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.

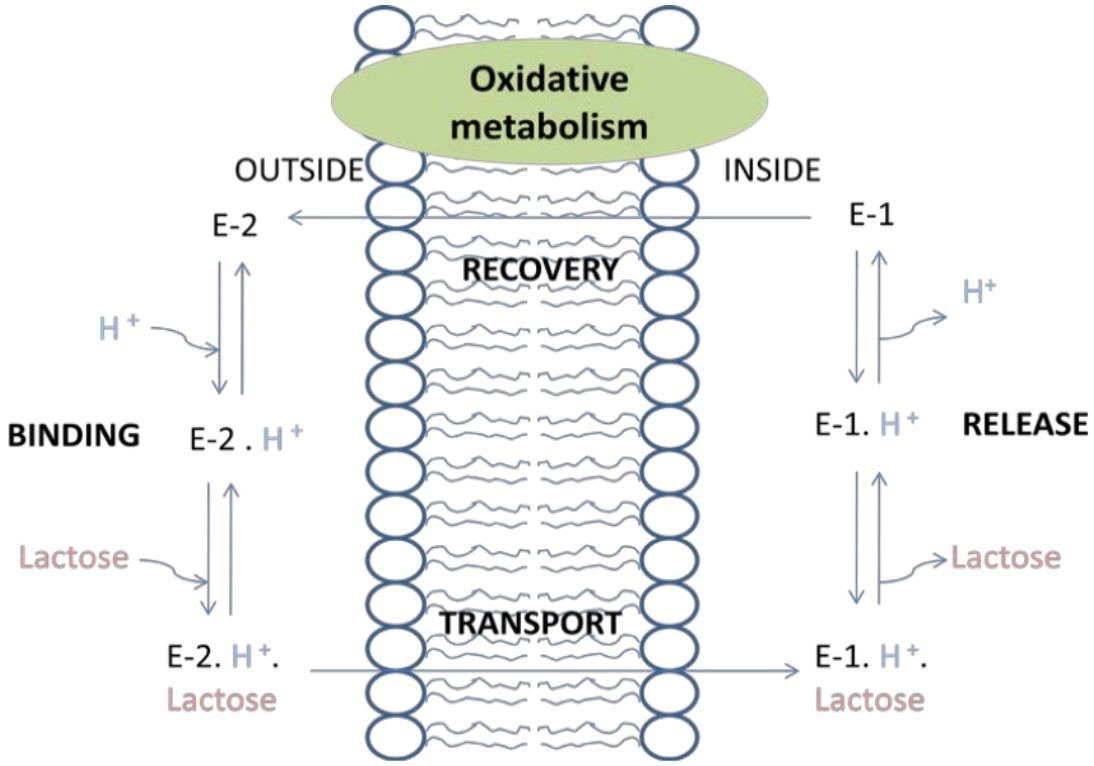


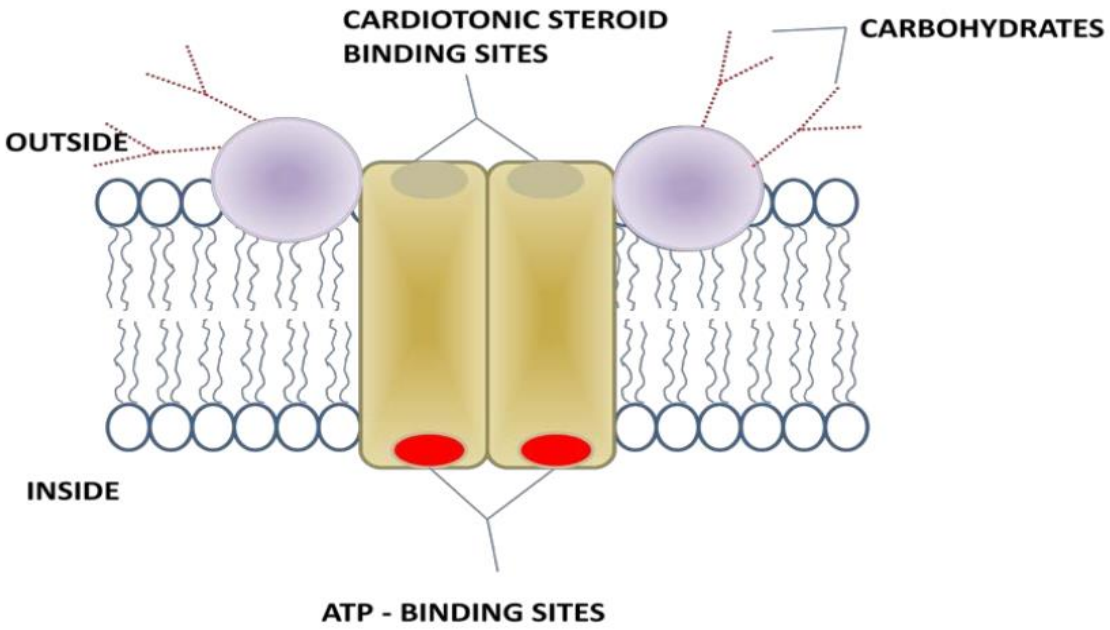
Figure 1: Schematic diagram for the cotransport of H^+ and lactose by lactose permease in *E.Coli*. H^+ binds first to E-2 outside the cell, followed by lactose. They are sequentially released from E-1 inside the cell. E-2 must bind to lactose and H^+ in order to change the conformation to E-1, thereby 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:

$3\ Na^+(in) + 2\ K^+(out) + ATP + H2O \rightarrow 3\ Na^+(out) + 2\ K^+(in) + ADP + Pi$

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.

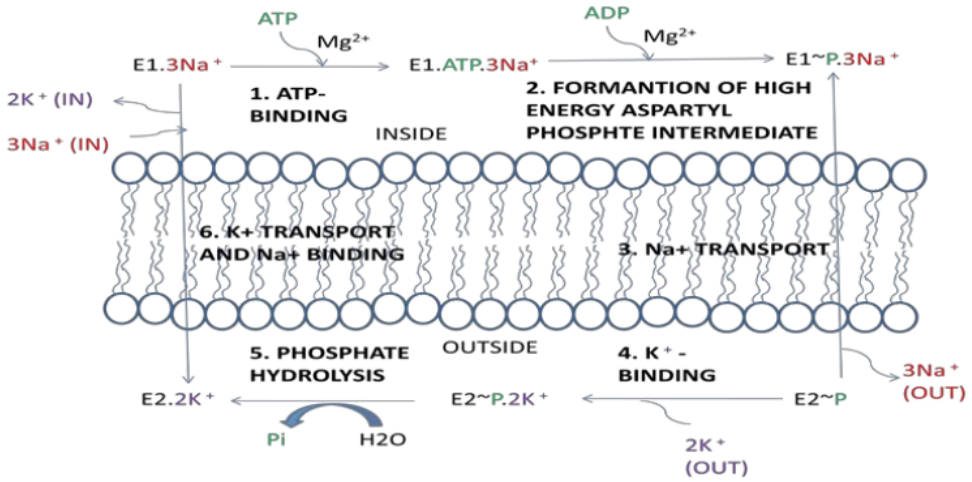


Figure 3: Scheme for the transport of Na^+ and K^+ by the Na^+/K^+ ATPase.

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.

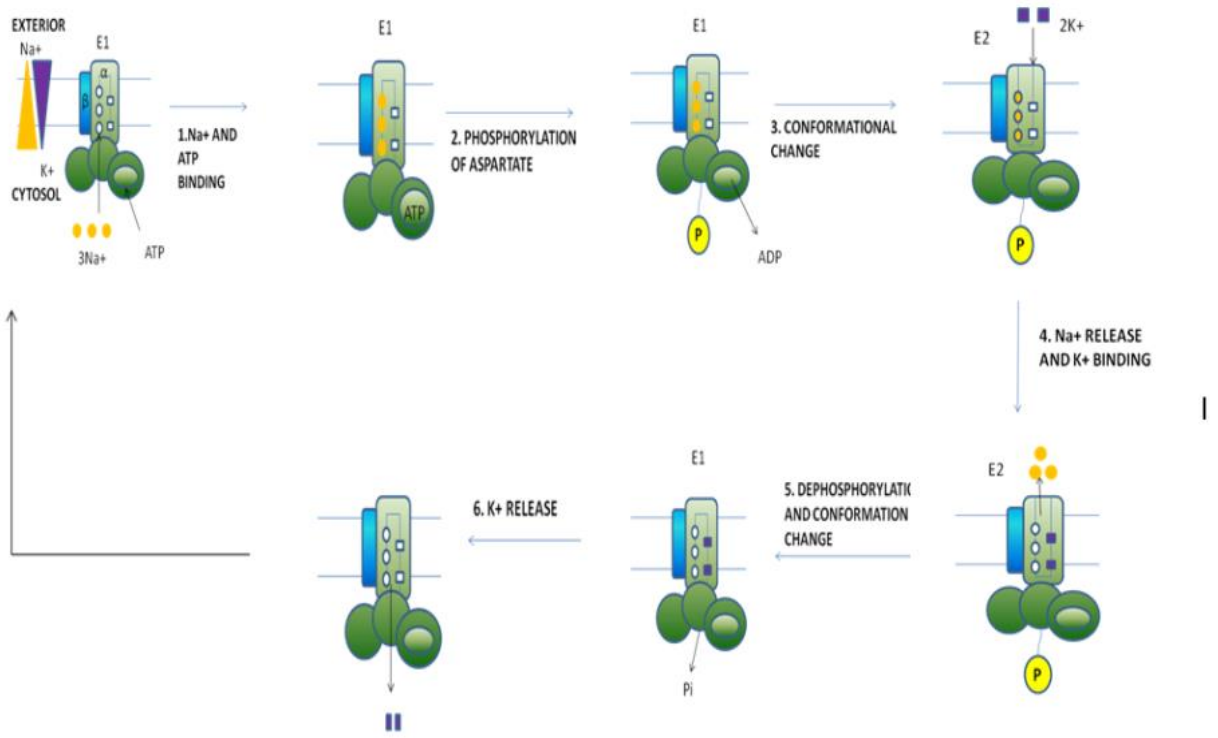


Figure 4: Operational model of the Na^+/K^+ ATPase in the plasma membrane. Only one of the two catalytic α subunits of this P-class pump is depicted. It is not known whether just one or both subunits in a single ATPase molecule transport ions. Ion pumping by the Na^+/K^+ ATPase involves phosphorylation, 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.

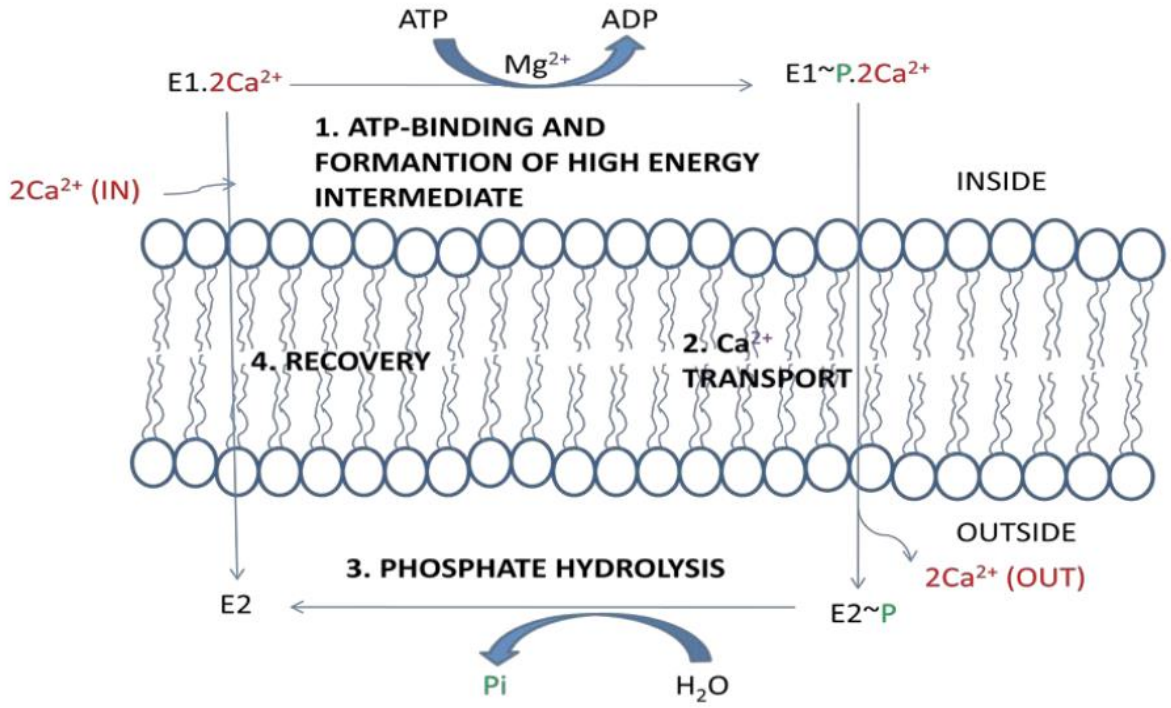


Figure 5: Scheme for the active transport of Ca^{2+} by the Ca^{2+} ATPase. Here (*in*) refers to the cytosol and (*out*) refers to the outside of the cell for plasma membrane Ca^{2+} ATPase or the lumen of the endoplasmic reticulum (or sarcoplasmic reticulum) for the Ca^{2+} ATPase of that membrane.

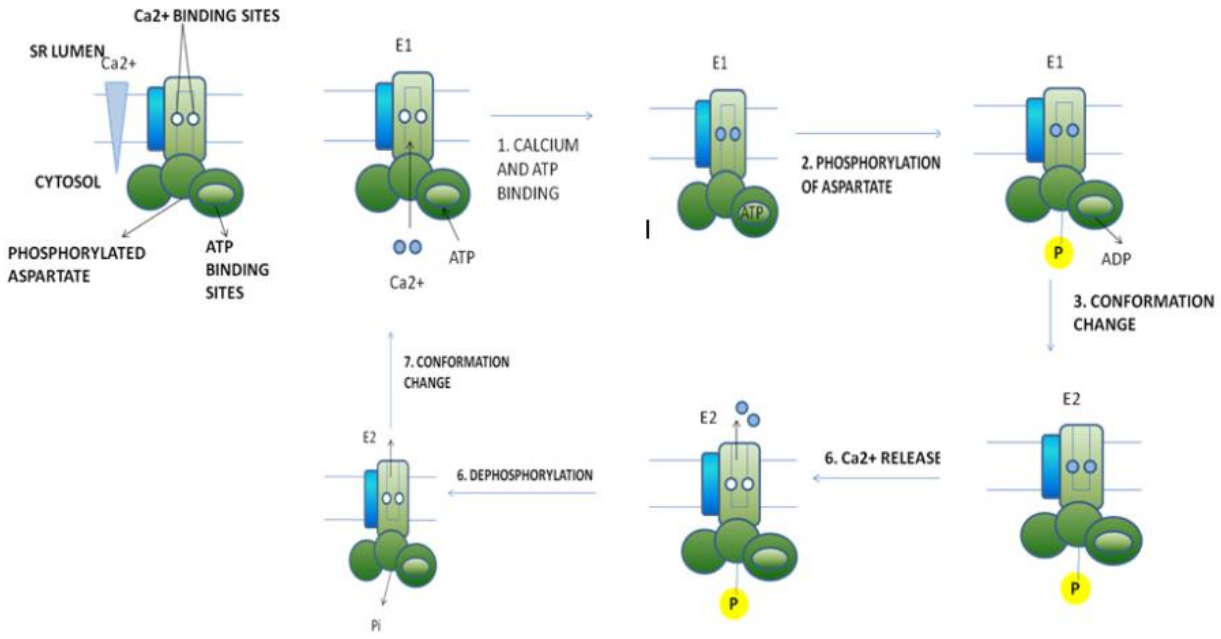


Figure 6: Operational model of the Ca^{2+} ATPase in the SR membrane of skeletal muscle cells. Only one of the two catalytic α subunits of this P-class pump is depicted. E1 and E2 are alternative conformations of the protein in which the Ca^{2+} binding sites are accessible to the cytosolic and exoplasmic 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.