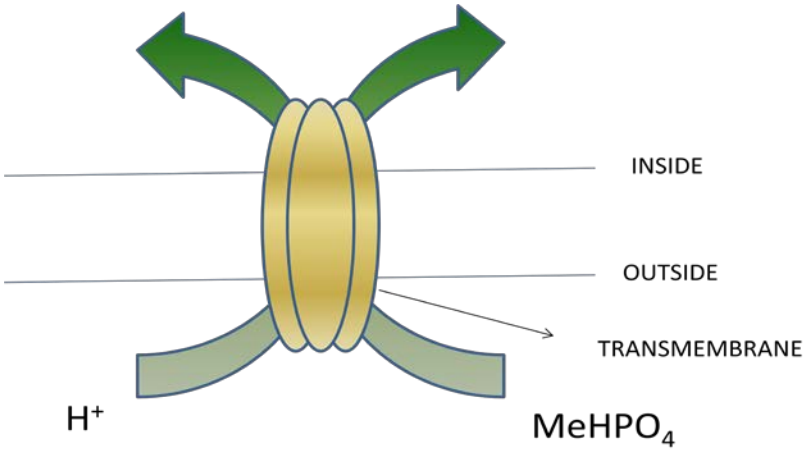

Figure 2: Phosphate transport by low affinity pit

In contrast, protein specific transport (Pst) is a periplasmic protein-dependent transporter. It consists of four subunits: a phosphate-binding protein located in the periplasmic space, two cytoplasmic associated proteins that contain six membrane spanning helices and a dimeric ATP binding protein. It operates as a primary transport mechanism i.e. unidirectional phosphate transport is coupled to a chemical reaction**.** Phosphate is transported in the form of $H_2PO_4^-$ and HPO_4^{2} in Pst system and has a relatively high substrate affinity.

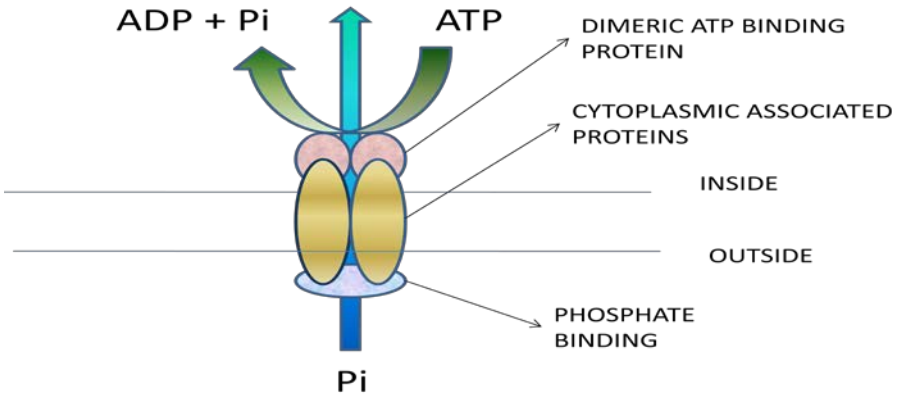

Figure 3: Phosphate transport by ATP dependent high affinity Pst system

Besides, phosphate also enters the cell in the form of esters such as *sn*-glycerol-3-phosphate, glucose-6-phosphate or mannose-6-phosphate. Other organic phosphate compounds may diffuse through the outer membrane before hydrolysis in the periplasm by phosphatases - allowing transport of Pi into the cytoplasm. Pi linked antiport systems of *sn*-glycerol-3-phosphate (GlpT) and glucose-6-phosphate (UhpT) mediate the translocation of organo-phosphate compounds across the cell membrane. Phosphate is also accepted as an analogue of organo-phosphate by these exchange systems; the affinity for phosphate is lower than for the organo-phosphate. PhoE pores are formed in *E. coli* cell membranes during phosphate limitation and have a preference for anions such as phosphate and phosphate-containing nutrients, facilitating the unspecific entry of phosphate into the cytoplasm by diffusion.

2. Arsenic transport:

It was studied that auqaporins facilitate the diffusion of metalloids such as arsenic (As) and antimony (Sb). The trivalent forms of these metalloids are structurally similar to glycerol at neutral pH and hence enter cells through aquaporins.

3. Magnesium transport:

Transport of Mg^{2+} into the cell is problematic, in spite of their largest hydrated radius, smallest ionic radius, and highest charge density. Transport systems for Mg^{2+} have been characterized well in *Salmonella typhimurium*. The CorA transport system is expressed constitutively and is the major Mg^{2+} transporter in Eubacteria and Archaea. It consists of three transmembrane domains, a large periplasmic domain, and no sequence homology to other proteins. The MgtE Mg^{2+} transporter also lacks sequence homology to other proteins, and it is unclear if Mg^{2+} transport is its primary function. The MgtA and MgtB Mg^{2+} transporters have sequence homology to P-type ATPases and closely related to the mammalian Ca^{2+} ATPases than to the prokaryotic P-type ATPases. Both transporters mediate Mg^{2+} influx with, rather than against its electrochemical gradient. Unlike CorA and MgtE, the MgtA and MgtC/MgtB loci are regulated, being induced by the two-component regulatory system PhoP/PhoQ. PhoQ is an Mg^{2+} membrane sensor kinase that phosphorylates the transcription factor PhoP under Mg^{2+} - limiting conditions. This factor then induces transcription of MgtA and MgtCB.

4. In hyperthermophillic Archaea, only tranporters of ABC type are useful in uptake of carbohydrates (e.g. glucose, cellobiose, maltotriose, arabinose, trahalose). This reflects an adaptation to the extreme habit, enabling organisms to acquire all available sugars very effectively.

Interesting facts:

- Transport system of a cell depends upon the substrate requirements of the cell, the bioavailability of the substrate, environmental conditions and membrane permeability.
- Phosphate can be transported either by low affinity pit or ATP dependent high affinity Pst system.
- In spite of largest hydrated radius, smallest ionic radius, and highest charge density of Mg^{2+}, its transport into the cell is problematic.
- Only tranporters of ABC type are useful in uptake of carbohydrates in hyperthermophillic Archaea.

Questions:

1. Transport of solutes across cells depends upon:
   a. Substrate requirements of the cell and bioavailability of the substrate.
   b. Environmental conditions and membrane permeability.
   c. Metabolic features and physiological state of the organism.
   d. All of the above.
2. The type of transport without any energy input in the cell is called:
   a. Passive transport
   b. Active transport
   c. Osmosis
   d. Plasymolysis
   e. Turgor pressure
3. Which of the following pieces of evidence would suggest that a substance entered a cell via active transport as opposed to passive transport?
   a. The substance moved from a high concentration to a low concentration.
   b. ATP was required for transport.
   c. The substance moved across the membrane via a carrier protein.
   d. None of the above.

4. What are the functions associated with cytoplasmic membrane which has role in transport mechanism of cell?
5. What are the composition of outer membrane and its functions that has role in transport mechanism of cell?
6. What is the classification of transport mechanism in the cells? Explain with example.
7. Explain the transport mechanism of phosphate in the cell.

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Module 3 Lecture 7

Endocytosis and Exocytosis

Endocytosis: Endocytosis is the process by which cells absorb larger molecules and particles from the surrounding by engulfing them. It is used by most of the cells because large and polar molecules cannot cross the plasma membrane. The material to be internalized is surrounded by plasma membrane, which then buds off inside the cell to form vesicles containing ingested material.

The endocytosis pathway is divided into 4 categories:

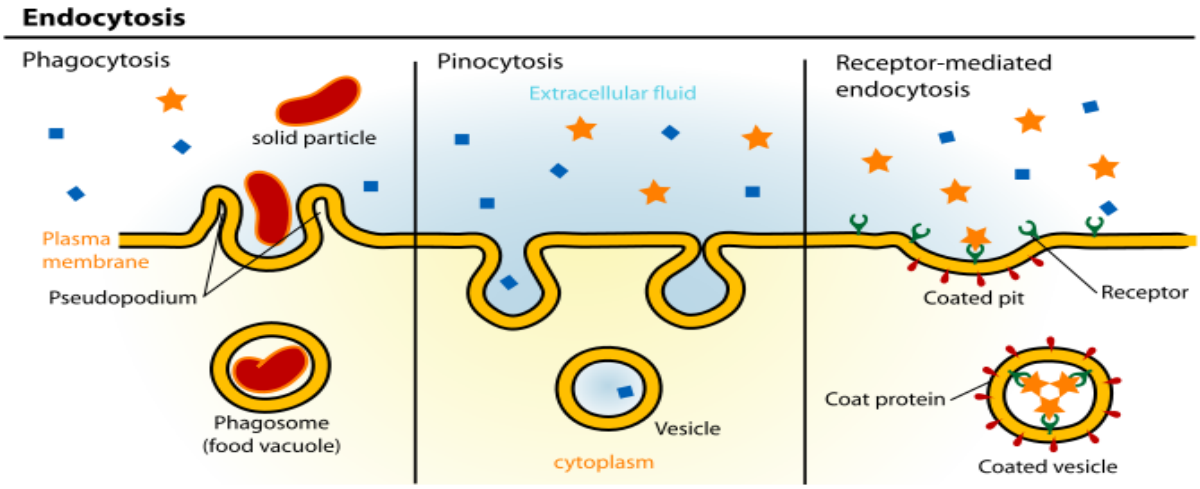

Figure 1: Types of endocytosis process (4th pathway is not shown in above figure)

1. **Phagocytosis:** Phagocytosis is the process by which certain living cells called phagocytes engulf larger solid particles such as bacteria, debris or intact cells. Certain unicellular organisms, such as the protists, use this particular process as means of feeding. It provides them part or all of their nourishment. This mode of nutrition is known as phagotrophic nutrition. In amoeba, phagocytosis takes place by engulfing the nutrient with the help of pseudopods, that are present all over the cell, whereas, in ciliates, a specialized groove or chamber, known as the cytostome, is present, where the process takes place.

When the solid particle binds to the receptor on the surface of the phagocytic cell such as amoeba, then the pseudopodia extends and later surrounds the particle as shown in figure

2. Then their membrane fuses to form a large intracellular vesicle called phagosome. These phagosomes fuse with the lysosome, forming phagolysosomes in which ingested material is digested by the action of lysosomal enzymes. During its maturation, some of the internalized membrane is recycled to plasma membrane by receptor mediated endocytosis.

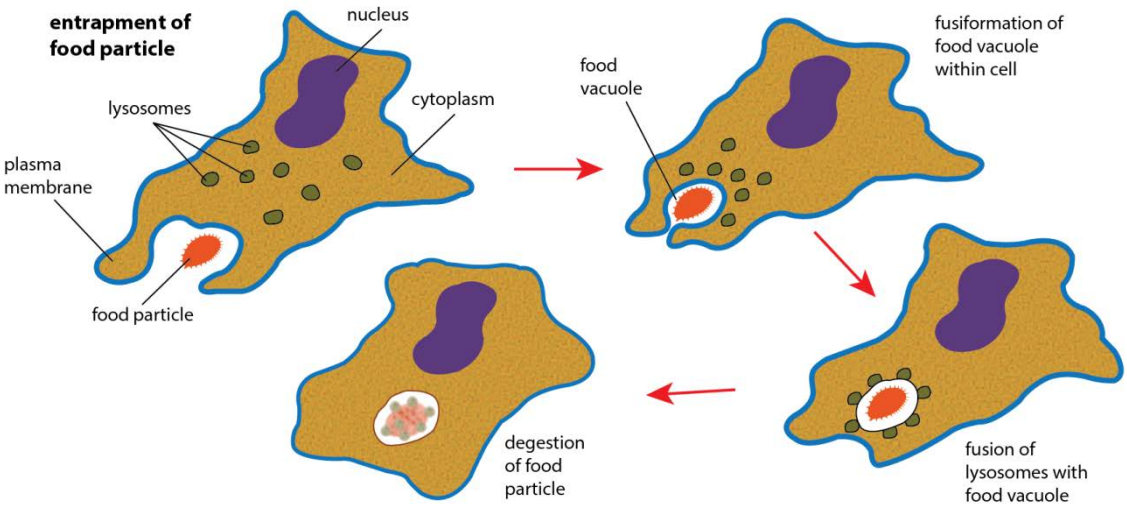

Figure 2: Example of phagocytic process for entrapment of food particle

Draw the above figure

The various phases of phagocytosis in amoeba for food capturing are:

- Adherence of the macromolecules to the receptor on the phagocytic cell
- Extension of pseudopodia and ingestion of microbe by phagocytic cell
- Formation of phagosome by the fusion of surrounding membrane
- Fusion of phagosome and lysosome to form phagolysosome
- Digestion of the ingested macromolecules by the acid hydrolytic enzymes in the lysosome
- Formation of residual body coating indigestible material
- Discharge of waste materials

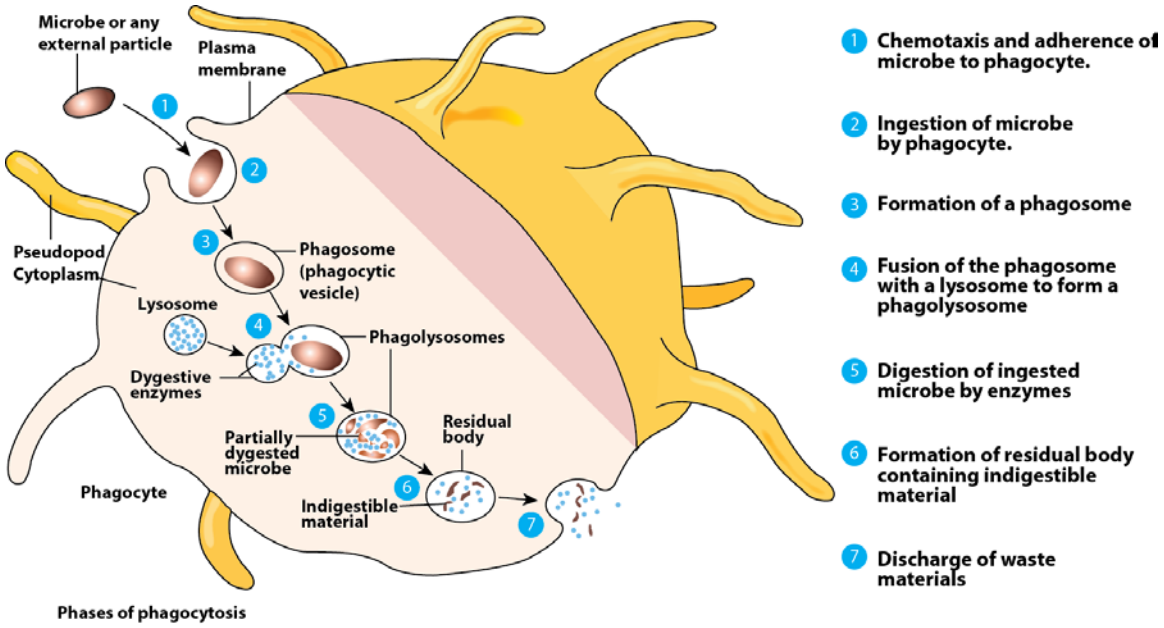

Figure 3: Phases of phagocytosis process Draw this figure

Other examples of phagocytosis include some immune system cells, that engulf and kill certain harmful, infectious micro-organisms and other unwanted foreign materials which in turn provides defence against invading micro-organism and eliminate damaged cells from the body. There are two types of phagocytes (WBC) in mammals: Macrophages and Neutrophils. These WBC acts as defence system by eliminating micro-organisms from infected tissues. In these cells, the engulfment of foreign material is facilitated by actin-myosin contractile system. It allows the cell membrane to expand in order to engulf the particle and then contract immediately, ingesting it. Macrophages also remove dead cells.

Steps of phagocytosis in the immune system:

The WBC cells are activated in the presence of certain bacterial cells, inflammatory cells or other foreign bodies. It includes the following steps:

- Phagocytes get activated by the presence of certain particles around them. As soon as they detect a foreign particle, the phagocytes produce surface glycoprotein receptors which increase their ability to adhere to the surface of the particle.
- The phagocyte slowly attaches to the surface of the foreign particle. The cell membrane of the phagocyte begins to expand and forms a cone around the foreign particle.

- The cell membrane surrounds the foreign particle to create a vacuole, known as phagosome or food vacuole. The phagosome is then passed into the cell for absorption.
- The lysosomes break the food vacuole or phagosome, into its component materials. The essential nutrients, if any, are absorbed in the cell, and the rest is expelled as waste matter. In case of the immune system, the cell creates a peroxisome, a special structure that helps the body to get rid of the toxins.

2. **Clathrin-mediated endocytosis:** Clathrin-mediated endocytosis is also known as receptor mediated endocytosis. It is the process of internalizing molecules into the cell by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being internalized (**Jackson et al.**).

Phases of clathrin-mediated endocytosis:

- Macromolecules (as ligands) bind to the specific cell surface receptors
- Then the receptors are concentrated in specialized regions of plasma membrane and clathrin and adaptor protein binds to these receptor forming clathrin-coated pits
- These pits bud from the membrane and form clathrin-coated vesicles containing receptors, proteins and ligands
- Then these vesicles fuse with early endosomes, in which the contents are sorted for the transport to lysosomes and receptors and proteins are recycled to plasma membrane

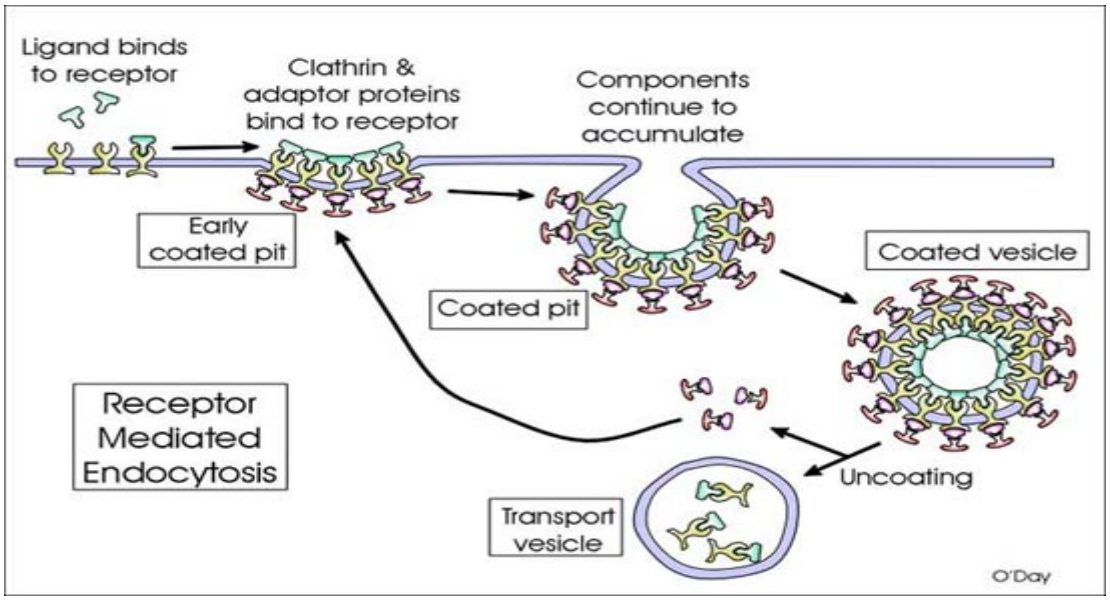

Figure 4: Phases of clathrin-mediated endocytosis

The example for clathrin mediated endocytosis is uptake of cholesterol by the mammalian cells. Here cholesterol is transported through the blood stream in the form of lipoprotein or LDL. The LDL particle consists of phospholipid bilayer, esterified and non-esterified cholesterol and Apo B protein as shown in figure 4. It was first demonstrated by Michael Brown and Joseph Goldstein in which uptake of LDL requires the binding of LDL particle to the specific cell receptor. Later it was found that it is concentrated in clathrin coated pits and internalized by endocytosis. Then receptor is recycled to plasma membrane and LDL is transported to lysosome and cholesterol is released for use by the cell.

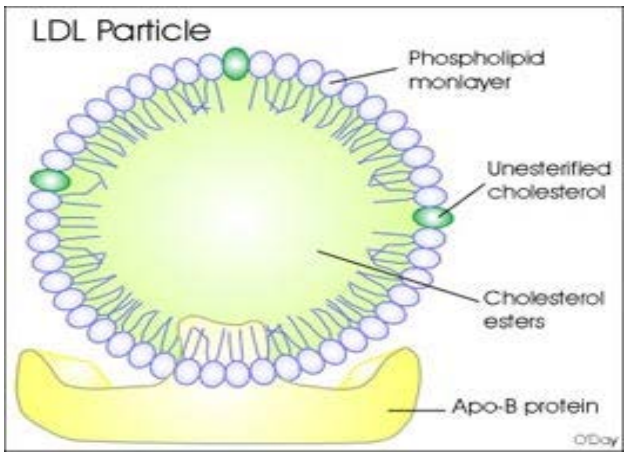

Figure 5: LDL particle or low density lipoprotein

Phases for receptor mediated endocytosis for cholesterol uptake involves:

- Receptor Binding & its activation: Here LDL receptor binds to Apo-B protein on the LDL particle
- Coated Pit Formation: Clathrin forms cage around forming endosome
- Clathrin-Coated Vesicle Budding
- Uncoating of the Vesicle
- Early Endosome associates with other vesicles
- Formation of CURL (Compartment for Uncoupling of Ligand and Receptor) or Late Endosome
- Recycling of the Receptor to the cell surface
- Fusion of Transport Vesicle with Lysosome
- Digestion of the LDL to Release Cholesterol

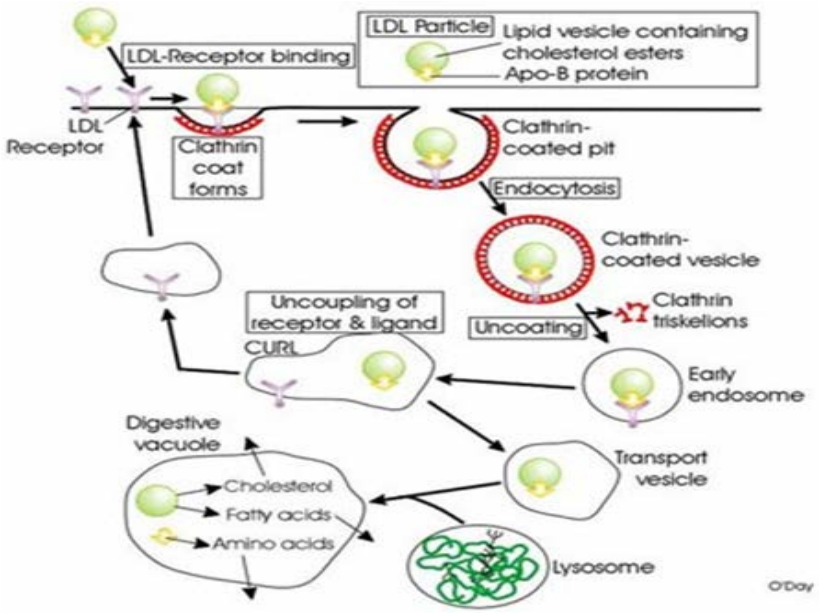

Figure 6: Receptor mediated endocytosis: Cholestrol uptake

In patients suffering from familial hypercholesterolemia, having high levels of cholesterol in serum and hence suffer from heart attacks early in life. Because these patients are unable to internalize LDL from the extracellular fluids, result in high accumulation of cholesterol. Normal individual possess LDL for transport of cholesterol but familial hypercholesterolemia results from inherited mutation in LDL receptor. These mutations can happen in two ways.

Either the patients simply fail to bind with LDL, demonstrating that a specific cell surface receptor is required for uptake of cholesterol. Or the patients are able to bind with LDL but are unable to internalize it. Because they are unable to concentrate in coated pits, demonstrating that coated pits in receptor plays an important role for cholesterol uptake. This mutation lies in the cytoplasmic tail of the receptor and can be subtle as the change of a single tyrosine to cysteine.

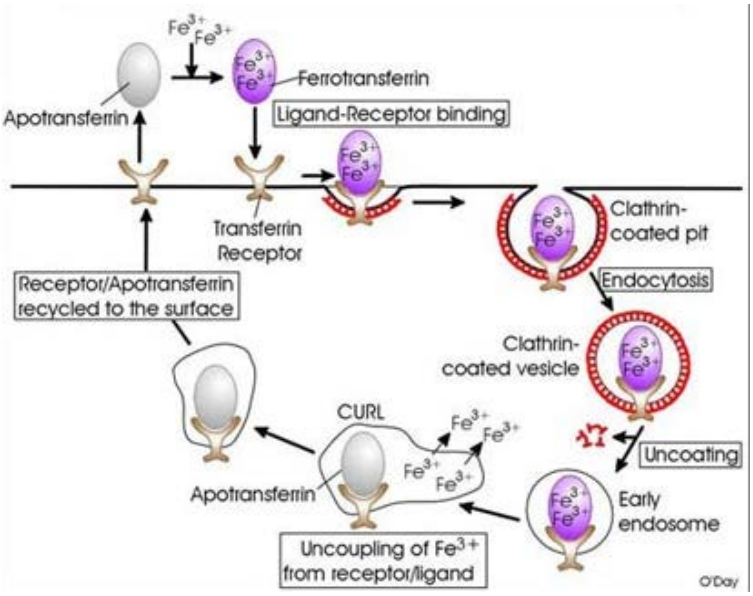

Figure 7: Example for receptor mediated endocytosis for ion uptake

3. Caveolae:

Caveolae is a pathway which is independent of clathrin- endocytosis process and involves in the uptake of molecules in small invaginations of the plasma membrane (50 to 80 mm diameter). These are enriched in lipid rafts of cholesterol, phospholipid and sphingolipids and possess a distinct coat formed by a protein called caveolin **(**cholesterol binding protein**)**. It is abundant in smooth muscle, type I pneumocytes, fibroblasts, adipocytes, and endothelial cells (**Parkar et al., 2009**).

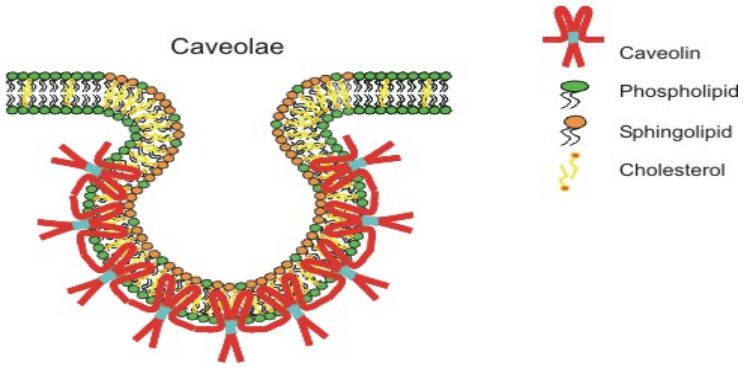

Figure 8: Structure of caveolae

Cells mostly use caveolae for the selective uptake of molecules as small as folate to full size proteins such as albumin and alkaline phosphatase. Many studies have shown that caveolae-mediated uptake of materials is not limited to macromolecules. In certain cell-types, viruses as simian virus 40 and even entire bacteria as some specific strains of *E. Coli* are engulfed and transferred to intracellular compartments in a caveolae-dependant fashion.

4. Macropinocytosis: The process of uptake of fluids in large vesicles (0.15 to 5 µm in diameter) is called macropinocytosis.

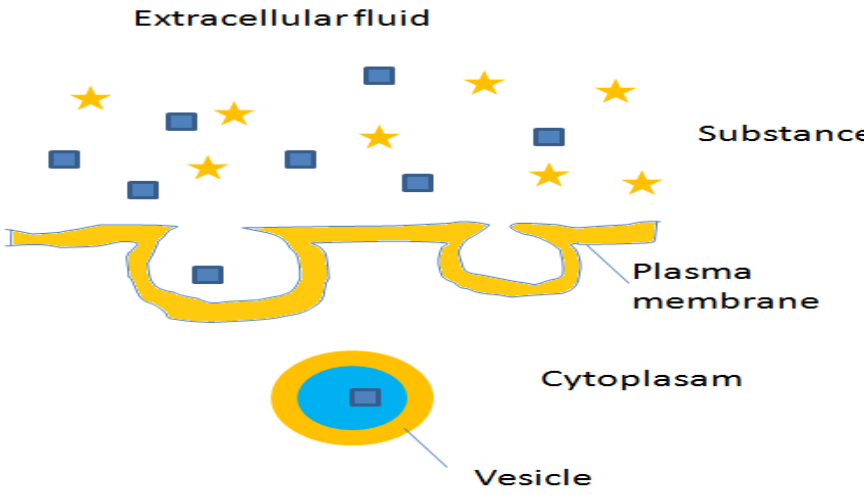

Figure 9: Diagram depicting Pinocytosis

Macropinocytosis is a different phenomenon from phagocytosis.

Macropinocytosis can uphold for particle uptake and involves the uptake of large amounts of fluid and solutes which is non- specific in nature. The receptors that trigger macropinocytosis have other physiological roles and are present on many cell types. These include growth factor receptors that activate common signalling pathways and involve global activation of the actin cytoskeleton resulting in plasma membrane ruffling and the formation of lamellipodia or blebs over the entire surface of the cell. In contrast, phagocytosis is particle-driven, and it depends on receptor interactions over the entire surface of the ingested particle. The receptors that trigger phagocytosis are usually specialized for interaction with the surface components of relevant cargo particles and actin modifications are localized to the phagocytic cup that forms around the particle.

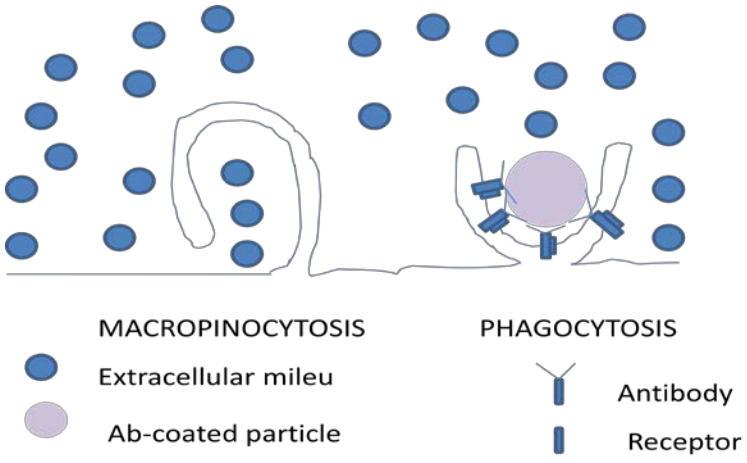

Figure 10: Differences between macropinocytosis and phagocytosis

Protein Trafficking in Endocytosis:

After internalization, clathrin-coated vesicles rapidly shed their coats and fuse with early endosomes which maintain an acidic internal pH and are located in periphery of the cell. This acidic pH leads to the dissociation of many ligands from receptors within early endosome compartment and hence serves as sorting compartment, from which molecules taken up by endocytosis are either recycled to the plasma membrane or transported to lysosomes for degradation. Later, ligands and membrane proteins for degradation are transported to late endosomes which are mediated by movement of large endocytic

carrier vesicles along microtubules. The late endosomes are more acidic than early endosomes and fuse with transport vesicles carrying hydrolases from Golgi apparatus. Late endosomes mature into lysosomes when they acquire a full complement of lysosomal enzymes and become acidic. Hence the endocytosed materials are degraded by action of acid hydrolases (**Cooper et al., 2000)**.

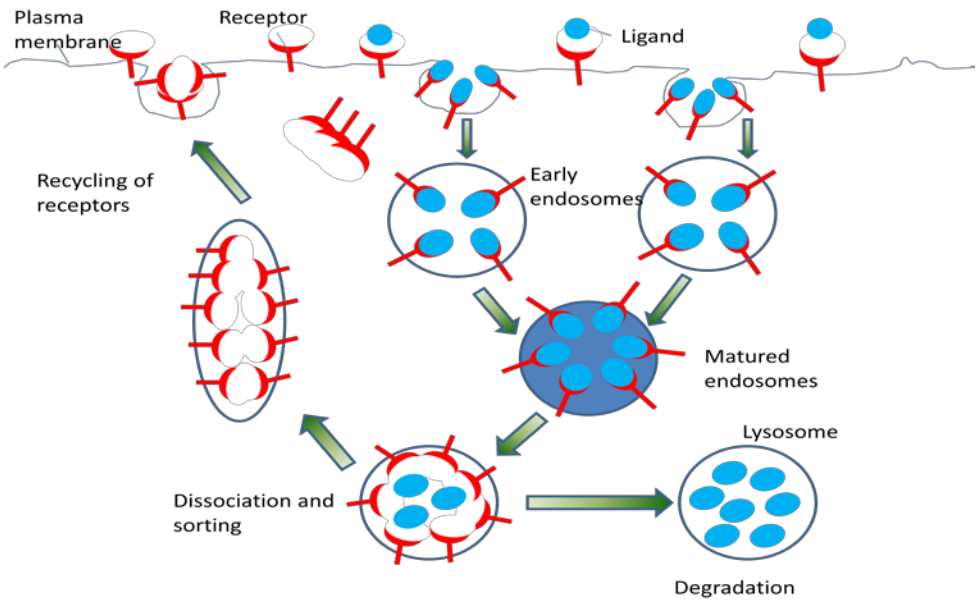

Figure 11: Protein trafficking during endocytosis. The figure shows the recycling of the plasma membrane and degradation of ligands and other membrane proteins in lysosome

The most common example for protein trafficking is recycling of synaptic vesicles. As when an action potential arrives at the terminal of most neuron signals, the fusion of synaptic vesicles with the plasma membrane releases neurotransmitter that carry signal to post synaptic cells. The empty synaptic vesicles are then recovered by plasma membrane in clathrin-coated vesicles, which fuse with early endosomes. The synaptic vesicles are then regenerated directly by budding from endosomes. They accumulate a new supply of neurotransmitter and recycle to the plasma membrane and ready for next cycle of synaptic transmission.

Transcytosis:

The process of transfer of internalized receptor across the cell to opposite domain of the plasma membrane is called transcytosis. It occurs in polarized cells or epithelial cells mostly. It is used for protein sorting and also a mean for transport of macromolecules across the epithelial cell sheets.

Example: Transport of Abs from blood to other secreted fluids. The Abs bind to the receptor on basolateral surface and then transcytosed along with their receptors to apical surface. The receptors are then cleaved and release Abs into extracellular secretions.

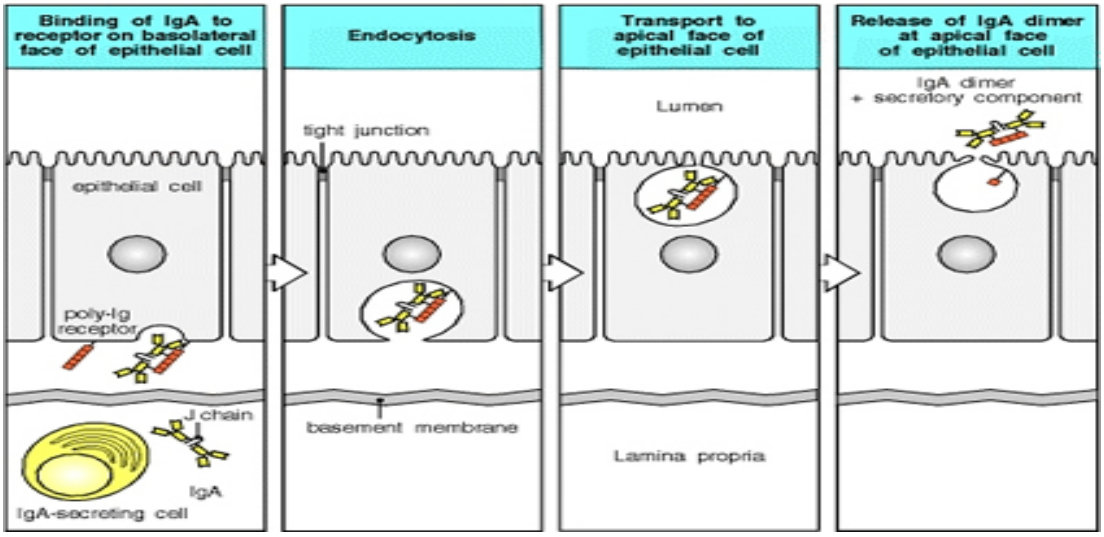

Figure 12: Process of transcytosis of IgA, an immunoglobulin, within an epithelial cell.

Exocytosis:

The process by which the cells direct the contents of secretory vesicles out of the cell membrane is known as exocytosis. These vesicles contain soluble proteins to be secreted to the extracellular environment, as well as membrane proteins and lipids that are sent to become components of the cell membrane. It is the final step in the secretory pathway that typically begins in the endoplasmic reticulum (ER), passes through the Golgi apparatus, and ends at the outside of the cell. Some of the examples include secretion of proteins like enzymes, peptide hormones and antibodies from cells and release of neurotransmitter from presynaptic neurons.

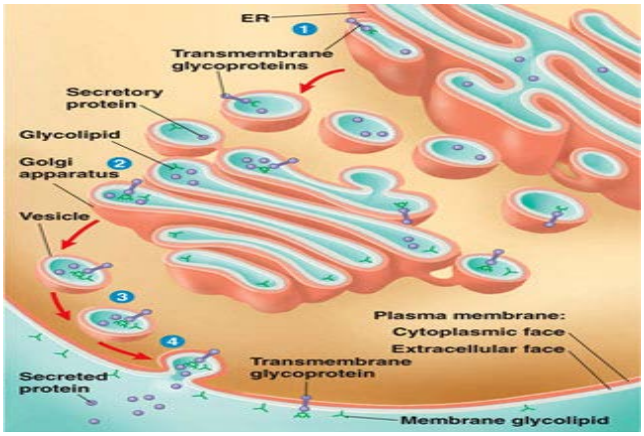

Figure 13: Diagram depicting Exocytosis process

Types of exocytosis: Exocytosis are of two types. Constitutive exocytosis and Regulated exocytosis

1. **Constitutive exocytosis: S**ecretory materials are continuously released without requirement of any specific kind of signal.
2. **Regulated exocytosis:** Regulated exocytosis requires an external signal, a specific sorting signal on the vesicles for release of components. It contains a class of secretory vesicles that fuse with plasma membrane following cell activation in presence of signal. Examples of regulated exocytosis are secretion of neurotransmitter, hormones and many other molecules

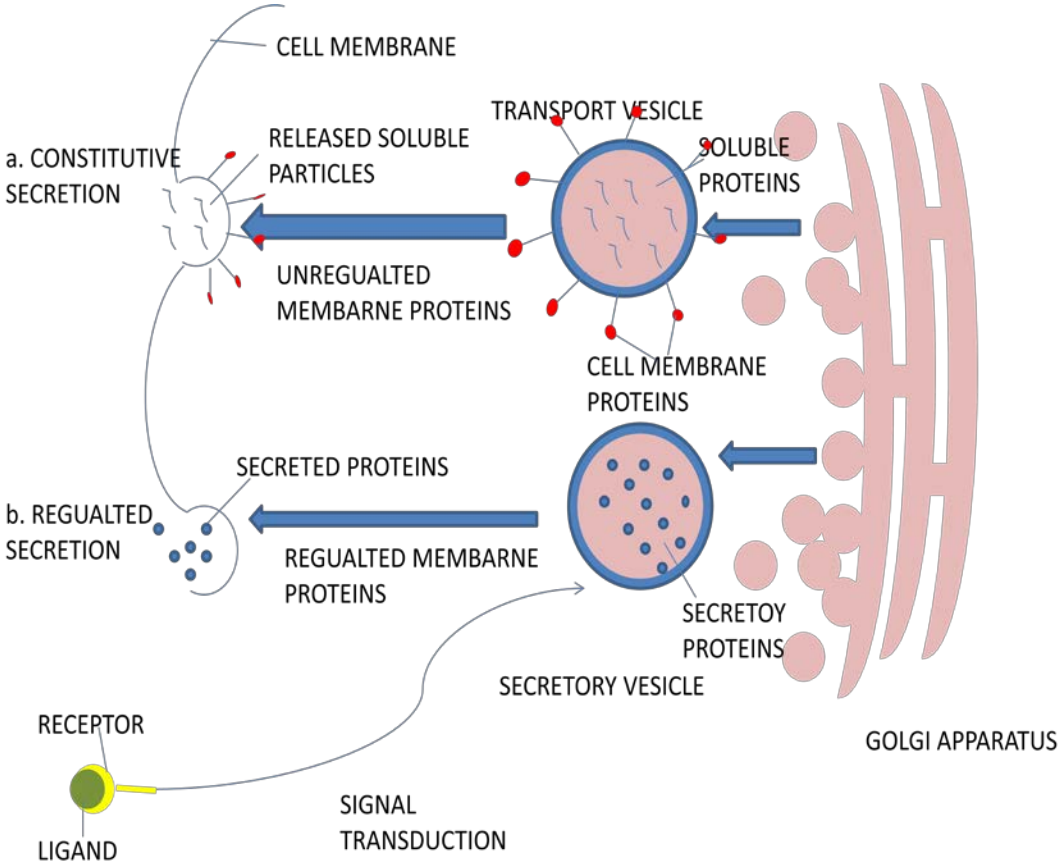

Figure 14: Types of exocytosis. (a) Constitutive secretion and (b) Regulated secretion

Steps in exocytosis:

Vesicles are used to transport the proteins from the Golgi apparatus to the cell surface area using motor proteins and a cytoskeletal track to get closer to cell membrane. Once these vesicles reach their targets, they come into contact with tethering factors that can restrain them. Then the process of **vesicle tethering** distinguishes between the initial, loose tethering of vesicles from the more stable, packing interactions. Tethering involves links over distances of more than about half the diameter of a vesicle from a given membrane surface (>25 nm). The process of holding two membranes within a bilayer's distance of one another (<5-10 nm) is called **vesicle docking.** Stable docking indicates the molecular interactions underlying the close and tight association of a vesicle with its target may include the molecular rearrangements and ATP-dependent protein and lipid modifications, needed to trigger bilayer fusion called **vesicle priming.** It is mostly takes place before exocytosis and used in regulated secretion type of exocytosis but not used in constitutive secretion. It is followed by **vesicle fusion** which includes merging of the vesicle membrane with the target and hence there is release of large biomolecules in the extracellular space with the help of some protein complex.

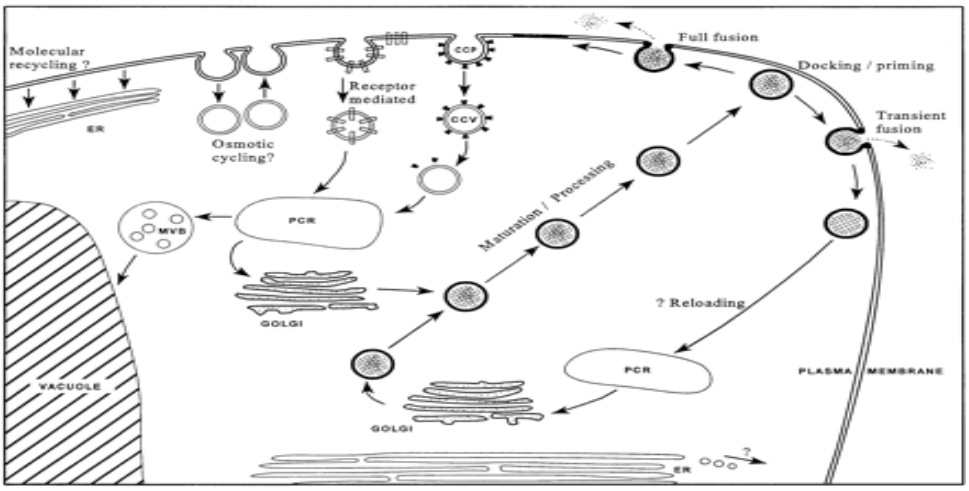

Figure 15: Sequence of Exocytosis and Endocytosis: Transport, Docking, Fusion, Content Release, and Recycling.

Interesting facts:

- Many cells in the body use exocytosis to release enzymes or other proteins that act in other areas of the body like secretion of the hormones glucagon and insulin, or to release molecules that help cells communicate with one another more directly through the products that they secrete like neurotransmitters.

- The immune system also uses exocytosis to communicate information between cells.

- Both endocytosis and exocytosis involve active transport, that is, energy must be expended to move particles against the concentration gradient.

- Both endocytosis and exocytosis involve the formation of vesicles: endocytosis forms them in order to take particles into the cell via the cell membrane and involves a reduction in cell membrane area, as part of the membrane is pinched off to form a vesicle; exocytosis forms them in order to expel things from the cell via the cell membrane and results in an increase in cell membrane, as the vesicle wall joins that of the cell membrane and is incorporated into it. Thus, the two processes also serve to balance each other.

- The vesicle fusion is driven by SNARE proteins process of merging the vesicle membrane with the target one resulting in release of large biomolecules in the extracellular space.

- Ca2+ might control the exocytotic/endocytotic balance in plants.

- Synaptotagmin I (syt1) is required for normal rates of synaptic vesicle endo- and exocytosis.

Questions:

1. The transport method of neurotransmitters between nerve cells is:
   a. Exocytosis
   b. Passive transport
   c. Receptor-mediated endocytosis
   d. Active transport
2. Which of the following membrane activities does NOT require the expenditure of energy by the cell?
   a. Active transport
   b. Osmosis
   c. Endocytosis
   d. Exocytosis
   e. Synthesis of more membrane
3. The membrane transport mechanism used when an amoeba engulfs a bacterial cell is called:
   a. Carrier-mediated facilitated diffusion
   b. Exocytosis
   c. Phagocytosis
   d. Pinocytosis
   e. Sodium-potassium pump
4. A person has a genetic disease that prevents the phospholipids in the plasma membrane of the white blood cells from freely fusing with the other membranes within the cell. How would this disease affect phagocytosis?
   a. Lysosomes would not be formed
   b. Facilitated diffusion would not occur
   c. Lysosomes would be formed lacking hydrolytic enzymes
   d. The phagocytic vacuole would not fuse with the lysosome
   e. Endocytosis would not occur

5. Pinocytosis:
    a. Is engulfment of large particles by the cell
    b. Occurs in protozoans and algae but not in more complex organisms
    c. Involves the specific binding of molecules to receptors on the cell surface
    d. Is the nonspecific uptake of fluids by pinching inward of the plasma membrane
    e. Is movement of molecules against the concentration gradient through a permeable membrane
6. Receptor-mediated endocytosis:
    a. Is a passive process
    b. Involves only membrane transport proteins
    c. Brings about the selective uptake of materials by enclosing them in membranous vesicles
    d. Does not require energy
    e. Is most likely to be found in cells that release large amounts of hormones
7. Exocytosis is a process by which cells
    a. Pass substances out of the cell in vesicle
    b. Pass substances out of the cell through the membrane by osmosis
    c. release substances directly into the extracellular fluid through a pore
    d. Release substances directly into the extracellular fluid through a pit
    e. Identify substances in the environment
8. A cell engaged in phagocytosis must be
    a. Engulfing a live organism
    b. Acquiring a liquid
    c. Engulfing a dead organism
    d. Transporting bulk dissolved nutrients
    e. Transporting bulk solid material

9. Coated pits are required for which type of membrane transport?
    a. Receptor-mediated endocytosis
    b. Phagocytosis
    c. Pinocytosis
    d. Exocytosis
10. Certain white blood cells are designed to engulf and destroy microbes such as bacteria. The process used to achieve this would be:
    a. Phagocytosis
    b. Receptor-mediated endocytosis
    c. Pinocytosis
    d. Exocytosis
11. What is the primary factor that determines if endocytosis or exocytosis would be needed to move a substance across the cell membrane as opposed to diffusion or osmosis?
    a. the chemical properties of the molecules being moved
    b. the size of the molecules being moved
    c. the differences in concentration of the substance inside and outside the cell
    d. whether water is being moved across the membrane
12. In endocytosis, a transport vesicle is derived from _____.
    a. plasma membrane
    b. ribosomes
    c. Golgi complex
    d. Lysosomes
13. Low density lipoproteins (LDL's or bad cholesterol) are taken up "in-bulk" into the cytoplasm of a cell. This process is an example of
    a. Endocytosis
    b. Exocytosis
    c. Molecular transport
    d. Oosmosis
    e. Diffusion

14. How the phagocytosis takes place in the immune system of cells?
15. Describe the uptake of cholesterol by the mammalian cells using receptor-mediated endocytosis.
16. What is protein trafficking? Explain with an example.
17. What is transcytosis? Explain with an example.
18. What are the two types of exocytosis? Explain with a schematic diagram.

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Module 3 Lecture 8

Entry of virus in cells:

Introduction:

The interactions between viruses and cells are complex, in spite of their simple structure and components. Viruses utilize a number of cellular processes which contains cellular proteins to enter into the cells. Some viruses are able to cross plasma membrane into cytosol by endocytic uptake, vesicular transport via cytoplasm and transport to the endosomes and other intercellular organelles. These processes are associated with clathrin-mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, or other mechanisms. Generally viruses are attached to the cell surface of proteins, carbohydrates and lipids. The interaction of viruses receptors are specific and require at least 3 point for interaction, which results in activation of cellular signalling pathways. The viruses enter the cell by endocytic mechanism. After the viruses go in the lumen of endosomes or the endoplasmic reticulum, they obtain signals which are in the form of being exposed to low pH, proteolytic cleavage, and the initiation of viral proteins, which results in modifications in the viral proteins, and then they are able to penetrate the vacuolar membrane. After they penetrate the vacuolar membrane, they pass the viral genome, the caspid, or the viral particle that is kept together into the cytosol. Afterwards, the majority of RNA viruses replicate at a variety of positions within the cytosol. In contrast, most DNA viruses continue through their passage towards the nucleus.

Viral entry into the cell:

Viruses enter the cell, which is covered by a phospholipid bilayer and acts as a cells natural barrier to its surrounding. The process by which this barrier is crossed depends upon the type of virus. There are 4 types of viral entry into the cell:

1. Attachment or Viral Adsorption: The viral receptors attached to the complementary receptors on the cell membrane.
2. Membrane Fusion: The cell membrane is punctured and later attached with the unfolding viral envelope.
3. Entry via Pore formation: An opening is established for the entry of viral particles.

Viral Penetration: The viral capsid or genome is injected into the host cells cytoplasm directly.**Entry through endocytosis:**

The type of entry through endocytosis is carried out by bacteriophages infection that form coliphages T4 and T2 and are hypodermic syringes with a tail that is capable of contracting. Endocytic vesicles carry viruses from the outer edges to the perninuclear area of the host cell, where the conditions for infection are changed and the distance is minimized towards the nucleus. Also the maturation of endosomes has slowly changing conditions, like lowering of pH or the switching of a redox environment, which enables viruses to detect their position within a cell and the passage and allows the endosomes to utilize this information to put a time of penetration and uncoating.

Viruses as Endocytic Carriers:

This type of viral entry is mostly observed in animal viruses. When these viruses attach to cells, they do not become disfigured. Rather the plasma membrane changes shape according to the shape of the virus. The outer layer of viruses is coverer proteins that attaches to receptors and formed an icosaherdral grid or as spike glycoprotein that span the entire viral envelope. The single interactions with the receptors are weak, but interaction with many different receptors increases the activity and results the binding to cells almost impossible to reverse. Lipid raft functions to control the signalling, fluidity and receptor functions on the membrane. These are rich in cholesterol and sphingolipids.

Viruses that use the Caveolar/Raft-dependent pathways form primary endocytic vesicles are dependent on cholesterol, lipid rafts, and complex signalling pathways.

Attachment Factors and Receptors:

Some viruses bind to defined endocytic receptor, like transferrin and low-density lipoprotein receptors, but viruses attach to the majority of other molecules like carbohydrate moieties with different functions like cell to cell recognition, ion transportation and attachment to the extracellular matrix. The difference between attachment factors and virus receptor is that the attachment factor simply attached to the viruses and hence focused on the top area of the cell, while virus receptors modify the viruses, promote cellular signalling or activate penetration. The situation of attachment receptor is common in influenza and polyomaviruses, where the attachment is very specific and deals with lectin domains. The other example is HIV-1 because two receptors Adenoviruses 2 and 5 are necessary to promote conformational modifications to assist fusion and encourage endocytosis.

Entry by macropinocytosis:

Viruses belonging to vaccinia, adeno, picorna and other virus families enter by macropinocytosis, an endocytic mechanism involved in fluid uptake. The virus particles first activate signalling pathways that trigger actin-mediated membrane ruffling and blebbing. This is followed by the formation of large vacuoles (macropinosomes) at the plasma membrane, internalization of virus particles and penetration by the viruses or their capsids into the cytosol through the limiting membrane of the macropinosomes.

Examples for entry of viruses into the cells on the basis of types of viruses:

1. Bacterial virus entry:

Bacteriophages attack bacterial cells and inject their genomes through specific receptor sites which include lipopolysaccharides, cell wall proteins, teichoic acid or flagellar or pilus proteins and also contain specific attachment proteins on the bacteria. The phage tail fibres are the attachment sites and bind reversibly to lipopolysaccharides and outer membrane protein OmpC. And then the base plate then settles down onto the surface and binds tightly to it which results in conformational changes in the short tail fibres, which then later contracts, pushing the tail core through the cell wall, in an ATP-driven process and is aided by a lysozyme activity associated with the tip of the tail tube.

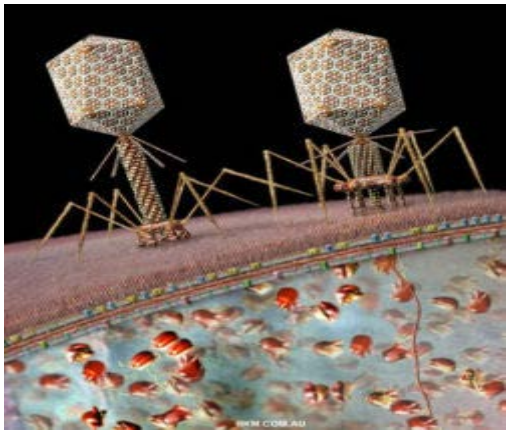

Figure 1: Enterobacteria phage T4 or viruses with 34-170 kbp dsDNA genomes, isometric heads and contractile tails - infects the gram-negative bacterium E coli

Phage lambda or enterobacteria phage λ is a tailed phage with an isometric head which attaches to the maltose receptor on the surface of the *E.coli* cell via the J protein in the tail tip. Although the tail is non-contractile, a DNA injection mechanism allows entry of DNA into the cell, via a sugar transport protein (PtsG) in the inner membrane, leaving the capsid behind.

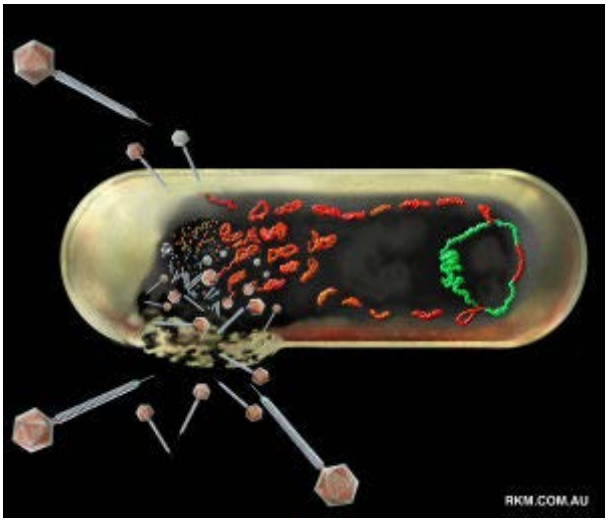

Figure 2: Lambda phage infecting *E.coli*

MS2 phage is an isometric single-stranded RNA-containing virus which infects *E.coli*. And the phage attaches to the F pili of *E.coli* via its single attachment or a protein. The A protein is covalently linked to the 5'-end of the genomic RNA; binding pilin causes cleavage of protein and releases it from the capsid. Thus, when the pilus is retracted into the cell, protein and RNA are pulled with it, leaving the empty capsid outside.

2. Animal cell entry:

Animal viruses enter into the cell by direct cell membrane fusion and entry via endocytotic or other vesicle. Direct membrane fusion involves direct attachment to the cell surface via **binding to a specific receptor.** Here the virion membrane fuses with the cell membrane, and the virion nucleoprotein complex is delivered into the cell cytoplasm directly. An example is HIV entry process. Here the virion attachment protein **gp120** attaches initially to the **CD4** protein on a helper T-cell. The gp120 then undergoes conformational change due to binding and binds the **accessory receptor CCR-5,** a **chemokine**. **gp41**, a cleavage product of a **gp160** precursor, and a part of the **"spike protein"** of the viral membrane is then able to bind into the cell membrane, via a

hydrophobic domain. A condensation of the gp41 structure results in formation of a "6 helix bundle" and causes close juxtaposition of cell and viral membranes, which promotes membrane fusion and nucleoprotein entry into the cell.

Fusion Protein mode of entry: All enveloped viruses follow fusion mode of entry, whether they fuse with the cell membrane directly or with the membrane via an internalised vesicle. This is mediated by three identified classes of envelope glycoproteins:

Class I fusion proteins

These fusion proteins are most common in retroviruses, myxoviruses, coronaviruses and paramyxoviruses. The "spikes" are composed of three identical protein subunits, largely alpha-helical in structure and assembled as trimers which are later on cleaved into two pieces. The carboxy-terminus of one piece is anchored to the viral membrane and the new amino terminus has a characteristic stretch of 20 hydrophobic amino acids.

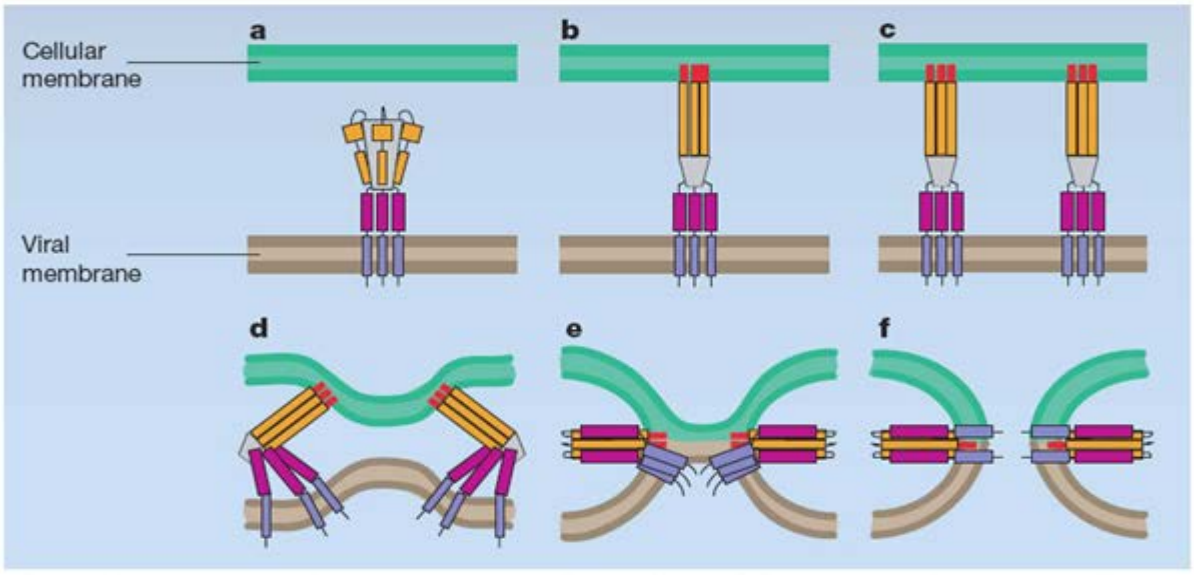

Figure 3: Proposed mechanism for membrane fusion by class I fusion proteins. (a)The metastable conformation of a trimeric fusion protein: with helical domain in orange, helical domain B in pink and the transmembrane domain in purple. (b)After binding to a receptor on the cellular membrane or an exposure to low pH found in intracellular compartment, the protein forms an extended conformation and hydrophobic fusion peptide (red) inserts into the target membrane. (c)Several trimers thought to be involved in this mechanism. (d)Protein refolding begins. The free energy thereby released causes the membrane to bend towards each other. (e)Formation of restricted hemifusion stalk allows the lipids in the outer leaflets of membrane to mix. (f)Protein refolding completes, forming the most stable form of fusion protein with fusion peptide and transmembrane domain anti-parallel to each other but in the same membrane.

Class II fusion proteins

They are found in dengue, tick-borne encephalitis, yellow fever and other flaviviruses, and Semliki Forest virus. This class of fusion proteins have a β-sheet-type structure and are not cleaved during biosynthesis. The proteins have three principal domains: Domain I begin at the amino terminus, domain II contains the internal fusion loop and domain III is at the carboxy terminus.

The dimeric protein binds to few cellular receptors for virus internalisation. The acidic pH inside endosomes causes domain II to swing upward, allowing monomers to rearrange laterally. The fusion loop inserts into the host-cell membrane, enabling trimer formation of the viral glycoprotein. Domain III shifts and rotates to create contacts, bending the membrane. The formation of further contacts leads to unrestricted hemifusion and the most stable form of the protein.

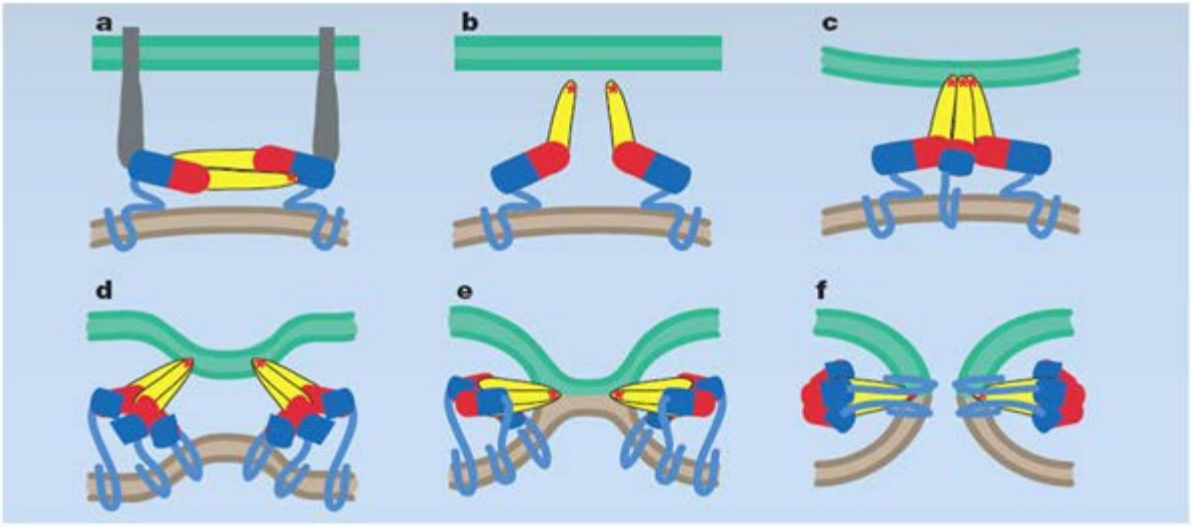

Figure 4: Proposed mechanism for fusion by class II proteins. (a)The dimeric E protein binds to a cellular receptor (grey) and the virus is internalized to endosomes. Membrane fusion release the virus into the cell body, takes place within endosomes. Domain I is in red, domain II in yellow and domain III in light and dark blue. (b)The acididc pH inside the endosomes cause domain II to swing upward and permit E monomers to rearrange laterally. (c)The fusion loop (red dot) inserts into the outer leaftlet of the host-cell membrane, enabling trimer formation. (d)The formation of trimer contacts extends from the top of the molecule. Domain III shifts and rotates to create contacts, bending the membrane. (e)The formation of further contacts leads to unrestricted hemifusion. (f)The final most stable form of the protein.

Class III fusion proteins

These proteins are characteristic of rhabdoviruses and vesicular stomatitis viruses. They form trimers of hairpins as a fusion structure by combining two structural elements. The post-fusion trimer has a central α-helical trimeric core; however, the fusion domains have two fusion loops at the tip of an elongated β-sheet. Most **non-enveloped viruses**, such as dsDNA adenoviruses and ssRNA picornaviruses, enter cells via vesicles. The former appear to enter via an endocytotic vesicle. In later, the capsid becomes rearranged as receptor binding induced structural transitions, whereby the VP4 internal protein is externalised and the virion surface becomes more lipophilic, and interacts with a vesicle membrane to form a pore so as to allow exit of the RNA into the cytoplasm.

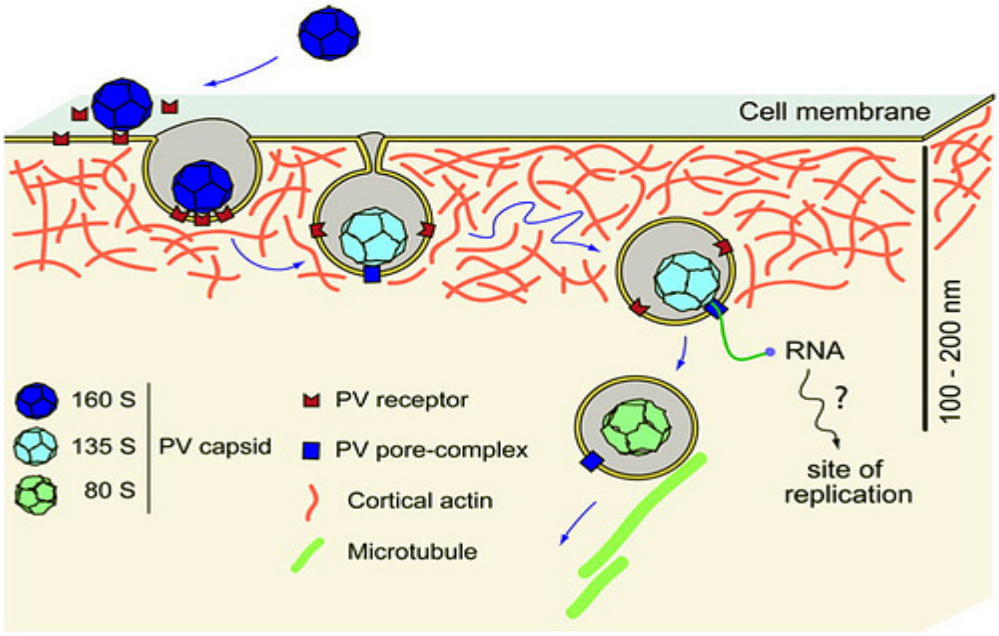

Figure 5: Poliovirus entry into live cells

Nuclear targeting:

A final result in the infection is that DNA genomes end up in the nucleus except for Poxviridae, Phycoviridae and some Baculoviridae and that RNA genomes end up in the cytoplasm except for myxoviruses. These are the sites where the respective viruses may be expected to replicate their genomes.

Adenoviruses are transported by means of the hexon protein of the partially degraded naked capsid which is released into the cytoplasm. This reaches a nuclear pore and allows escape of viral DNA plus certain viral polypeptides into the nucleus.
Parvoviruses enter host cells by receptor-mediated endocytosis, escape from endosomal vesicles to the cytoplasm, and then replicate their DNA in the nucleus.
Herpesviruses generally enter by fusion with the cell membrane by a process that involves several envelope glycoproteins acting together and the core particles migrate to nuclear pores, and release DNA there.
Poxviruses have "intracellular" single-enveloped and "extracellular" double-enveloped forms which enter by **direct cell fusion** (pH-independent) or **lysosomal vesicle fusion** (pH-dependent). Once core virions are in the cytoplasm, they uncoat further to expose a nucleoprotein complex which is first transcriptionally and later, replicationally, active.

3. Plant cell entry:

Every cell in plant is separated from every other cell by thick cell walls, whose dimensions are far larger than the size of the average virion. So plant cells are inaccessible to viruses. These plant cells interconnect only via specific discontinuities in the cellulose walls. These act as gated intercellular channel, which limit the passage of both molecules and virions between cells. Plant viruses possess ssRNA + ve **sense, non-enveloped** and **do not specifically interact with host cell membranes.** The mechanisms employed to enter cells appear to be passive carriage through breaches in the cell wall, followed by cell-to-cell spread in a plant by means of specifically-evolved "movement" functions, and then spread via conductive tissue as whole virions.

The mode of transmission of viruses affects their concentration and localisation in plants. For example, mechanically transmitted viruses (eg: bromoviruses, tobamoviruses) tend to reach very high concentrations in most tissues by non-specific means. Whereas viruses which are introduced into plants via insect vectors with piercing mouthparts tend to be limited in their multiplication to phloem elements, which are preferred target tissues for insect feeding. Consequently, these viruses (eg: luteoviruses, geminiviruses) reach only very low concentrations in whole plants.

Entry of virus on the basis of morphological structure:

The naked virus enters either via translocation (i.e. crosses cell membrane intact directly); genome injection (attachment to the cell surface and releases its genome which penetrates the cytoplasm via a pore in the plasma membrane) or receptor mediated endocytosis. Whereas the enveloped virus enters the cell via receptor mediated endocytosis or membrane fusion.

Interesting facts:

- Some viruses are able to cross plasma membrane into cytosol by endocytic uptake, vesicular transport via cytoplasm, and transport to the endosomes and other intercellular organelles. These processes are associated with clathrin-mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, or other mechanisms.
- HIV-1 uses two receptors Adenoviruses 2 and 5 are necessary to promote conformational modifications to promote the fusion and encourage endocytosis.
- The bacteriophage tail fibres are the attachment sites and bind reversibly to lipopolysaccharides and outer membrane protein OmpC.
- Phage lambda is a tailed phage which attaches to the maltose receptor on the surface of the *E.coli* cell via the J protein in the tail tip.
- Animal viruses enter into the cell by direct cell membrane fusion and entry via endocytotic or other vesicle.
- Neuclear targeting is the result of final destination in the infection of viruses. In some, DNA genomes end up in the nucleus except for Poxviridae, Phycoviridae

and some Baculoviridae and that RNA genomes end up in the cytoplasm except for myxoviruses.

- The mechanisms employed to enter virus into the plant cells appear to be passive carriage through breaches in the cell wall, followed by cell-to-cell spread in a plant by means of specifically-evolved "movement" functions, and then spread via conductive tissue as whole virions.
- Entry of the dengue virus to mammalian cells can occur via receptor-mediated endocytosis in clathrin coated pits.

Questions:

1. How do viruses gain entry to a host cell?
    a. by dissolving a piece of the host cell membrane
    b. by binding to a receptor site on the host cell
    c. by binding to an antibody site on the host cells
    d. all of the above
2. How the dengue viruses enter the cells?
    a. Receptor-mediated endocytosis
    b. Binding to the cell surface receptor
    c. Phagocytosis
    d. Pinocytosis
3. Which receptor plays an important role for the entry of retoviruses?
    a. Glycoproteins
    b. Integrins
    c. Hexon
    d. Chemokine
4. Entry of HIV is an example of
    a. Macropinocytosis
    b. Endocytosis
    c. Attachment factors and receptors
    d. Vesicles

5. Describe the different modes of entry of viruses across the plasma membrane of the cells. Explain with an example.
6. What is fusion protein mode of entry?
7. What is nuclear targeting? Explain with an example.

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Module 3 Lecture 9

Entry of toxins into the cells

Toxins are poisonous substances produced by certain microbes. And the ability to produce toxins by which many pathogens produce disease is known as “toxigenesis”. Toxins may be carried far from the site of invasion by the blood or lymph. Various toxins may cause fever, cardiovascular disturbances, diarrhea, and shock. They can inhibit protein synthesis, destroy blood vessels, and disrupt the nervous system. Most of the toxins are bacterial origin whereas some toxins are also produced by some fungi as a competitive resource. The toxins, named mycotoxins, deter other organisms from consuming the food colonised by the fungi.

In response to the presence of a toxin, the body produces antibodies called antitoxins, which will combine with the toxin and make it harmless. The active toxins are treated by heat or expose to chemicals such as formaldehyde. This makes them harmless but still able to trigger the immune response that causes the production of antibodies. The inactivated toxins are called toxoids, and are used for vaccinations. Diphtheria and tetanus vaccines are prepared this way.

Bacterial Toxins:

There are two main types of bacterial toxins, lipopolysaccharides, which are associated with the cell wall of Gram-negative bacteria, and proteins, which are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. The cell-associated toxins are referred to as endotoxins and the extracellular diffusible toxins that are secreted by bacteria are referred to as exotoxins. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides that act enzymatically or through direct action with host cells and stimulate a variety of host responses.

The production of the toxin is specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while nonvirulent strains do not, and the toxin is the major determinant of virulence (e.g. tetanus and diphtheria).

Usually the site of damage caused by a toxin indicates the location for activity of that toxin. Some protein toxins have very specific cytotoxic activity. For example, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in necrosis. Bacterial protein toxins are strongly antigenic. Protein exotoxins are inherently unstable. In time they lose their toxic properties but retain their antigenic ones.

A plus B Subunit Arrangement

Many toxins act intracellularly and consist of two components: subunit A which is responsible for the enzymatic activity of the toxin and subunit B is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

Pore forming toxins:

Lipids are hydrophobic molecules which are essential constituents of membranes in the cells, whereas bacterial toxins are mainly hydrophilic proteins. All bacterial toxins interact first with their target cells by recognizing a surface receptor, which is either a lipid or a lipid derivative, or another compound but in a lipid environment. When bound to the receptor, some toxins act locally at the cell membrane, triggering pore formation across the lipid bilayer to release cell nutrients or kill target by disturbing their membrane. In contrast, other toxins enter cells and modify an intracellular target. These active toxins are trapped into endocytotic vesicles and follow different steps to access into the cytosol. One of the example is Staphylococcus which secretes pore forming toxins, to alter the host cells and trigger a release of nutrients useful for their growth.

Pore forming toxins use two mechanisms to form pores in the cell membrane, according to the structural domain building the channel:

- Insertion of amphipathic α-helices or α-PFT

Represented by colicins and staphylococcus δ-toxins

- Insertion of amphopathic β-hairpins organized in β-barrels or β-PFT

These are hydrophilic proteins rich in β-sheets and most of the bacterial PFT belong to this class. β-PFTs bind to a cell surface receptor, oligomerize and one or two β-hairpins of individual monomers associate into a β-barrel structure which inserts into the lipid bilayer and creates a channel.

Example: β-PFTs forming large pores: cholesterol dependent cytolysins (CDCs)

CDCs, such as *Clostridium perfringens* PFO (perfringolysin), recognize cholesterol as a receptor. The molecule is rich in β-sheets and is hydrophilic.

PFO pore formation includes the binding of water-soluble PFO monomers to cholesterol in lipid bilayers, which is mediated by the short hydrophobic undecapeptide loop at the tip of the domain 4. Domains 1, 2, and 4 fit into L-shaped forming a cylindrical structure. Interaction of domain 4 with cholesterol induces a conformational change of domains 3, which are rotated from domains 2 and form a belt in the outside face of the cylinder which promotes in the exposure of hydrophobic residues and the insertion of a transmembrane β-barrel into the lipid bilayer. And hence larger pores are formed which contain 35-50 monomers.

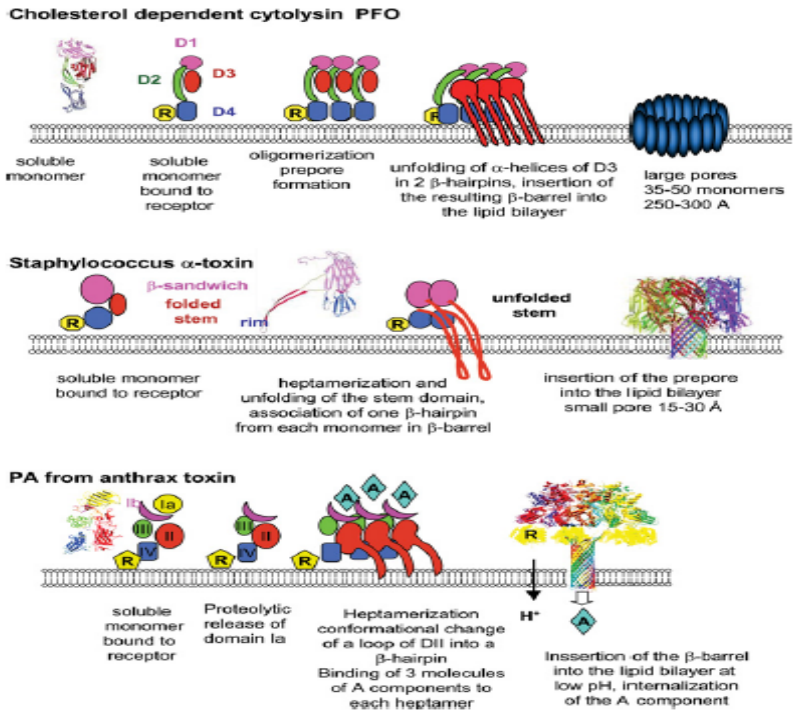

Figure 1: Pore formation of three β-PFTs. Pore formation can occur in the following ways: large pores induced by PFO, an example of a CDC; small pores resulting from heptamerization of *Staphylococcus* α-toxin; and pores formed by the binding component of anthrax toxin (PA) through endosome membrane, permitting the internalization of the corresponding enzymatic components. All the three types of β-PFTs show a common mode of β-barrel formation and subsequent insertion into lipid bilayers. Please draw this figure

Receptor mediated endocytosis:

The bacterial toxin consist of two components: one component (subunit A) which is responsible for the enzymatic activity of the toxin and the other component (subunit B) which is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are two mechanisms of toxin entry into target cells:

1. Direct entry:

The B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

2. Receptor mediated endocytosis:

Here the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of receptor-mediated endocytosis. The toxin is further internalized in the cell in a membrane-enclosed vesicle called an endosome. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

In both cases, a large protein molecule must insert into and cross a membrane lipid bilayer, either the cell membrane or the endosome membrane. This activity is based in the ability of most A+B or A/B toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways.

Example: Diphtheria Toxin

The diphtheria toxin is produced by *Corynebacterium diphtheriae*. It is a bacterial exotoxin of the A/B prototype. It has two parts: subunit A, contains the enzymatic activity for inhibition of elongation factor-2 involved in host protein synthesis and subunit B, is responsible for binding to the membrane of a susceptible host cell. The B subunit possesses a region T (translocation) domain which inserts into the endosome membrane thus releasing the enzymatic component into the cytoplasm. In vitro, the native toxin is produced in an inactive form which is activated by the proteolytic enzyme

trypsin in the presence of thiol. The diphtheria toxin enters its target cells by either direct entry or receptor mediated endocytosis.

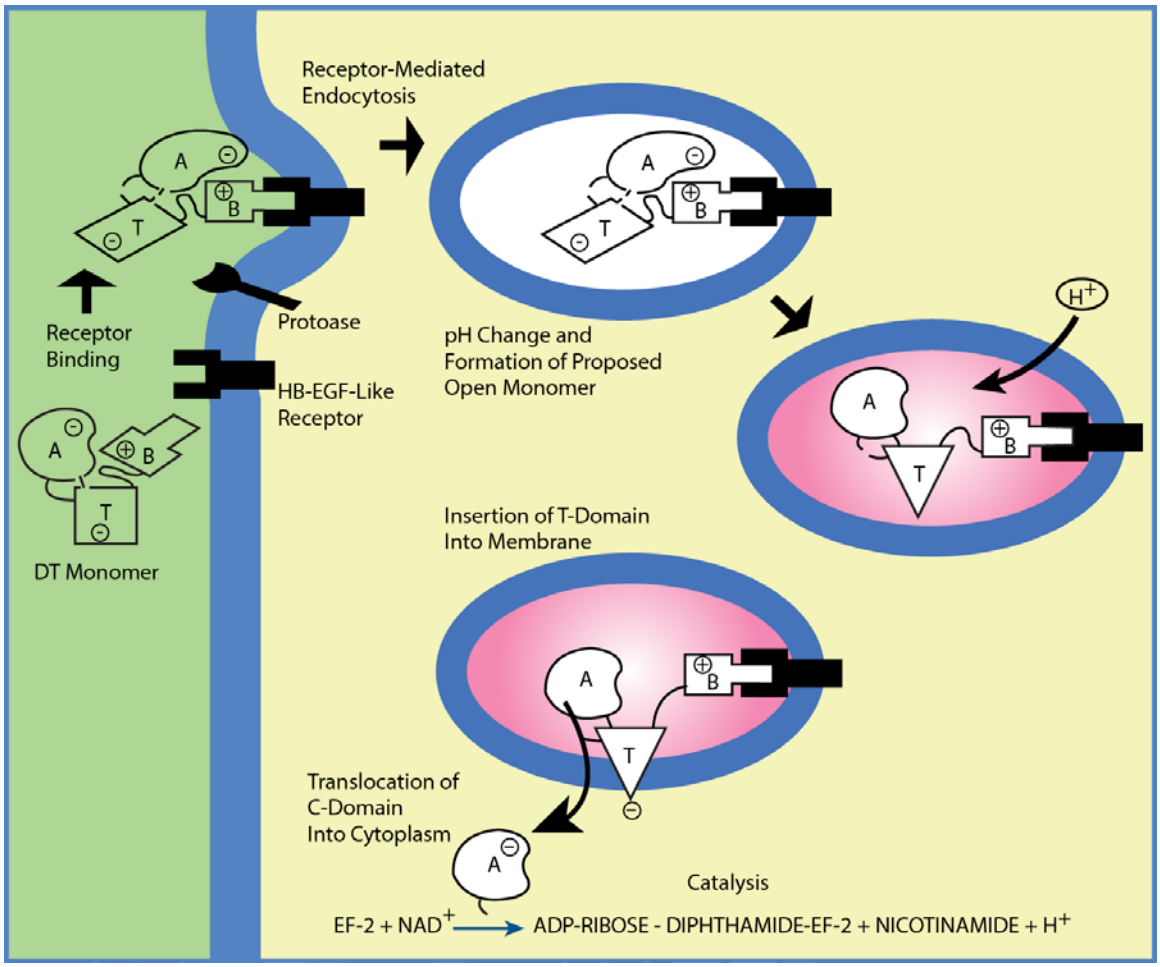

Figure 2: Entry and activity of diphtheria toxin (Dtx) in susceptible cells. The B domain of the toxin binds to a cognate receptor on a susceptible cell. The toxin is taken up in an endosome by receptor mediated encocytosis. Acidification of the endocytic vesicle allows unfolding of the A and B chains exposing the hydrophobic T domain of the toxin. The T domain inserts into the endosome membrane translocating the A fragment into the cytoplasm where it regains its enzymatic configuration. The enzymatic A component utilizes NAD as a substrate. It catalyzes the attachment of the ADP-ribose portion of NAD to elongation factor (EF-2) which inactivates its function in protein synthesis. Redraw this figure

Clathrin independent endocytosis of ricin and Shoga toxin:

Plant toxin ricin binds to both glycolipids and glycoproteins with terminal galactose all over the cell surface and is therefore localized to all types of membrane invaginations and the toxin is internalized by all endocytic mechanisms. Ricin has been localized in clathrin-coated pits, but is still endocytosed when this pathway is blocked.

Clathrin-independent endocytosis is different from uptake by caveolae and macropinocytosis. For instance, clathrin-independent endocytosis occurs on the apical side of polarized cells, whereas caveolae are localized in the basolateral domain. Clathrin-independent endocytosis of ricin occurs when uptake from caveolae and clathrin-dependent endocytosis are inhibited by extraction of membrane cholesterol. Removal of cholesterol leads to the disappearance of caveolar and inhibits formation of invaginated clathrin-coated pits (**Sandvig et al.,2000).**

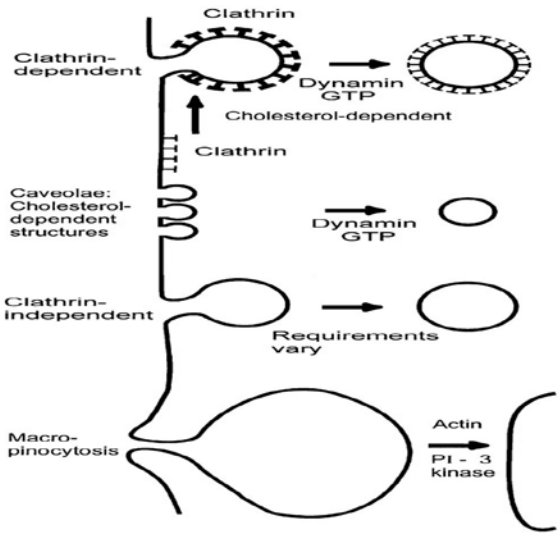

Figure 3: Structures proposed to be involved in endocytosis.

On the other hand, Shiga toxin is endocytosed preferentially by the clathrin-coated pathway, although it is bound to a glycolipid receptor (globotriasylceramide; Gb3).

Bacterial toxin transport using macromolecular syringes:

The pathogens inject their toxins into the cytosol of host cells through bacterial transport machines that function as macromolecular syringes are either bacterial flagella or conjugative pili and facilitate the direct passage of toxin effectors from bacterial donor cells into eukaryotic cells by processes of Type III or Type IV secretion mechanisms or by constructing large pores within the plasma membrane of target cells that function as for direct effectors delivery.

As the first step in cellular entry, AB toxins bind to one or more plasma membrane surface receptors. They incorporate two discrete and essential functional components that vary in physical arrangement but are generally conserved in terms of function. Thus, toxins “A fragments” are the active moiety that can modify intracellular target molecules by one of the enzymatic activities, including ADP-ribosylation, UDP-glucosylation, or proteolysis. Meanwhile, the “B fragments” serve as delivery vehicles for their A components by binding to plasma membrane surface receptors and facilitating translocation of the A components into the cytosol through available portals.

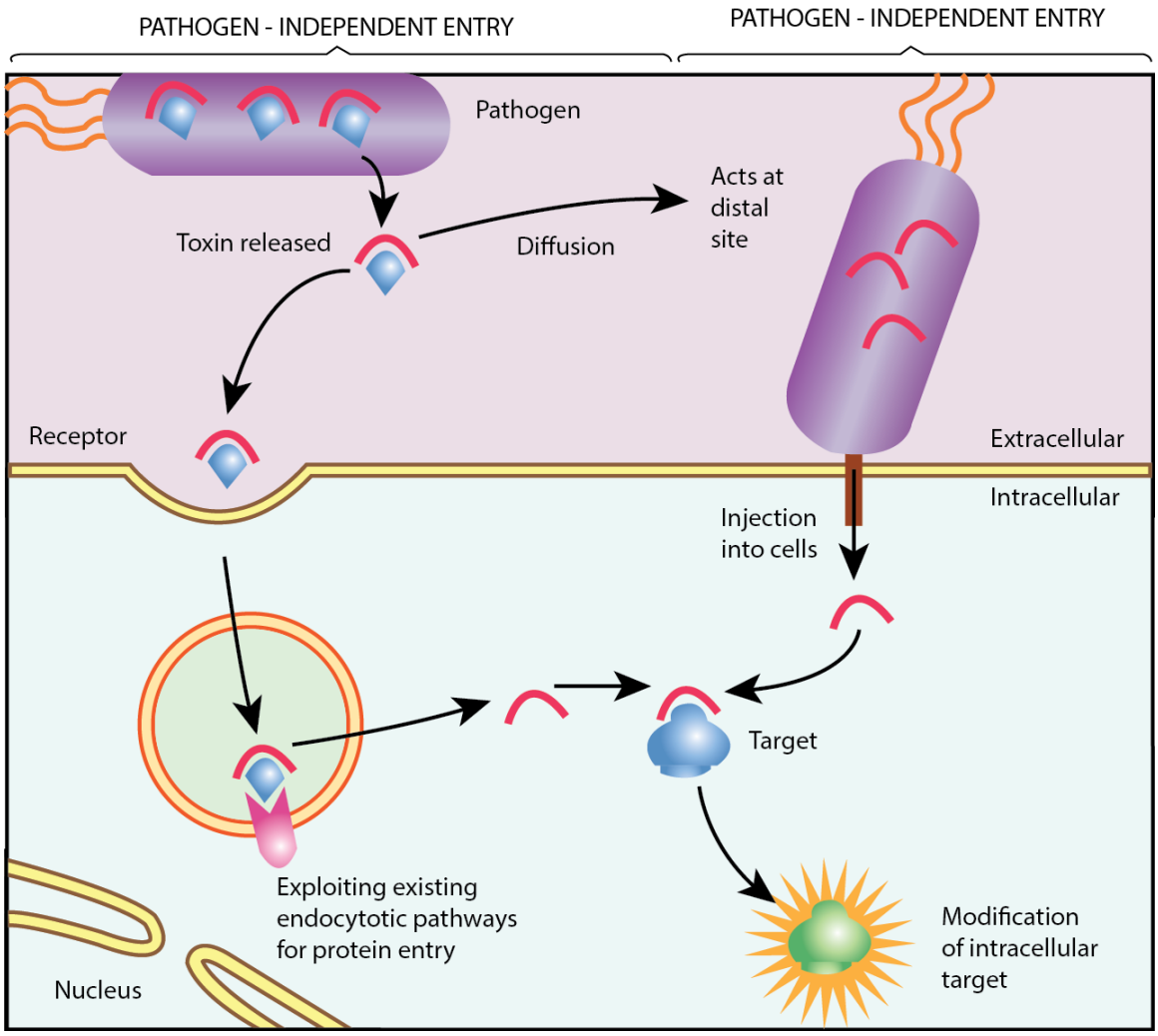

Figure 4: Intracellularly acting bacterial toxins access their substrates within host cells by one of several mechanisms. Some gram-negative pathogens directly inject toxin effectors through flagellar- or pilus-adapted transport machines into eukaryotic cells by Type III or IV secretion systems, respectively. Alternatively, bacteria release intracellular-acting toxins, also called AB toxins, into the host environment where they act locally or diffuse to act distally to the site of colonization. AB toxins commonly exploit endocytic pathways that eukaryotic cells use for importing proteins. Finally, *Bordetella* adenylate cyclise toxins directly enter the cytosol from the plasma membrane. Redraw this

Toxins Exploit the Acidic Environment of Endosomal Compartments

Some AB toxins like diphtheria, anthrax and the botulinum neurotoxins, exploit the drop in pH to between 5.0 and 6.0 as endocytic vesicles are trafficked from the plasma membrane into the cell. This acidification results in the insertion of B fragments into the membrane and the formation of ion-conducting channels. Partially unfolded A fragments use these B fragment-derived channels as conduits into the cytosol.

Toxins Exploit the Sec61 Retro-Translocon in the Endoplasmic Reticulum

The second group of AB toxins includes cholera toxin, shiga toxin, and *Pseudomonas aeruginosa* exotoxin A and exploit the degradation pathway for misfolded proteins. The secretion pathway is at least partially reversible to the fate of nascent proteins for secretion, enabling several bacterial toxins to travel this “retrograde” pathway from the plasma membrane to the ER lumen through pores to the cytosol. Within the lumen of the ER, the A fragments are transported through an existing membrane complex whose primary protein is Sec61. The B fragments of these toxins bind to receptors to facilitate the trafficking of catalytic A fragments to the ER.

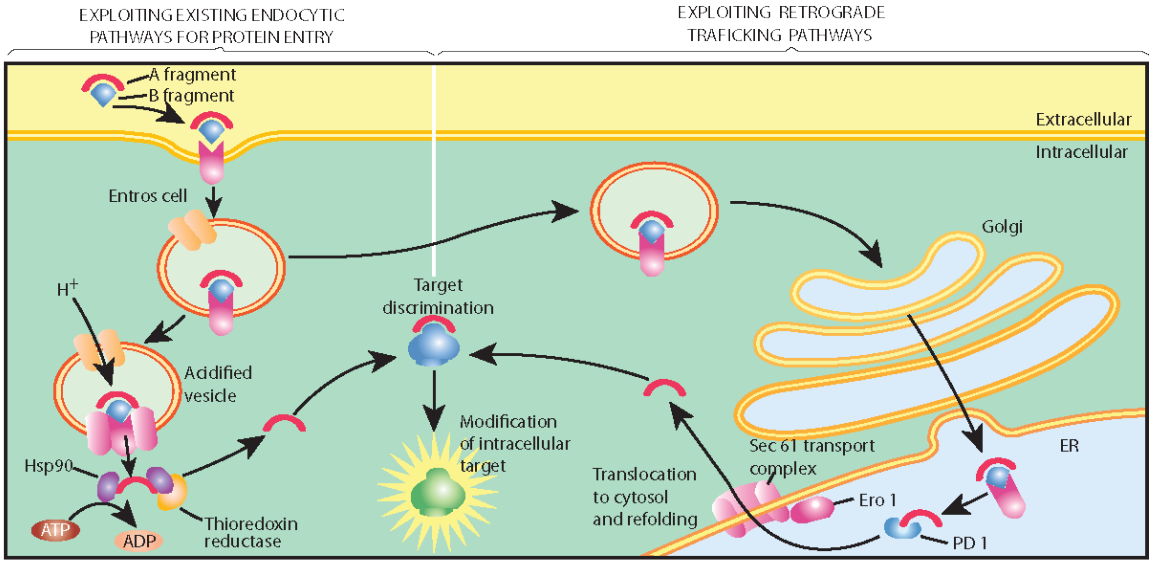

Figure 5: Entry of AB toxins into target cells. Some toxins use host endocytic pathways that cells use for degrading exogenous proteins within lysosomes. Redraw this

Entry of toxin with influx of Ca^{2+}:

In the absence of $Ca^{2+,}$ cells were not sensitive to the toxic proteins like abrin and modeccin and also the sensitivity to ricin and diphtheria toxin was reduced. Calcium deprivation leads to negative effect on the binding and endocytosis of these toxins. Some studies indicate that Ca^{2+} is involved in the entry mechanism for abrin, modeccin, and ricin, possibly as a Ca^{2+} flux together with the toxin.

The plant toxins abrin, modeccin, and ricin consist of two polypeptide chains connected by a disulfide bond. B-chain binds the toxins to cell surface receptors while A-chain enters the cell and inhibits protein synthesis.

Diphtheria toxin is synthesized as one polypeptide chain which is cleaved by proteolytic enzymes into an A- and a B-fragment. “A fragment” possesses enzymatic activity and inactivates components of the protein-synthesizing machinery. Recently it was shown that low pH is required for entry of diphtheria toxin into the cytosol before transport of the A-fragment across the membrane. The low pH induces exposure of a hydrophobic domain in the B fragment that forms ion permeable channels across the membrane and interacts with the membrane lipids and hence facilitates entry of the A-fragment (**Sandvig et al., 1982**).

Interesting facts:

- “A” denotes to the active protein and “B” dentotes to the binding protein in AB toxin.
- Some toxins like diphtheria, anthrax and the botulinum neurotoxins, exploit the drop in pH for its entry as endocytic vesicles are trafficked from the plasma membrane into the cell.
- Some toxins like cholera toxin, shiga toxin, and *Pseudomonas aeruginosa* exotoxin A exploit the sec61 retro-translocon in the endoplasmic reticulum for its entry.
- Calcium deprivation leads to negative effect on the binding and endocytosis of these abrin, modeccin, ricin and diphtheria toxin. The entry mechanism for these toxins depends on Ca^{2+} flux together with the toxin.

Questions:

1. Which of the following is not an A-B exotoxin?
    a. Streptolysin O
    b. Staphylococcus aureus enterotoxin
    c. Cholera toxin
    d. Diphtheria toxin

e. Tetanus toxin

2. All of the following are true of A-B exotoxins except:
   a. The B portion of the toxin binds to surface receptors on host cells.
   b. They consist of two polypeptide components.
   c. They are only produced by gram-negative bacteria.
   d. The A portion of the toxin is the active component.
   e. Many exotoxins are A-B toxins.
3. Which of the following bacterial toxins binds to nerve cells, preventing chemical communication between nerve and muscle cells?
   a. Diphtheria toxin
   b. Erythrogenic toxin
   c. Staphylococcal enterotoxin
   d. E. coli endotoxin
   e. Botulinum toxin
4. Which is true of endotoxins?
   a. They increase blood pressure.
   b. They are produced by gram-positive bacteria.
   c. They are disease-specific.
   d. They are released upon cell lysis.
   e. They are proteins.

5. In a bacterial exotoxin:
   a. The A subunit allows the toxin to bind to the surface of specific host cells.
   b. The A subunit is part of the outer bacterial membrane, released when the bacterialcell dies.
   c. The A subunit is able to interfere with a specific host cell activity, once it has beentaken into the host cell.

d. We expect the A subunit for all exotoxins to operate in the same manner.

6. What effect does botulin toxin have on the body?
   a. It causes excitation of neurons.
   b. It stimulates the removal of fluid from the tissues.
   c. It stimulates increased intestinal motility.
   d. It paralyzes the muscles of the respiratory tract.
   e. It prevents gastrointestinal motility.
7. Where are the target cells of diphtherotoxin located?
   a. The throat
   b. The skin
   c. The skeletal muscles
   d. The lungs
   e. The heart and nervous system
8. What does the A in AB toxin stand for?
   a. Active
   b. Agglutination
   c. Adhesion
   d. Accumulation
   e. None of these is correct.
9. Which bacterium produces the hemolysin streptolysin?
   a. *S. pyogenes*
   b. *S. aureus*
   c. *C. diphteriae*
   d. *C. botulinum*
   e. *C. tetani*
10. Which toxin contains LPS and triggers fever?
    a. Endotoxin
    b. Exotoxin
    c. Both
    d. None

11. CDCs regonize:
    a. Cholesterol as a receptor
    b. Carbohydrates moieties as a receptor
    c. Glycolipids receptor
    d. Glycoproteins
12. How the toxins enter into the cell?
13. What are macromolecular syringes?
14. What are the functions of A- and B-fragments in A/B toxin for the entry of toxin into the cell?
15. What are the two mechanisms that are used by pore forming toxins for the formation of pores in the cell membrane? Explain with an example.

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