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.

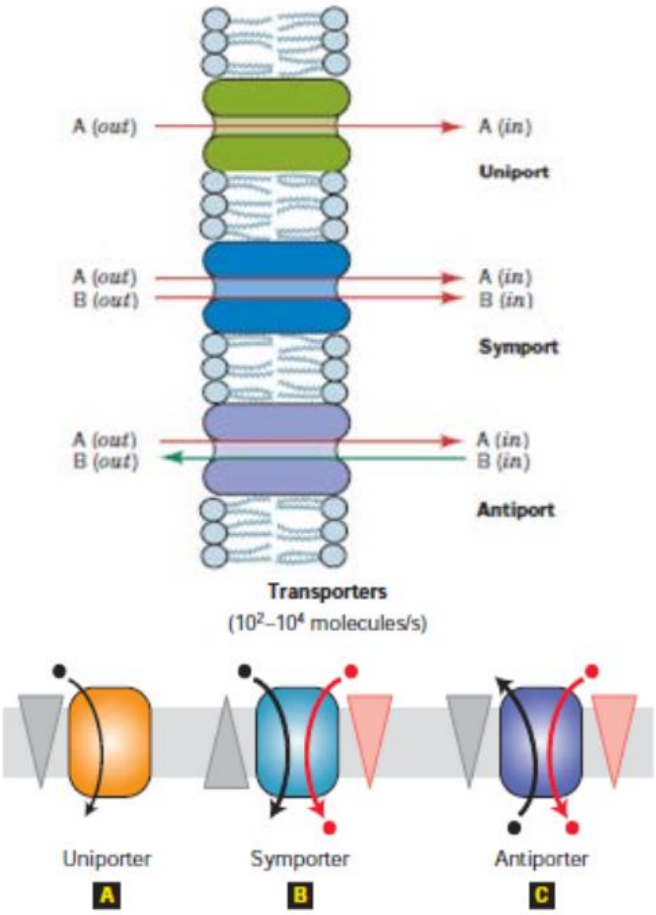

Figure 1: Transporters, which fall into three groups, facilitate movement of specific small molecules or ions.(A) Uniporters transport a single type of molecule down its concentration gradient. Cotransport proteins (symporters (B), and antiporters (C)) catalyze the movement of one molecule againstits 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

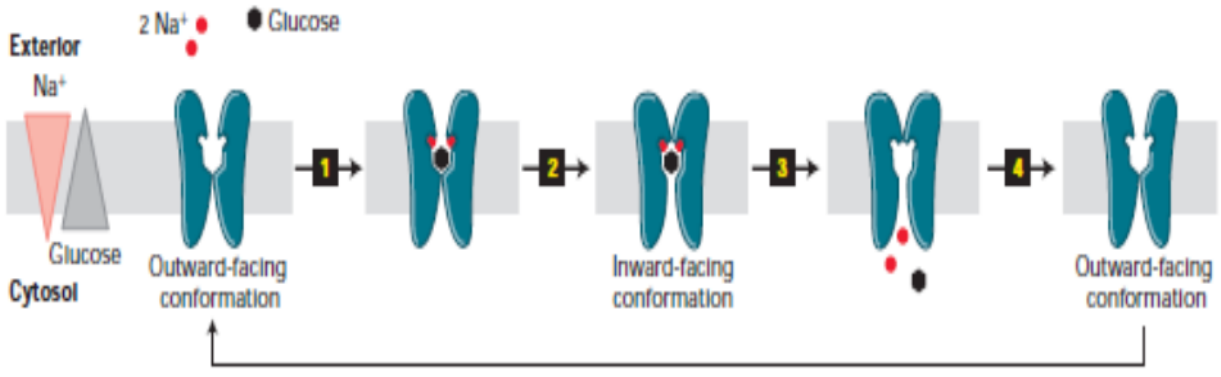

Figure 2: Operational model for the two-Na^+/one glucose symporter.

The overall reaction is:

$2Na^+$ (out) + Glucose (out) $\leftrightarrow$ $2Na^+$(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 ΔG of about -3 kcal per mole of Na^+ transported.Thus the ΔG 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) $\leftrightarrow$ $3Na^+$ (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 $\approx 2 * 10^{-7}$ 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.

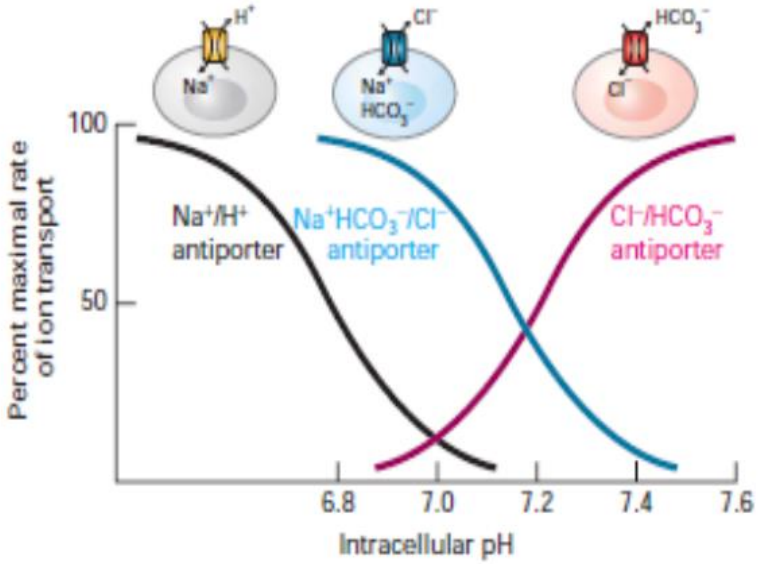

Figure 3: The activity of membrane transport proteins that regulate the cytosolic pH of mammalian cells changes with pH.Direction of ion transport is indicated above the curve for each protein

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.

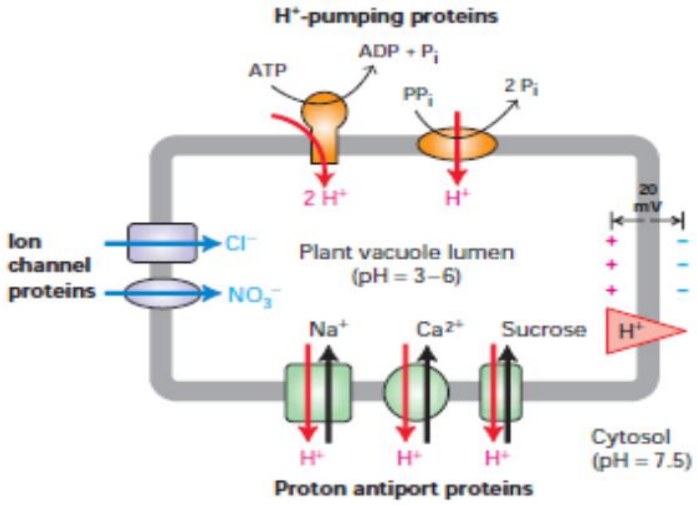

Figure 4: Accumulation of ions and sucrose by the plant vacuole.The vacuolar membrane contains two types of proton pumps (orange): a V-class H^+ ATPase (*left*) and a pyrophosphate-hydrolyzing proton pump (*right*) that differs from all other ion-transport proteins and probably is unique to plants. These 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.