P-type ATPases at a glance

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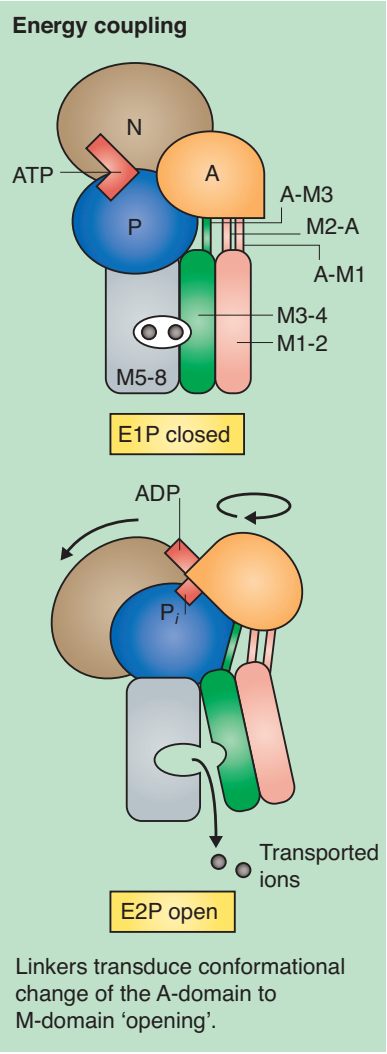
In the poster that accompanied this article the centre panel, entitled ‘Structural and functional features’ contains the sub-panel ‘Energy coupling’ in which the bottom structure should not be labelled ‘E1P open’ but ‘E2P open’.

The correct version of this panel is as follows:



The correct version of the poster is available for downloading at http://jcs.biologists.org/content/124/2/157/suppl/DC2

The authors apologise for this error.

P-type ATPases at a glance

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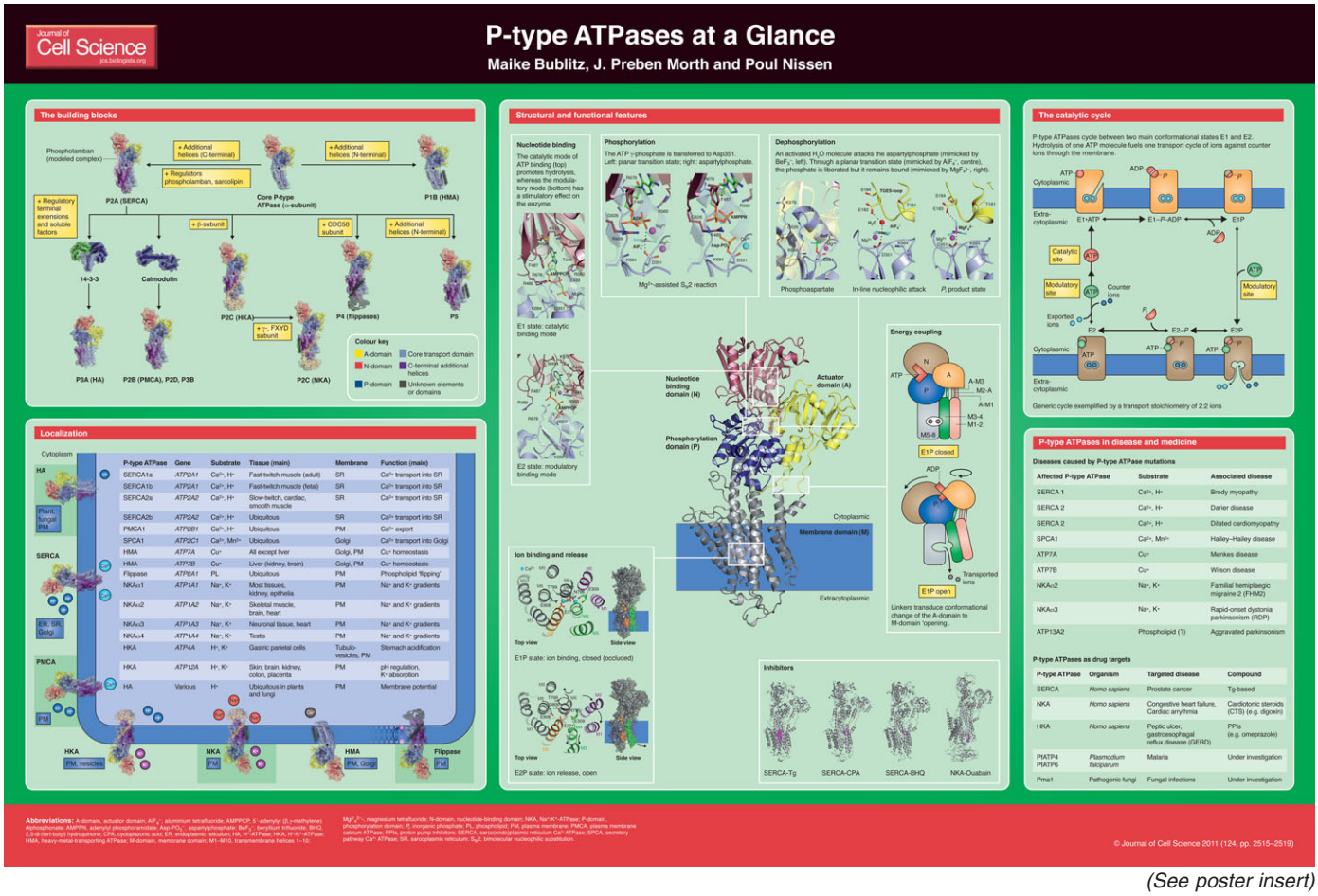
Introduction

P-type ATPases are a large family of integral membrane transporters that are of vital importance in all kingdoms of life. In eponymous distinction from the other main classes of transport ATPase – the F_0F_1 (F-), the vacuolar (V-) and the ATP-binding cassette (ABC-) type – the P-type ATPases form a phosphorylated (P-) intermediate state during their ion transport cycle (Pedersen and Carafoli, 1987). Members of this family generate and maintain crucial (electro-) chemical gradients across cellular membranes, by translocating cations, heavy metals and lipids.

The Na^+/K^+-ATPase (NKA; expressed in animals) and the plasma membrane H^+-ATPase (HA; expressed in plants and fungi) maintain the essential plasma membrane potential in all eukaryotic cells, which is based on different ion concentrations on the intra- and extracellular sides of the membrane. This electrochemical gradient fuels central cellular processes, such as the secondary transport of metabolites, and it also provides the basis for electrical excitation in neurons. The gastric H^+/K^+-ATPase (HKA) acidifies the stomach lumen and the heavy metal ATPases (HMA) are required for trace metal homeostasis and detoxification in both prokaryotes and eukaryotes. The Ca^{2+}-ATPases of the sarco(endo)plasmic reticulum (SERCA), the plasma membrane (PMCA), and the secretory pathway (SPCA) are crucial for muscle function, Ca^{2+} signaling and Ca^{2+} transport into secretory vesicles. The P4-type ATPases or flippases are thought to translocate phospholipids between lipid bilayer leaflets (Kuhlbrandt, 2004; Palmgren and Nissen, 2010).

Availability of structural information on P-type ATPases has increased drastically within recent years. This has led to the elucidation of hallmark intermediate conformations along the reaction cycle, thereby providing the structural basis for ATP hydrolysis and ion transport (reviewed in Bublitz et al., 2010). Furthermore, it has promoted advances in our understanding of pathophysiology because dysfunction of human P-type ATPases (SERCA, NKA or Cu^+-ATPases) can cause severe diseases, such as heart failure, rapid-onset dystonia parkinsonism or Wilson disease, respectively, and NKA and HKA are well-established targets for clinically important drugs. The importance of these enzymes in cancer cells and many pathogens also offers novel drug strategies.

This Cell Science at a Glance article and the accompanying poster summarize the key features of the P-type ATPases. The following sections provide an overview of the main building blocks and functions of the P-type



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ATPases and outline our current understanding of the catalytic cycle, as learned from structual analysis of SERCA in different conformations. The contribution of single domains to the concerted action of energy transduction is pointed out, leading to a generalized model of P-type ATPase catalysis. Finally, implications of P-type ATPases within a medical context are addressed; these include P-type-ATPase-targeting drugs for therapeutic use as well as the ongoing efforts to explore P-type ATPase in pathogenic organisms.

P-'typical' ATPase building blocks

Since the discovery of the NKA (Skou, 1957), our knowledge of the P-type ATPase family has grown considerably and it is now divided into five main classes (P1, P2, P3, P4 and P5) with subfamilies (P1A, P1B, etc.) (Palmgren and Axelsen, 1998). P-type ATPases are widespread across many species from bacteria to humans. The primary structure of a P-type ATPase is generally identified by conserved sequence motifs in the cytoplasmic domains. The overall cytoplasmic domain arrangement includes a nucleotide-binding (N), a phosphorylation (P) and an actuator (A) domain (Toyoshima et al., 2000). The transmembrane (M) domain has a central core of six α-helices – a common feature of all known P-type ATPases. P-type ATPases with one predicted transmembrane α-helix in addition to this core unit are found only in prokaryotes, and include the bacterial K^+-extruding Kdp complex with its P1A-type KdpB subunit (Greie and Altendorf, 2007) and also certain P1B-ATPases that are predicted to transport heavy metal ions, such as Cd^{2+} or Hg^{2+} (Axelsen and Palmgren, 1998). Most heavy metal ATPases, however, have two (Kuhlbrandt, 2004) or three (Hatori et al., 2007) additional transmembrane helices – inserted at the N-terminal side of the core domain – that are involved in metal recognition. Classes P2–P5 comprise larger enzymes with a C-terminal extension to the core domain (Karlish et al., 1992) that is often composed of four additional α-helices; this can, however, vary in certain family members, as the SERCA isoform 2b, for example, has five additional helices (Campbell et al., 1992).

The main transport subunit (often called the α-subunit) is associated with additional subunits (referred to as β- and γ-subunits) in members of the classes P2C and P4 (Poulsen et al., 2008; Zhou and Graham, 2009). Members of the least-characterized class, P5, often have additional helices at a position that is N-terminal to the core region – similar to members of class P1. P3A-type H^+-ATPases possess extensions at both the N- and C-terminal ends. The N-terminal extension may function as a pH sensor, whereas the C-terminal part [also called the regulatory (R-) domain] is autoinhibitory and can be released by phosphorylation or when binding to regulating factors (Kuhlbrandt et al., 2002; Portillo, 2000). A growing number of such allosteric modulators and scaffolding proteins are being identified – including 14-3-3 protein, which interacts with the plant H^+-ATPase (Jahn et al., 1997) and calmodulin, which interacts with PMCA (Enyedi et al., 1989). Membrane-bound regulators include phospholamban and sarcolipin, which regulate SERCA (Traaseth et al., 2008) and the regulatory γ-subunit of NKA, which belongs to the family of FXYD proteins.

Localization

The human P-type ATPases reside in all cellular membranes. Several of the cation pumps act in the plasma membrane (e.g. NKA, PMCA and flippases), whereas members of the SERCA-like subfamily P2A are most abundant in the membrane of the endoplasmic or sarcoplasmic reticulum. SERCAs are also expressed in the Golgi complex, together with the Cu^+-ATPases and SPCA (van Baelen et al., 2004). The human Cu^+-ATPase relocalize from the Golgi to the plasma membrane when Cu^+ efflux is required (Lutsenko et al., 2008). Similarly, the gastric HKA is stored in an intracellular membrane system within the parietal cells of the gastric epithelium, which fuses with the plasma membrane to acidify the stomach lumen (Shin et al., 2009).

Most P-type ATPases are found as various cell-type-specific isoforms that emerge from alternative splicing or separate, but similar, genes. SERCA1, for example, is expressed as the 1a isoform in the adult fast-twitch muscle and as the 1b isoform – with an extended C-terminus – exclusively in fetal and regenerating tissue (Zador et al., 2011). SERCA2a is mainly found in cardiac and slow-twitch muscle, whereas SERCA2b is ubiquitously expressed (Wuytack et al., 2002). Similarly, the NKA α-subunit appears in four isoforms, of which α1 is expressed in most tissues, α2 and α3 in tissues including those of the heart and brain, and α4 in testis only (Kaplan, 2002). Of the two human genes that encode HKA, one is expressed only in gastric epithelial cells, the other one is found in skin, brain, colon and placenta (van Driel and Callaghan, 1995).

Having noted the importance of membrane transport processes that are mediated through P-type ATPases – in both general housekeeping and in highly specialized secretory, excitatory or contractile tissues – we now explain how these ion pumps fulfill their various tasks by using surprisingly similar molecular mechanisms.

The catalytic cycle

To achieve active transport of cations against an electrochemical gradient, a transporter must prevent the formation of an open passage across the entire membrane because this would lead to a rapid back-flow of ions, thereby destroying any charge or concentration gradient. To overcome this problem, the P-type ATPases function according to an 'alternating-access' model (Jardetzky, 1966). In this model, the access pathways to both sides of the membrane are transiently closed – thereby occluding the ions – before opening to either side in an alternating manner. The actual ion translocation is accomplished through extensive conformational changes that are driven by ATP hydrolysis. Overall, the ATPase alternates between two conformational regiments, the so-called E1 and E2 states. The E1 states are associated with autophosphorylation by ATP and have a high affinity for the cation that is to be expelled from the cytoplasm. The E2 states are associated with autodephosphorylation, have a lower affinity to these cations and may bind counterions instead (Albers, 1967; Post et al., 1969). Phosphorylation by ATP of an aspartate residue in the ion-bound E1 state forms the high-energy E1P.ADP state, which promotes a subsequent conformational change to the functionally distinct lower-energy E2P state. This conformational change is associated with the opening of an extracytoplasmic exit pathway from the ion-binding sites, and a distortion of these sites lowers the affinity for the E1-bound cations. Binding of counter-transported ions to the cation-binding sites (or other events that stimulate closure of the exit pathway) induces re-occlusion and dephosphorylation of the transporter (E2P to E2). The counterions are then released during transition to the E1 state.

Crystal structures of SERCA1a in several key conformational states reveal that this enzyme can bind a nucleotide throughout the entire reaction cycle, either in a catalytic (E1) or a modulatory (E2) mode (Clausen et al., 2011; Jensen et al., 2006). The next section describes the nucleotide-binding domain in more detail.

Nucleotide binding

Accurate positioning of the ATP molecule for hydrolysis or stimulatory effects is crucial for the correct function of ATPases. ATP is bound in a conserved pocket in the N-domain, with the phosphate moiety pointing towards the reactive aspartate residue in the P-domain. The two modes of ATP binding – catalytic or modulatory – take place in the same pocket, but differ slightly (Jensen et al., 2006). In the modulatory mode, the γ-phosphate is 9 Å away from the phosphorylation site; the catalytically important Mg^{2+} site (see below), which is close

to the γ-phosphate, is unoccupied. Instead, another Mg^{2+} is bound, coordinated by the α- and β-phosphates. In both SERCA1a and the NKA, ATP accelerates the transition from the E2 to the E1 state (Forbush, 1987; Mintz et al., 1995). In SERCA1a, ATP also stimulates the transitions from E2P to E2 (dephosphorylation), and E1P to E2P (luminal gate opening) (Champeil et al., 1988; Lund and Moller, 1988). In the catalytic mode, the ATP molecule is brought closer to the phosphorylation site by coordination with Mg^{2+}, which places the aspartic acid side chain so that it is accessible for in-line nucleophilic attack and phosphoryl transfer (Sorensen et al., 2004).

Phosphorylation and dephosphorylation

Phosphorylation and dephosphorylation reactions follow the scheme of a bimolecular nucleophilic substitution (S_N2). Structural studies on phosphoryl transfer are possible by using fluorinated aluminium (AlF_4^-), beryllium (BeF_3^-) and magnesium (MgF_4^{2-}) ions. These mimic the geometries of a planar pentavalent transition state of phosphoryl transfer, the covalent phosphorylated E2P ground state and the P_i-bound product state of dephosphorylation, respectively. The low-turnover substrate adenylyl imidodiphosphate (AMPPNP), was successfully used to solve the structure of the high-energy E1P state, which completed the snapshots of reaction intermediates along the E1–E2 catalytic cycle (Olesen et al., 2004; Olesen et al., 2007; Sorensen et al., 2004; Toyoshima et al., 2004; Toyoshima and Mizutani, 2004).

The Mg^{2+} cofactor and a conserved lysine residue at both sides of the reactive aspartate side chain stabilize its conformation that is prone to in-line attack of the ATP γ-phosphate and delocalize the negative charge to reduce electrostatic repulsion. Another Mg^{2+} stabilizes the leaving ADP group by interacting with the α- and β-phosphates (Sorensen et al., 2004). The γ-phosphate transfer breaks the ATP-mediated link between the N- and the P-domain, allowing the N-domain to move away, thereby exposing the nucleotide-binding site for ADP release and, eventually, ATP binding. The A-domain then rotates into the emerging space and interacts with the phosphorylated P-domain, thereby protecting the aspartyl-phosphoanhydride against spontaneous hydrolysis. Owing to this conformational change, the conserved Thr–Gly–Glu–Ser (TGES) motif of the A-domain is placed above the phosphorylation site. The subsequent dephosphorylation is mediated by the threonine and glutamate residues of the TGES motif, which coordinate and activate one H_2O molecule for nucleophilic attack by abstracting a proton (Olesen et al., 2004). ATP binding stimulates the release of the leaving P_i group (Jensen et al., 2006), which causes the A-domain to disengage from the phosphorylation site, thus initiating the return to the E1 state.

Having understood the events that take place at the phosphorylation site, one important question arises: how is the energy gained from ATP hydrolysis and the accompanying domain rearrangements in the cytoplasmic region coupled to a powerful 'push' of ions through the membrane?

Energy coupling

ATP hydrolysis, and ion binding and release take place at sites that are ~50 Å apart, which makes it necessary to couple the free energy release from the hydrolysis of the phosphoanhydride bond at one site to the 'uphill' ion transport at the other site.

Of central importance for this energy coupling are the three linker regions that connect the A-domain with the transmembrane helices M1, M2 and M3 (A-M1, M2-A and A-M3, respectively) (Toyoshima et al., 2004). Several studies that have probed shortening, lengthening or proteolytic cleavage of these linkers in SERCA demonstrate a severe effect on the communication between the phosphorylation site and the ion-binding site, which causes impairment of pump function (Daiho et al., 2003; Daiho et al., 2007; Holdensen and Andersen, 2009; Lenoir et al., 2004; Moller et al., 2002). When phosphoryl transfer releases the tight connection between the N- and P-domains, and when the A-domain rotates into the emerging gap of the phosphoenzyme, this extensive (almost 120°) rotation exerts a pull on the three linkers. This, in turn, leads to a change in the tilt and the position of the attached transmembrane helices M1, M2, M3 and M4: they separate from the remaining transmembrane helices (M5, M6, M7, M8, M9 and M10) and open up the ion-exit pathway (Olesen et al., 2007). Furthermore, the P-domain moves up and down relative to the membrane. Simultaneously, these rearrangements distort the geometry in the ion-binding site, leading to a drastic decrease in affinity for the bound ion and, thereby, ion release.

Ion binding and release

The ions bind to the transmembrane domain, coordinated by negatively charged and polar residues, in a region between transmembrane helices M4, M5, M6 and M8. The number of transported ions varies: the fungal H^+-ATPase transports one H^+, SERCA transports two Ca^{2+} out versus two to three H^+ in, and the NKA transports three Na^+ out and two K^+ in (Inesi et al., 1978; Post and Jolly, 1957; Perlin et al., 1986; Yu et al., 1993).

The ion-binding sites of SERCA and NKA are surprisingly similar, and one can anticipate that coordination distances, and distribution and number of charged amino acid side chains have been adapted to the respective ions (Bublitz et al., 2010). It is probable that specificity of the transporter is also conferred through a gating mechanism and a selectivity filter at the ion entrance pathway (Einholm et al., 2007; Laursen et al., 2009; Morth et al., 2011). Ion release is triggered by distortion of the high-affinity coordination geometry through transmembrane helix movements; in SERCA, most notably a translation of M4 towards the luminal side and a rotation of M6. The simultaneous, tripartite separation of the segments M1–M2 and M3–M4 from M5–M10 opens up the exit pathway (Olesen et al., 2007).

Further elements can assist ion release: our recent electrophysiological study has shown that the NKA operates through a gated C-terminal pathway, supposedly to acquire a cytoplasmic H^+ that facilitates Na^+ release and occupies the vacated third ion-binding site during the counter transport of only two K^+ (Poulsen et al., 2010).

P-type ATPases in disease

Because most P-type ATPases serve crucial cellular processes, their malfunction is associated with several pathophysiological conditions in humans. Mutations in the Cu^+-ATPase-encoding genes *ATP7A* and *ATP7B* cause Menkes disease and Wilson disease, respectively (de Bie et al., 2007; Lutsenko et al., 2008). Defects in SERCA1 cause recessive Brody myopathy, and SERCA2 impairment is linked to Darier disease and heart failure (Brini and Carafoli, 2009). Severe neurodegenerative disorders are associated with mutations in the NKA (de Carvalho et al., 2004; Riant et al., 2005), and a recent study demonstrates an involvement of plasma-membrane-localized SPCA2 in store-independent Ca^{2+} signaling, which promotes carcinogenesis (Feng et al., 2010). Clearly, lowered ATPase activities that arise from mutations or dysregulation are difficult to tackle through drugs, but the in-depth understanding of mechanisms, such as stimulatory effects, might assist in finding alternative strategies to improve certain disease patterns.

Inhibitors and clinical drugs

A substantial number of P-type ATPase inhibitors are known and some of them have been crystallized together with their respective targets – such as the SERCA inhibitors thapsigargin (Tg), a sesquiterpene lactone from the plant *Thapsia garganica* (Toyoshima and

Nomura, 2002); cyclopiazonic acid (CPA), a secondary metabolite from certain fungi (Laursen et al., 2009); and the synthetic compound 2,5-di-(tert-butyl) hydroquinone (BHQ) (Obara et al., 2005). Ouabain is a cardiotonic steroid (CTS) that inhibits NKA (Ogawa et al., 2009; Yatime et al., 2010), and the inhibitory complex of HKA with the K^+-competitive acid blocker SCH28080 was recently analyzed by electron crystallography (Abe et al., 2011). The inhibitor-bound structures reveal three different drug-binding sites: CPA and BHQ occupy the same binding pocket in the proposed ion entry pathway at the cytoplasmic ends of helices M1–M4 of SERCA. Tg binds laterally to the M-domain, in a groove between M3, M5 and M7. Ouabain and SCH28080 bind to analogous sites in the NKA and the HKA, namely in the extracellular exit pathway close to transmembrane helices M4, M5 and M6.

The detailed understanding of ATPase-inhibitor interactions may serve applications of structure-based drug design, targeting, for example, crucial P-type ATPase activities in pathogens, parasites and cancer cells. In fact, inhibition of P-type ATPase is already applied for therapeutic purposes. The gastric HKA is the target for the specific inhibitor omeprazole to treat dyspeptic conditions, and NKA inhibition by cardiotonic steroids (such as digoxin) from *Digitalis* sp. is widely used in the treatment of congestive heart failure and arrhythmia. SERCA inhibition through a thapsigargin pro-drug strategy (Denmeade et al., 2003) is currently clinically tested as a strategy to cure prostate cancer by induced apoptosis (see the GenSpera website at www.genspera.com for more information). Evidently, high specificity in terms of target, isoform and tissue will crucially determine the performance of these drugs in practical use.

In addition to human targets, fungal, bacterial and protozoal P-type ATPases are now also being recognized as promising targets in the development of new antimicrobials, owing to their pivotal role for the host organism. Fungal plasma membrane H^+-ATPase and the SERCA-like PfATP6 and PfATP4 from *Plasmodium falciparum* (Cardi et al., 2010; Rottmann et al., 2010) – the causative pathogen of malaria – are examples of such attractive targets for the development of new drugs.

Perspectives

The P-type ATPases have crucial functions in the cell, and we already know a lot about their basic mechanism, although details of the energetics and single-molecule behavior are still to be revealed. Many members of this large family are still awaiting characterization at the basic level of function – such as the P4- and P5-ATPases, which are found in all eukaryotes and constitute more than half of the P-type ATPases encoded in humans. Another important aspect concerns participation of P-type ATPases at a higher level of structure and function of biomembranes, for example, the larger complexes of the NKA that are associated with receptor signaling transmitted by ouabain-like compounds (Liu and Xie, 2010), and the signalosome of H^+-ATPase in plants that responds to environmental cues (Gaxiola et al., 2007; Yang et al., 2010). Of note, P-type ATPases are also central players in metabolism, as they account for as much as 40% of the energy turnover in humans. P-type ATPases will continue to attract our attention in molecular cell biology and physiology, and will also be targets for applied research in biotechnology and medicine.

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