

ATPases, Ion-motive

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Ion motive ATPases allow ion(s) to accumulate on one side of a biological membrane at the expense of ATP hydrolysis. In this way active transport across biological membranes is mediated.

Introduction: Active Transport

Keynes and Hodgkin showed that nerve cell stimulation corresponds to an influx of Na^+ in the cell. In 1957 Skou (1997 Nobel Prize for chemistry) demonstrated that an ATPase activated by Na^+ and K^+ is responsible for this Na^+ movement through crab nerve membrane. The concept of a membrane ATPase transporting Na^+ led to the discovery of other ion motive ATPases such as the Ca^{2+} ATPase in skeletal muscle, the gastric H^+/K^+ ATPase, and later two groups of H^+ ATPases, one found in plasma membranes of fungi and plants and the other in vacuole membranes.

Ion transport into and out of a biological compartment is referred to as membrane transport. When the ions move down the electrochemical potential gradient this movement is referred to as passive or facilitated transport. When they move against the electrochemical potential gradient, the transport requires energy and is said to be active. In the latter case the energy is supplied by ATP hydrolysis in the cell and the two processes are intimately linked. They are coupled within the same protein, which is the locus of both ion transport and ATPase activity. These proteins, the so-called ion motive pumps, play a key role in maintaining one ion highly concentrated on one side of a membrane. For instance, the Na^+/K^+ ATPase maintains high K^+ and low Na^+ concentrations in the cell by expelling three Na^+ against the entry of two K^+ per cycle. There are two types of ion motive pumps: the P-type, which is phosphorylated by ATP during its cycle, and the V-type, which is not phosphorylated. Based on sequence homologies between the ion motive ATPases, molecular biology has evidenced numerous V-ATPases and P-ATPases – not only of the P_{II} type transporting Na^+ , K^+ , Ca^{2+} or H^+ , but also of the P_{I} type transporting Cu^{2+} , Cd^{2+} and Zn^{2+} . Note that the F-type ATPases, which are also membrane ATPases, although structurally very similar to the V-type, function *in vivo* as ATP synthases, i.e. they synthesize ATP at the expense of a preexisting H^+ gradient across the membrane.

Secondary article

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Structure of P-type ATPases

The primary sequences of the various P-ATPases have been established since the 1980s through sequencing of the encoding DNAs, and this has revealed many isoforms. Heterologous expression of the main ATPases has been conducted in various hosts and much information has been gathered from site-directed mutagenesis and chimaera constructions. These techniques are used to analyse whether a given amino acid or region of the ATPase is essential, participates, or simply does not interfere with a given property of the ATPase.

P-ATPases essentially comprise one polypeptide (α chain) responsible for both ion transport and ATP hydrolysis. Some have an additional glycosylated polypeptide (β chain) that is not involved in the pumping mechanism but is thought to be important for $\alpha\beta$ complex insertion in the membrane.

The α chains of the various P-ATPases comprise about 1000 amino acids (~ 100 kDa), with numerous conserved sequences and similar hydrophobic profiles. These profiles predict 10 transmembrane segments, an even number in agreement with the finding that N- and C-terminal amino acids are both found on the cytoplasmic side. The model in **Figure 1**, which was derived for the Ca^{2+} ATPase, shows the general features of the α chain (Green and Stokes, 1992). Seventy per cent of the mass is in the cytoplasm, 25% in the membrane and 5% in the space across the membrane. The catalytic site, which comprises the ATP-binding site and the phosphorylation site, is located in the large cytoplasmic loop between the fourth and fifth transmembrane segments, whereas the transport sites, i.e. the ion translocation sites, are buried in the membrane. The ATP-binding site and the phosphorylation site are two regions of high homology for the P family, particularly the sequences KGAPE in the ATP site and DKTGTLT, in which D is the phosphorylation site. Among the transmembrane segments, the sequence PEGL is characteristic of the fourth segment for the P_{II} type, which becomes CPX for the P_{I} type.

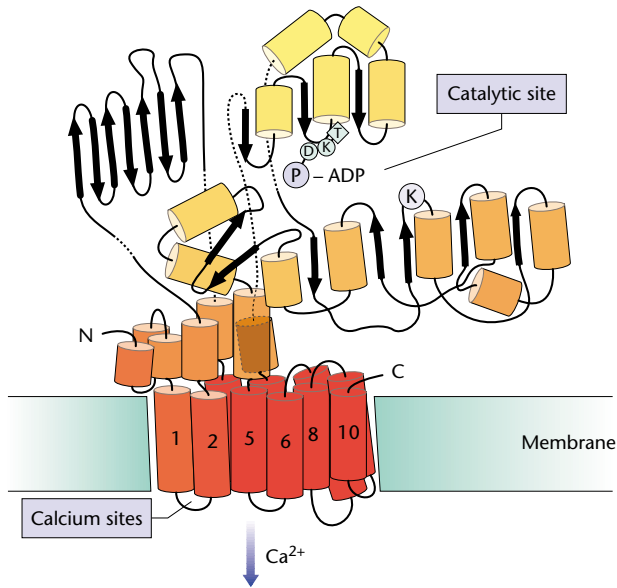


Figure 1 Ca^{2+} ATPase tertiary structure prediction, redrawn from Green and Stokes (1992). Note the phosphorylation site (D, aspartate) and the nucleotide site (K, lysine)

None of the P-ATPases has yet been crystallized in a form appropriate for X-ray diffraction. However, in the presence of vanadate and Mg^{2+} , P-ATPases form two-dimensional crystals that can be seen in vitreous ice. In the case of the Ca^{2+} ATPase, the two-dimensional crystals diffract at 1.4 nm and give the general shape of the molecule (Toyoshima *et al.*, 1993; see **Figure 2**). The intramembranous details in **Figure 2** are a tentative fit of the shape of the Ca^{2+} ATPase to the 10-helix model of **Figure 1**. Although not yet confirmed, the model in **Figure 2** shows that ATP hydrolysis and Ca^{2+} transport occur 4–5 nm apart. This implies a transduction of the energy provided by ATP

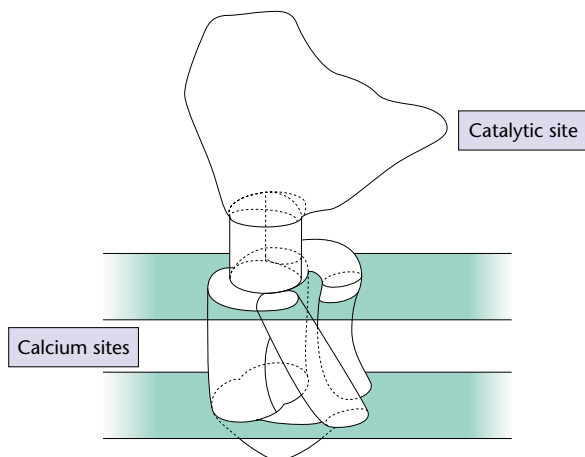


Figure 2 Ca^{2+} ATPase shape, redrawn from Toyoshima *et al.* (1993).

splitting from the phosphorylation site to the transport sites.

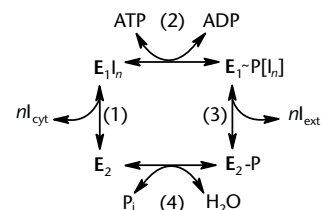
The β subunit is composed of about 300 amino acids. Starting from the N-terminal side, a short segment is located in the cytoplasm, followed by a unique transmembrane segment, while 80% of the β subunit is located in the extracellular space. This extracellular region comprises at least three glycosylated asparagines, representing a mass of about 10 kDa.

The membranous region of the P-ATPases is surrounded by phospholipids. Because the density of proteins in membranes varies from one membrane to another, the number of phospholipids surrounding each kind of ATPase varies, although a minimum is required for the ATPase to be functional. Nonionic detergents solubilize the ATPases in an active form. When the ATPases are totally delipidated the activity is lost, but this can be restored by adding phospholipids. Active ATPases can then be integrated into artificial membranes, leading to functional reconstituted proteoliposomes.

General Mechanism for Phosphorylation and Ion Translocation

The work of a P-ATPase is to transport an ion across a membrane from one compartment where it is diluted to another compartment where it is concentrated. This concentration work requires energy, which is provided by ATP hydrolysis. The two processes, ion movement and ATP hydrolysis, are intimately linked. This is depicted in **Scheme 1**, the so-called E_1/E_2 model, which represents the minimum number of events required to accomplish such a transport cycle.

In this scheme the number, n , of ions, I, transported per cycle is represented by nI . This scheme alternates vectorial steps, where the ion(s) bind to or dissociate from the ATPase, and chemical steps, where the ATPase changes its conformation. In this scheme E_1 has a high affinity for I and its binding sites are orientated towards the compartment of low concentration. After binding of nI (step 1), the ATPase can be phosphorylated by ATP (step 2). In the $E_1 \sim P[I_n]$ species the phosphate is covalently bound to the aspartate of the phosphorylation site (**Figure 1**) and the nI are said to be occluded. This means that they are in a state which



Scheme 1 E_1/E_2 model for ion transport.

impairs rapid exchange with bulk I on either side of the membrane. $E_1 \sim P[I_n]$ is unstable and changes its conformation in $E_2\text{-P}$ (step 3). During this change the affinity of the ATPase for I decreases, the transport sites are orientated toward the compartment of high concentration, and nI dissociate. In step 4, $E_2\text{-P}$ is hydrolysed and the ATPase is ready to start a new cycle.

An important feature of the P-ATPase cycle is its total reversibility. In practical terms two situations can be distinguished. In the presence of an ion gradient, ATP can be synthesized from ADP and inorganic phosphate (P_i) at the expense of the efflux of nI per ATP synthesized. ATP synthesis lasts as long as the ion gradient and the ADP and P_i pool allow the reverse cycle to work. In the absence of an ion gradient, ATP can be synthesized by describing, step by step, the cycle in the reverse direction. In the absence of I, when the ATPase is in the E_2 form, it is spontaneously phosphorylated by P_i in the presence of Mg^{2+} , i.e. step 4, which leads to the formation of $E_2\text{-P}$. The reverse cycle can proceed provided that the low-affinity transport sites are readily accessible, in other words if the experiment is conducted with fragments of membranes or leaky vesicles. In this case, I and ADP are added together so that nI bind to the low-affinity sites, thus inducing the formation of $E_1 \sim P[I_n]$, which in turn binds ADP and transfers its bound phosphate to ADP to synthesize ATP. The $E_1 \sim P[I_n]$ and $E_2\text{-P}$ species are chemically different in that $E_1 \sim P[I_n]$ only reacts with ADP to synthesize ATP. This is referred to as ADP sensitivity. The ability to be phosphorylated by P_i confers on the P-ATPases a sensitivity to vanadate, which forms a dead-end complex with E_2 . Vanadate is therefore a potent inhibitor of P-ATPases, but also of some non-P-ATPases, such as myosin, a soluble protein, and P glycoprotein, a membrane ATPase responsible for multidrug resistance.

This E_1/E_2 model, which has been such a fruitful working hypothesis, envisages two main conformations for the ATPase. E_1 is phosphorylatable by ATP and E_2 is phosphorylatable by P_i , the chemical switch between the two conformations being the occupancy of the high-affinity binding site(s) by nI . This is an oversimplification, as more than one conformational change occurs during the cycle. Jencks (1989) proposed that the chemical and vectorial events are interlocked like two gearwheels, so that neither process can take place unless the other occurs. Another limitation of the E_1/E_2 model is the absence of a counterion transported by the ATPase, for instance K^+ for the Na^+/K^+ and the H^+/K^+ ATPases.

Note that although many P-ATPases have been isolated, characterized, mutated and so forth, the mechanism of ion translocation has still not been elucidated. As will be mentioned below, some movements of the two K^+ through the Na^+/K^+ ATPase, as well as those of the two Ca^{2+} through the sarco(endo)plasmic reticulum ATPase 1 (SERCA 1), are sequential, as if the ions were crossing the membrane one after the other. Whether or not

the ions do cross the membrane sequentially is still an open question, but one that needs to be answered if one wants to propose a mechanism for ion translocation across the ATPase.

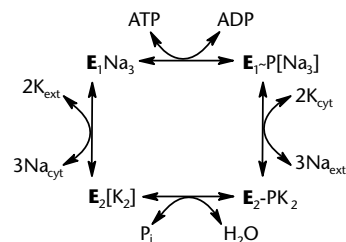
Whether the P-ATPases function as monomers or polymers is another unresolved question. In the case of the sarcoplasmic Ca^{2+} ATPases, for instance, there is no evidence that several molecules interact during the pump cycle and it has been shown that, after solubilization, the monomer is able to describe the whole cycle. This does not exclude the possibility of interactions of ATPases in the membrane to form *in situ* the pathway for ions to cross the membrane. In this regard it has been proposed by several authors that the Na^+/K^+ ATPase is an $(\alpha\beta)_2$ dimer. However, the authors do not agree on the functional characteristics induced by the dimer formation.

Na^+/K^+ ATPases

Na^+/K^+ ATPase is found in the plasma membrane of all higher eukaryote cells. For each cycle, three Na^+ are pumped out of the cell in exchange for two K^+ , thus generating Na^+ and K^+ concentration gradients and a membrane potential ($\Delta\psi$). Na^+/K^+ ATPase has important physiological roles. One is to maintain the resting potential in excitable cells; another is to ensure Na^+ and fluid reabsorption by the kidney tubules. Approximately one quarter of ATP consumed by humans at rest is hydrolysed by Na^+/K^+ ATPases. For a review, see Lingrel and Kuntzweiler (1994).

Cardiac glycosides, including ouabain, are specific inhibitors of the Na^+/K^+ ATPase. This specific inhibition has allowed the identification, localization and characterization of Na^+ pumps in various tissues. Ouabain, which totally inhibits ATPase activity, binds preferentially to the phosphorylated ATPase with an affinity higher than micromolar. Site-directed mutagenesis indicates that the binding site of ouabain is extracellular and interacts with more than one extracellular loop and at least one transmembrane segment.

Scheme 2 illustrates the exchange of three Na^+ for two K^+ . E_1 has millimolar affinity for cytoplasmic Na^+ . After phosphorylation of E_1Na_3 by micromolar concentrations



Scheme 2 Function of Na^+/K^+ ATPase.

of ATP in the presence of Mg^{2+} , the three Na^+ are occluded. Then one Na^+ is released in the extracellular space before the electrically compensated exchange on E_2-P of the two remaining Na^+ for two extracellular K^+ . After dephosphorylation, K^+ ions are occluded in the membrane and released in the cytoplasm. When studied in the reverse direction, K^+ dissociation from E_2-P toward the extracellular space shows that the two K^+ dissociate sequentially. Millimolar concentrations of ATP accelerate the cycle, in particular the rate of K^+ deocclusion.

Ca^{2+} ATPases

The Ca^{2+} ATPase from sarcoplasmic reticulum was simultaneously found by Ebashi and Hasselbach in 1962. Molecular biology has since revealed the presence of Ca^{2+} ATPases in almost all cells. In mammals the two main classes of Ca^{2+} ATPases are the sarco(endo)plasmic reticulum Ca^{2+} ATPases (SERCA) and the plasma membrane Ca^{2+} ATPases (PMCA). These two classes comprise numerous isoforms.

SERCA

SERCA 1

For fast-twitch muscle cells, long-term intracellular homeostasis maintains the calcium concentration below micromolar by means of the cytoplasmic membrane machinery (see below, PMCA). Muscle contraction is induced by Ca^{2+} spikes, i.e. transient increases in intracellular Ca^{2+} coming from the intrareticulum space and crossing its membrane through Ca^{2+} channels (ryanodine receptor). Relaxation of the muscle is effected by SERCA 1, the Ca^{2+} ATPase located in the reticulum membrane, which pumps two intracellular Ca^{2+} ions back into the reticulum for each ATP molecule hydrolysed. SERCA 1 comprises a single polypeptide α chain of 994 amino acids in the adult isoform. It represents more than 80% of the total proteins of the sarcoplasmic reticulum. It has long been widely accepted that SERCA 1 operates as a polymer (di, tri and tetramers have been proposed). It is now established that, after solubilization by nonionic detergents, the monomer can describe the whole cycle. This does not exclude specific or nonspecific interactions of SERCA 1 molecules in the native membrane, where SERCA 1 occupies nearly all the membrane surface.

Sarcoplasmic reticulum provides a natural model for studying the mechanism of ion motive pumps, as depicted in Scheme 1, where nI represents two Ca^{2+} . The question of the countertransport is still open, the most likely counterion being K^+ or H^+ , which would be equivalent to K^+ for the Na^+/K^+ ATPase (Scheme 2). E_1 has two Ca^{2+} sites with submicromolar affinity. This allows the Ca^{2+} ATPase to capture Ca^{2+} in the cytoplasm of a

muscle cell. Ca^{2+} binding is cooperative and sequential. In the presence of Mg^{2+} , micromolar ATP concentrations phosphorylate E_1Ca_2 to form $E_1 \sim P[Ca_2]$ where the two Ca^{2+} are occluded. Deocclusion is followed by sequential Ca^{2+} dissociation toward the intrareticulum space. E_2-P has a millimolar affinity for Ca^{2+} , allowing accumulation of millimolar Ca^{2+} in the reticulum. This means that in fast-twitch muscle cells SERCA 1 maintains a Ca^{2+} concentration ratio of 10 000 between the cytoplasm and the intrareticulum spaces. No direct regulation of SERCA 1 is known. Thapsigargin has been found recently to be a specific inhibitor of the SERCA family. For a review of SERCA 1, see Mintz and Guillain (1997).

SERCA 2

SERCA 2a includes the Ca^{2+} ATPases of cardiac, slow-twitch and smooth muscle sarcoplasmic reticulum. Its transport mechanism resembles that of SERCA 1 except that SERCA 2a is regulated by phospholamban (PL). PL is an intrinsic protein, formed by five identical polypeptides of 52 amino acids, that binds to the large loop of SERCA 2a – close to the phosphorylation site – and inhibits Ca^{2+} transport. This inhibition is released by PL phosphorylation, which occurs via three different kinases: cAMP-dependent, Ca^{2+} -calmodulin-dependent, and Ca^{2+} -phospholipid-dependent. All of these phosphorylations are reversible. For a review of SERCA 2, see Davis *et al.* (1990).

PMCA

PMCA was discovered by Schatzmann in 1966. Its structure and transport cycle resemble those of SERCA. Four isoforms of PMCA have been found. The role of PMCA is to ensure intracellular Ca^{2+} homeostasis at the resting Ca^{2+} concentration. PMCA seems less efficient than SERCA as only one Ca^{2+} appears to be extruded from the cell per ATP hydrolysed. Other differences arise from the regulation of PMCA by calmodulin (CaM), acid phospholipids and protein kinase. Compared to SERCA, PMCA has an additional tail of 100–200 amino acids located at the C-terminal; this is the locus of interaction with CaM. In the absence of Ca^{2+} or CaM, this tail folds over the catalytic part of the ATPase, possibly joining the two loops, thus inhibiting Ca^{2+} pumping. In this sense the CaM-binding site is considered as a repressor of the pump. As an illustration, cleavage of the C-terminal tail induces maximum activity of PMCA, which becomes insensitive to CaM. This C-terminal region also contains the site of phosphorylation by protein kinase. The putative site of regulation by acid phospholipids is thought to be located at the N-terminal end. No specific inhibitor is known for the PMCA family. For a review of PMCA, see Wuytack and Raeymaekers (1992).

H⁺/K⁺ ATPases

H⁺/K⁺ ATPase is mainly located in gastric parietal cells and an isoform has recently been found in the luminal membrane of the renal collecting duct. It is an $\alpha\beta$ complex exhibiting many homologies with the Na⁺/K⁺ ATPase. It carries an electroneutral exchange of H⁺ and K⁺. H⁺/K⁺ ATPase appears to be the most powerful ion motive pump as its gastric secretion of HCl induces an H⁺ gradient of 4×10^6 across the membrane of a parietal cell, which corresponds to pH < 1 in the secretory canaliculus. There is agreement that the transport stoichiometry is two H⁺ against two K⁺ in the absence of an ionic gradient across the membrane. However, transport of one H⁺ against one K⁺ has been proposed in the presence of a large gradient. This change in stoichiometry does not tally with Jencks' theory (see above) and is therefore still under debate. Another point is the apparent contradiction between the electroneutrality of the whole cycle and the sensitivity of transport to the membrane potential.

Gastric H⁺/K⁺ ATPase has been widely studied as a target for antisecretory drugs used for ulcer treatment. Thanks to the low gastric pH, omeprazole – the most powerful of these inhibitors – covalently reacts with the sulfhydryl group of cysteines that are accessible from the secretory canaliculus. For a review, see Wallmark *et al.* (1990).

H⁺ ATPase

It has been known since Pasteur's time that yeasts are responsible for acidification during alcoholic fermentation. In 1960, an ATPase was found to be responsible for this acidification. The P-type H⁺ ATPases of plants and fungi comprise a single α chain and are located in the plasma membrane. They transport H⁺ out of the cell, creating an H⁺ gradient and a $\Delta\psi$ that are used by numerous nutrients to enter the cell (sugars, amino acids, etc.). The transport cycle is described by Scheme 1, with nH representing one H⁺. Unlike the other P-ATPases, H⁺ ATPase needs millimolar concentrations of ATP to be phosphorylated. For a review, see Nakamoto and Slayman (1989).

Structure and Physiological Role of V-type ATPases

First found in vacuoles, V-ATPases are present in numerous organelles such as synaptic vesicles, chromaffin granules, plant and fungal vacuoles, lysosomes, Golgi complex, and even in the apical membrane of amphibian urinary bladder or kidney tubule cells. All V-ATPases

extrude H⁺ out of the cytoplasm at the expense of ATP hydrolysis, H⁺ being accumulated either outside the cell or in the internal space of the organelle. Note that V-ATPases are very different from P-type H⁺ ATPases as they are not phosphorylated during turnover and their architecture, which is complex and not totally elucidated, resembles that of F-ATPases (F₀F₁ ATP synthases found in eubacteria, mitochondria and chloroplasts: Boyer and Walker awarded the 1997 Nobel Prize for Chemistry). For a review, see Finbow and Harrison (1997).

In plants, vacuole membranes contain numerous V-ATPases that participate in lowering the intravacuolar pH to values as low as 2 in citrus fruits, for instance. As seen above for the H⁺ ATPase, V-ATPases create an H⁺ gradient and a $\Delta\psi$ that are used by solutes and ions to enter the organelle or the cell. For instance, the V-ATPase in the apical membrane of amphibian urinary bladder epithelial cells creates a $\Delta\psi$ which favours the entry of Na⁺ into the cell via the Na⁺ channel of the apical membrane. Na⁺ is then extruded across the basolateral side of the cell via the Na⁺/K⁺ ATPase. Another example is the accumulation of neurotransmitters into synaptic vesicles at the expense of the H⁺ gradient created by the V-ATPase. Another role of V-ATPases is the acidification of the intravesicular space. For instance, during receptor cycling via the endosomes, acidification of the intraendosomal space induces ligand dissociation, so that the receptor can be recycled toward the plasma membrane. V-ATPases are also responsible for the acidic activation of the hydrolytic enzymes trapped in lysosomes.

V-ATPases are composed of two domains: a transmembrane domain V₀ and a soluble and dissociable domain V₁, the whole molecule ranging from 700 to 900 kDa. Like F-ATPases, V-ATPases are composed of at least 10 different peptides, some of them existing as multiple copies in each V-ATPase molecule. V₁ can be dissociated from the membrane by mild chaotropic treatment. It is composed of at least eight subunits, among which both the A and B subunits are present in three copies. This soluble part of V-ATPases (about 550 kDa) is responsible for the ATPase activity. The A subunit comprises a nucleotide-binding sequence and is the locus of ATP hydrolysis. The B subunit also comprises a consensus sequence for a nucleotide-binding site which is thought to regulate the cycle. These A and B subunits are similar to the β and α subunits of F-ATPases and contain the six nucleotide sites. On dissociation of the V₁ headpiece, some additional subunits also dissociate. These subunits form a stalk that ensures the connection between V₁ and V₀. V₀ is essentially made of six copies of subunit c. Each subunit c is formed by four transmembrane helices, which are arranged in a membrane crown to form the H⁺ channel (Figure 3).

Although not much is known about the mechanism of V-ATPases, it is thought to be similar to that of F-ATPases. The main difference is in their physiological function, as the V-ATPases create an H⁺ electrochemical gradient,

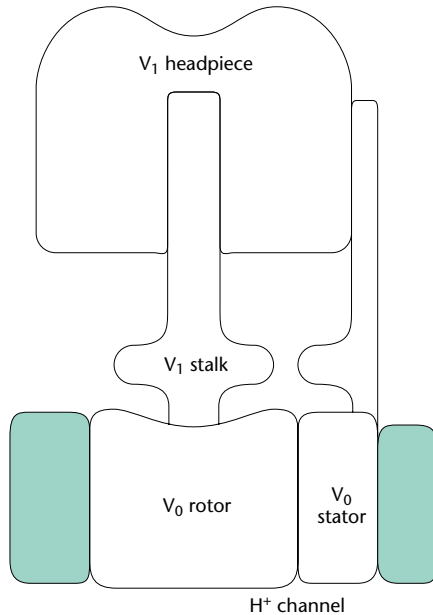


Figure 3 V-ATPase motor, redrawn from Boekema *et al.* (1997). ATP hydrolysis in V_1 headpiece induces rotation of the V_1 stalk which in turn induces rotation of the V_0 rotor. This mechanical energy is used for H^+ translocation.

whereas the F-ATPases are ATP synthases, using the same H^+ gradient to synthesize ATP. Despite these similarities, V-ATPases are not sensitive to oligomycin, a specific inhibitor of F-ATPases, nor are they sensitive to vanadate. V-ATPases are specifically inhibited by bafilomycin A_1 , which has been a powerful tool in investigating their presence in all systems. On the basis of similarities with F-ATPases, it is tempting to apply the rotational mechanism described for F-ATPases to V-ATPases. However, it is worth mentioning that F_0 contains 9–12 c subunits, each c being formed by two transmembrane helices and bearing one exchangeable H^+ , which leads to 9–12 H^+ translocated per cycle. On V_0 , each of the six c subunits also bears one exchangeable H^+ , leading to a lower coupling ratio between ATP hydrolysis and H^+ translocation.

Originally found in the vacuoles, V-ATPases are now described as ubiquitous ATPases found in all eukaryote

cells. This confers on V-ATPases an increasing importance among the ion motive pumps.

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