ATP dependent proton pumps

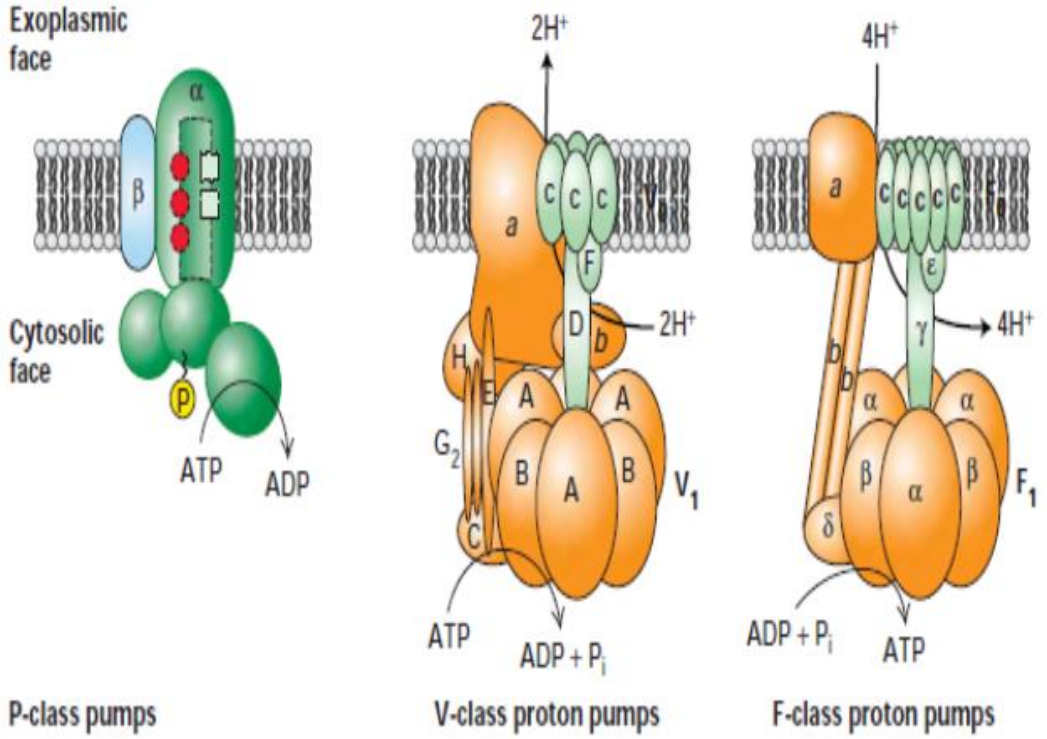
Proton pump

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:



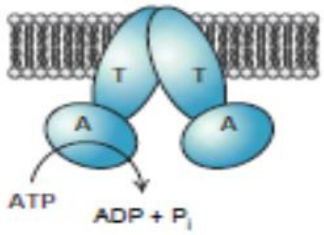


Figure 1: Different types of ATP dependent proton pumps

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 $\alpha\beta\alpha\beta\alpha\beta$. 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 $(\alpha\beta)3$ hexamer of the F_1 complex form a rigid structure anchored in the membrane. The rodlike b subunits form a stator that prevents the $(\alpha\beta)3$ hexamer from moving while it rests on the γ subunit.

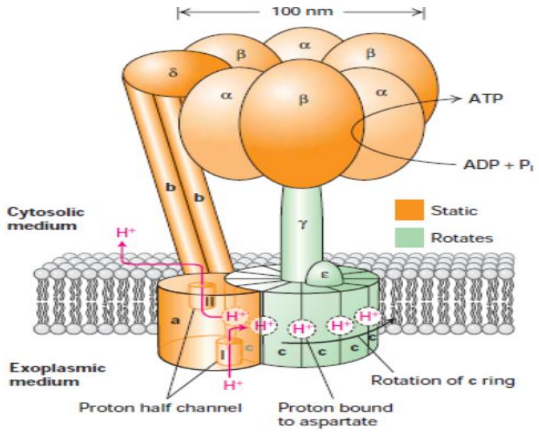


Figure 2: Model of the structure and function of ATP synthase (the F_0F_1 complex) in the bacterial plasma membrane.
The F_0 portion is built of three integral membrane proteins: one copy of a, two copies of b, and on average 10 copies of c arranged in a ring in the plane of the membrane. Two proton 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.

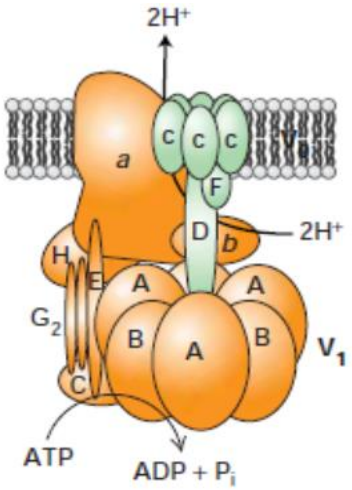


Figure 3: V-class proton pump

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.

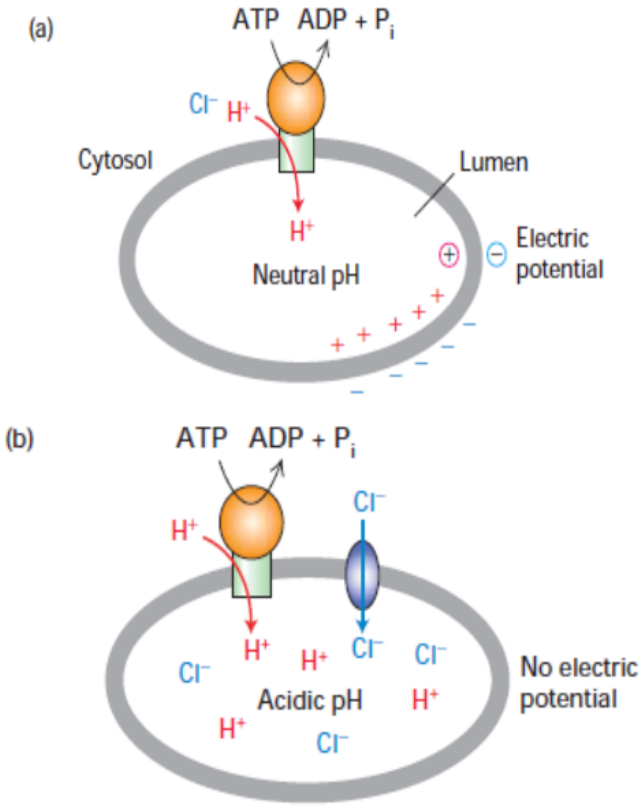


Figure 4: Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, 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.