REVIEWS

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Recent Insights into the Structure and Mechanism of the Sodium Pump

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The sodium pump (or Na-K-ATPase) is essential to the function of animal cells. Publication of the related calcium pump (SERCA) structure together with several recent results from a variety of approaches allow us to propose a mechanistic model to answer the question: "How does the sodium pump pump?"

The Sodium Pump in the P-ATPase Family

The Na-K-ATPase, also known as the sodium pump or Na/K pump, is the active transport system that is responsible for maintenance of the gradients of Na^+ and K^+ across the plasma membrane. Because these gradients provide energy for several essential cellular functions (control of membrane potential, cell volume, pH homeostasis, and many others), it is not surprising that this transport protein is present in all animal cells. The Na-K-ATPase is a member of the P-ATPase family, a group of integral membrane proteins characterized by a number of specific conserved sequences related to their ATP hydrolytic function and the presence of several (6–10) hydrophobic domains predicted to form transmembrane α-helices. Various types of P-ATPases are found in all forms of life from Archaea to Eukaryotes. Phylogenetic analysis of the available P-ATPase sequences have allowed the definition of five main groups, referred to as groups I–V (4), as illustrated in FIGURE 1.

According to the classification of Axelsen and Palmgren (4), the α-subunit isoform of the Na-K-ATPase (4 genes in human), together with the two isoforms of the H-K-ATPase, form the subgroup IIc of the P-ATPases. The subgroups IIa and IIb include the two main types of calcium pump, the sarcoplasmic-endoplasmic reticulum calcium ATPases (SERCA) and the plasma membrane calcium ATPases (PMCA).

Subgroup IIc P-ATPases, the Na-K- and H-K-ATPases, are characterized by the presence of an associated ~40-kDa glycoprotein designated as their β-subunit. The cotranslational association of the β-subunit is absolutely required for the maturation, targeting, stability, and functional expression of these proteins (12). No cation transport function has been recorded for a group IIc P-ATPase α-subunit expressed alone without a β-subunit. The high degree of homology of the proteins

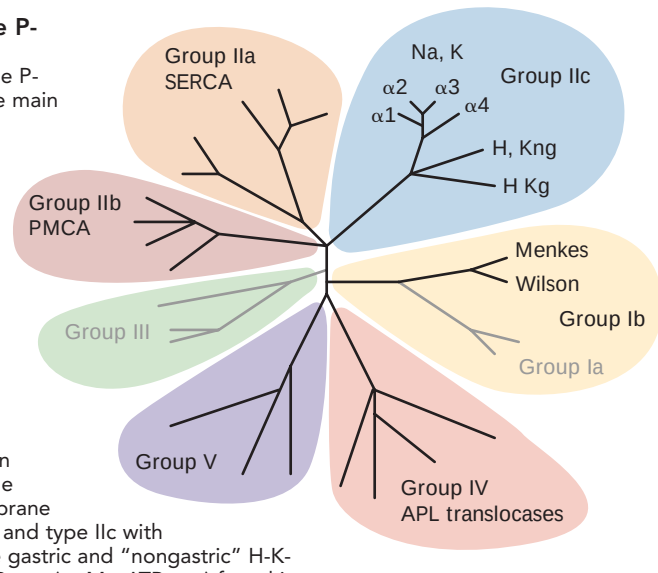


FIGURE 1. **Schematic phylogenetic tree of the P-ATPase family**
This simplified and schematic phylogenetic tree of the P-ATPase family illustrates the relationship between the main branches of this family. A more complete version of this tree can be found in the P-type ATPase Database internet site (http://biobase.dk/~axe/Patbase.html) maintained by K. B. Axelsen. The branches represent the genes present in the human genome (black lines) and, in addition, groups Ib and III (gray lines), which are not found in animal cells. Group I includes the B subunit of the bacterial KDP system (Ia) and a very large family (Ib) of cation pumps able to transport various metal ions (Cu^{2+}, Ag^+, Cd^{2+}, etc.). The human genome contains 2 group Ib ATPases that are both known to transport copper (the Menkes and Wilson proteins). Group II includes the sarcoplasmic-endoplasmic reticulum calcium pumps (SERCA; group IIa; in human 3 SERCA genes + 2 other genes corresponding to the secretory pathway calcium pumps), the plasma membrane calcium pump (PMCA; group IIb, 4 genes in human), and type IIc with the 4 isoforms of the Na-K-ATPase α-subunit and the gastric and "nongastric" H-K-ATPase α-subunits. Type III P-ATPases are proton ATPases (or Mg-ATPases) found in yeast, plant, and protozoa but not in multicellular animals. Up to 14 group IV genes have been found in the human genome (although some of them might be pseudogenes), and one of these genes has been characterized as an aminophospholipid (APL) transporter or "flippase" in protozoa and mammals, but very little is known about their function. No functional data are available concerning the group V P-ATPases.

in group IIc makes their comparison very useful in the quest to understand the nature of the cation-selectivity mechanism (5, 6).

Recently, a group of seven small proteins, called "FXYD" proteins from the sequence of a conserved motif, has been identified (40). One of these proteins (FXYD2) had indeed been known as the γ-subunit of the Na-K-ATPase, but most members of this group have now been shown to be associated with the α/β-subunit of Na-K-ATPase and to modulate some aspect of its function (subject reviewed in Ref. 9).

Principles of Na/K Pump Function

The P-ATPase name comes from the observation of a transient phosphorylation during the functional cycle: the γ-phosphate of ATP is transferred to an aspartate residue (D376) that belongs to a phosphorylation site motif DKTGT present throughout the whole family. The P-ATPases have also been named E1–E2 ATPases because they can be found in two main conformations, E1 and E2, which differ in many aspects. In the case of the Na-K-ATPase, for which the most extensive studies have been performed in this regard, the E1 and E2 conformations differ, for instance, in affinity for Na^+ and K^+, sensitivity to ADP and ATP, intrinsic fluorescence, and sensitivity to proteolysis. There are in fact several definitions of the E1 and E2 conformations, depending on which type of test is performed, and these definitions may not fully overlap (37). FIGURE 2 illustrates the principle of cation transport by the Na-K-ATPase according to the "alternative-access" or "gated-channel" model and indicates the E1 and E2 conformations. As we will see below, the full functional cycle may include many more states or substates.

The aim of this review is first to summarize a few of the most significant findings in recent years, findings that may shed some light on our understanding of the Na-K-ATPase structure-function relationship (this summary will obviously not be exhaustive because of space constraints, and, I am afraid, it will be personally biased in the choice of what is most significant). Together, these recent data allow us to draw a structural-functional model that will be presented in a second part, and a third part will summarize some of the most important questions that presently remain open.

Recent Key Findings

SERCA high-resolution structure and homology modeling of the Na/K pump

One of the most important advances in the field has been the disclosure of a high-resolution structure of SERCA by Toyoshima and his collaborators [Protein Data Bank (PDB) code 1EUL] (43). SERCA was crystallized in the presence of a high concentration of calcium, and two calcium ions are present in the protein, defining the two calcium-binding sites. This was soon followed by a second structure of the same protein in a different conformation (PDB code 1IWO) (44) in which SERCA was crystallized in the presence of its inhibitor thapsigargin. Very recently, the structure of a third conformation of SERCA associated with

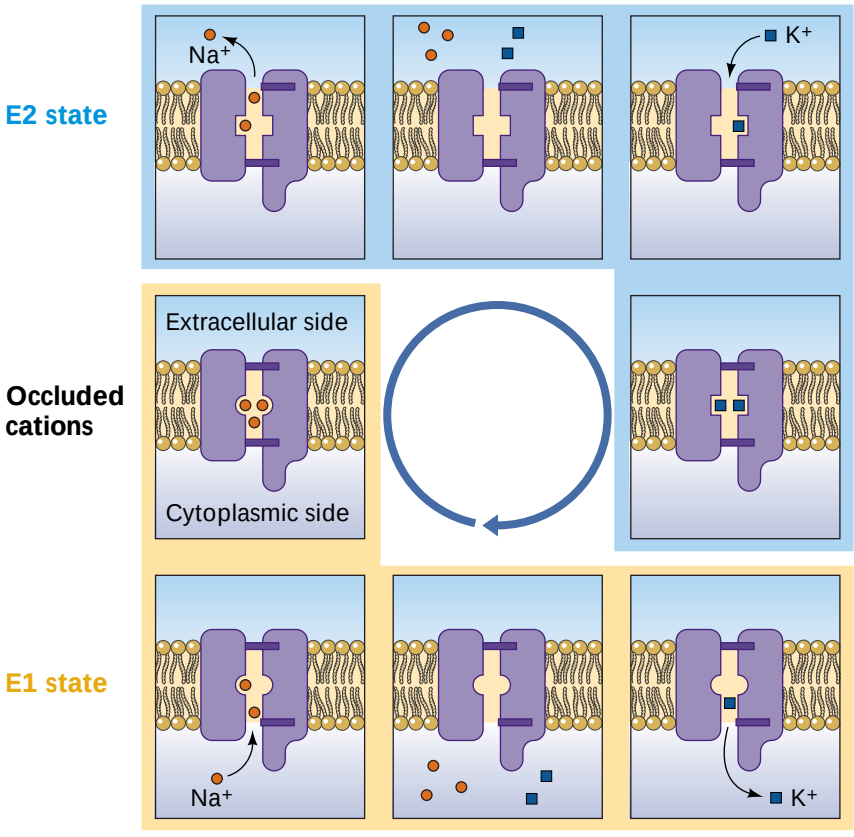
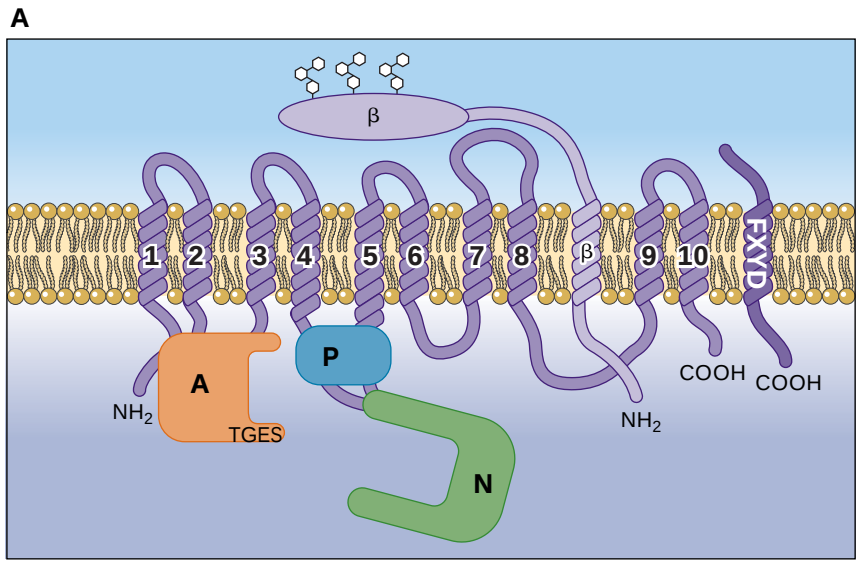
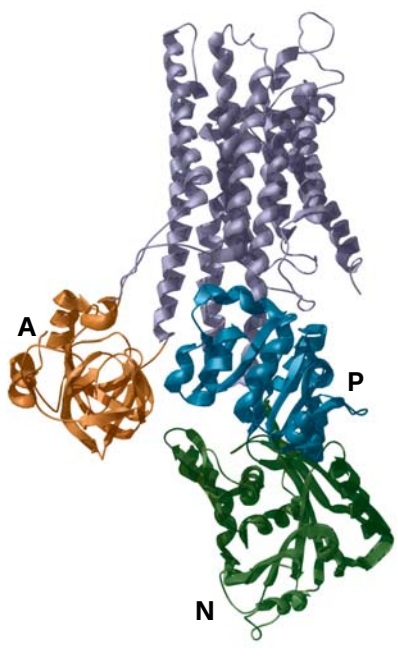


FIGURE 2. **Principle of the alternating-access model of the Na/K pump**
This scheme illustrates the principle of the alternating-access model of an ion pump initially proposed by Läuger (24). The protein is embedded in the membrane (shown with the extracellular side on *top* and the cytoplasmic side at *bottom*). The binding sites for the cation are located in the transmembrane part of the protein and are accessible alternately from the cytoplasmic side (E1 conformation; *bottom*) and from the extracellular side (E2 conformation; *top*) of the membrane. Access to the binding sites is controlled by two "gates" illustrated by the small horizontal dark bars, one for the extracellular side and one for the cytoplasmic side. Conformations with the two gates closed may also exist and correspond to states in which Na^+ or K^+ ions are "occluded." In addition to the opening and closing of the gates, there is a change of the cation-binding site structure that entails a large modification of the selective affinity for cation (Na^+ and K^+ high affinity, symbolized by the round and square shapes of the cation-binding sites, respectively).

either a nonhydrolyzable analog of ATP (PDB codes 1T5S and 1VFP) or with ADP + aluminum fluoride (ADP-AlF_4; PDB code 1T5T) has been published independently by two groups (38, 42). The availability of these structural data is remarkable considering that they concern a mammalian protein for which a considerable amount of biochemical and physiological data is available. In contrast, most of the other integral membrane proteins that have been crystallized originate from prokaryotes, and often little physiological data are available for these prokaryote homologs [and in one case, what was thought to be a Cl^- channel turned out to behave rather as a secondary active Cl^-/H^+-coupled transport system! (1)].

All of these structural data have caused a tremendous amount of interest in the field not only of SERCA but also of the related P-type ATPases; the relatively high degree of homology between SERCA and the group IIc P-ATPases (Na-K- and H-K-ATPases) makes it possible to use homology modeling to obtain credible structures of this group of proteins, and several of these models have been published in the past two years (6, 22, 27, 30, 34, 39). The degree of homology, and therefore the reliability of the model, varies considerably along the sequence. Many parts of the intracellular actuator (A), nucleotide-binding (N), and phosphorylation (P) domains (FIGURE 3) are highly homologous, and, except for a few short loops located at the periphery of these domains, the model can be considered reliable for these domains. In contrast, the extreme NH_2- and COOH-terminal parts of the protein are highly divergent between type IIa and IIc





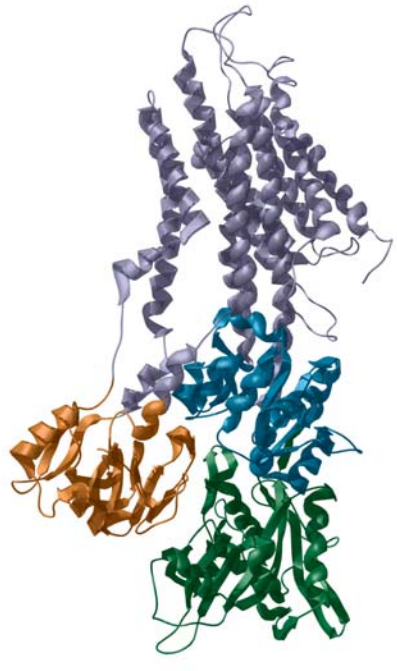


FIGURE 3. **General features of the P-IIc ATPase structure**
A: structure of the Na-K-ATPase. Ten transmembrane segments are represented in an "unfolded" disposition, but they actually form a bundle around the 3 central 4th, 5th, and 6th transmembrane segments. Color coding shows the actuator (A; orange) domain, which is constituted by the NH_2-terminal segment preceding the 1st transmembrane segment and the intracellular loop between the 2nd and 3rd transmembrane segments. It contains the TGES motif that is involved in the dephosphorylation step. The phosphorylation (P; blue) domain is made up of the proximal and distal parts of the large intracellular loop between the 4th and 5th transmembrane segments. In its initial section, it contains the DKTGT motif with the aspartyl residue D376, on which the phosphate of ATP is transiently transferred. The nucleotide (N; green) domain is made up of the main part of the large intracellular loop and forms a kind of pocket for ATP binding. The β-subunit has a single transmembrane segment, a short intracellular NH_2 terminal, and a large extracellular domain with several glycosylation sites (3 are shown). In this scheme the β-subunit transmembrane segment is located close to the 7th and 8th transmembrane segments because the early part of the extracellular domain of the β-subunit is known to interact with a conserved SYGQ motif located in the relatively large extracellular loop between the 7th and 8th transmembrane segments. The Na-K-ATPase α- and β-subunits may also associate with a third subunit belonging to the "FXYD" protein family. *B* and *C*: 3-dimensional structures of 2 conformations of SERCA [PDB codes 1EUL (*B*) and 1IWO (*C*)].

groups and even between different isoforms of the Na-K-ATPase, and not much can be predicted about their structure. Concerning the transmembrane segments, the sequences of the first three hairpins (transmembrane segments 1–2, 3–4, and 5–6) can be aligned rather easily and thus also provide a reliable homology structure. The two last transmembrane hairpins (transmembrane segments 7–8 and 9–10) are less conserved, and the alignments between SERCA and type IIC P-ATPases are not without ambiguities. Furthermore, the presence of an additional transmembrane segment provided by the β-subunit, most probably closely associated with the last four transmembrane segments, makes the structure predictions less reliable for this part of the protein. Similarly, the extracellular loops are poorly conserved between the IIa and IIc groups (especially the third extracellular loop, which is longer in the P-IIc group), and the presence of the large extracellular domain of the obligatorily associated β-subunit makes the structure prediction rather hazardous for the entire extracellular part of the protein.

The overall architecture of SERCA and related P-ATPases was known from previous work (low-resolution structure of Na-K-ATPase, SERCA, and H-ATPase obtained by cryoelectron microscopy), but our understanding has been greatly improved and refined by the SERCA high-resolution structure obtained by X-ray crystallography. The architecture of SERCA revealed by the structures published by Toyoshima and his collaborators (43, 44) are illustrated in FIGURE 3A, which shows a scheme of the main functional domains of the P-ATPases that could be identified: the 10 transmembrane helices and the main domains A, N, and P of the cytoplasmic part of the protein.

FIGURE 3, B AND C shows two structures published by Toyoshima and collaborators. FIGURE 3B (1EUL) is thought to be in the E1 conformation, with the ion-binding sites loaded with two calcium ions. The three intracellular domains (identified with the same color coding as in FIGURE 3A) are rather widely separated from each other. The other structure (1IWO; FIGURE 3C) has no calcium ions inside and is complexed with a molecule of thapsigargin; it is thought to represent the E2 conformation. Comparison with the first structure shows very large movements of the intracellular domains, resulting in a much more compact configuration of the intracellular part of the protein: the N domain has made a ~90° rotation around its attachment point, with the P domain and the A domain making a ~100° rotation around a horizontal axis so that parts that were completely exposed in 1EUL are now closely apposed to the P domain. Large movements can also be detected in the transmembrane part of the protein; in particular, a kink has appeared close to the middle part of the first transmembrane helix and the whole helix has moved toward the intracellular side by more than one helix turn. The cation-binding sites are reorganized following rather complex movements of all of the transmembrane segments that are described in detail in the original paper and a recent review (41, 44).

A large amount of data obtained by site-directed mutagenesis, chemical derivatization, or site-directed proteolysis is available for the Na-K- and H-K-ATPases, most of which had been obtained before the publication of the high-resolution structure of SERCA. These data can now be interpreted in view of the models obtained by homology. A large part of the conclusions that have been drawn from these data are compatible with the structural model and indeed provide strong support for at least the general architecture of the model, such as the central position of the fourth, fifth, and sixth transmembrane segments around the cation-binding sites and the role of several negatively charged residues in these binding sites, as well as the fine structure of the phosphorylation site and the ATP-binding domain (20). The phosphorylation site and the cation-binding sites will be described in the following paragraphs.

Phosphorylation site

One of the two structures published by Sorensen et al. (38), the ADP-AlF_4–bound form (1T5T), is thought to mimic the transition state of the phosphoryl transfer reaction leading to the E1~P state of SERCA. The other structure crystallized by Sorensen et al. (38) (1T5S) and the one published by Toyoshima and Mizutani (42) (1VFP) are very similar and represent the same conformational state of SERCA. In the ADP-AlF_4 complex, aluminum fluoride occupies the position of the γ-phosphate of ATP but cannot actually be transferred to D351, and thus the reaction cycle is arrested at a very specific step (see FIGURE 5). It is thus possible to observe very precisely the relationship between the bound nucleotide, Mg^{2+} ions, and the phosphorylation site. This structure of the ADP-AlF_4–bound E1~P SERCA is in excellent agreement with the predictions made by Patchornik et al. (29, 30) on the basis the Fe^{2+}-catalyzed cleavage of the Na-K-ATPase about the relationship between the γ-phosphate of ATP, the Mg^{2+} ion, and several highly conserved motifs of the P domains. The structure of the phosphorylation site is illustrated in FIGURE 4.

Metal-catalyzed oxidative cleavage also predicted a close proximity between the highly conserved TGES motif (E183 in SERCA) in the A domain with the bound nucleotide phosphate in the E2-P conformation (29, 30), and this was initially difficult to reconcile with the first SERCA structure (1EUL)

which showed a conformation in which these two domains are distant. The second structure (1IWO) demonstrated that the TGES motif is brought from a distant position in the E1 conformation (~30 Å) to a much closer position (~10 Å) to the phosphorylation site (D351 in SERCA) in the E2 conformation. Confirming earlier results obtained with the yeast proton pump (31), a mutation in this motif of SERCA was recently shown (8) to result in a considerable slowing down of the autophosphatase activity (phosphate release from the E2-P state), a step that is initiated by binding of extracellular K^+ to the E2-P form in the Na-K-ATPase (*step 7* in FIGURE 5). Together, these data demonstrate convincingly that the TGES domain that is brought into close contact with the phosphorylation site in the E2 conformation participates in the K^+-triggered dephosphorylation step. How the occupancy of the cation site by K^+ may be transmitted to the A domains remains to be explained.

Cation sites

The first crystallized form of SERCA (1EUL) contains two Ca^{2+} ions, and analysis of this high-resolution structure has clearly defined the position of two cation-binding sites roughly at the level of the middle of the membrane. Site I is located in a space between transmembrane segments 5 and 6, and site II is largely formed by a short unfolded region of transmembrane segment 4 and resides between transmembrane segments 4 and 6 (43). The Na-K-ATPase should provide a binding site to accommodate three Na^+ ions according to its well-accepted stoichiometry of 3 Na^+/2 K^+/1 ATP. In a detailed analysis of the Na-K-ATPase model, Ogawa and Toyoshima (27) propose that two sodium ions occupy two sites homologous to the calcium sites I and II of SERCA and predict the position of a third sodium site at about the same level in the membrane but closer to transmembrane segment 9. The three-dimensional structure of these sites and schematic views of the contribution of the fourth, fifth, sixth, eighth, and

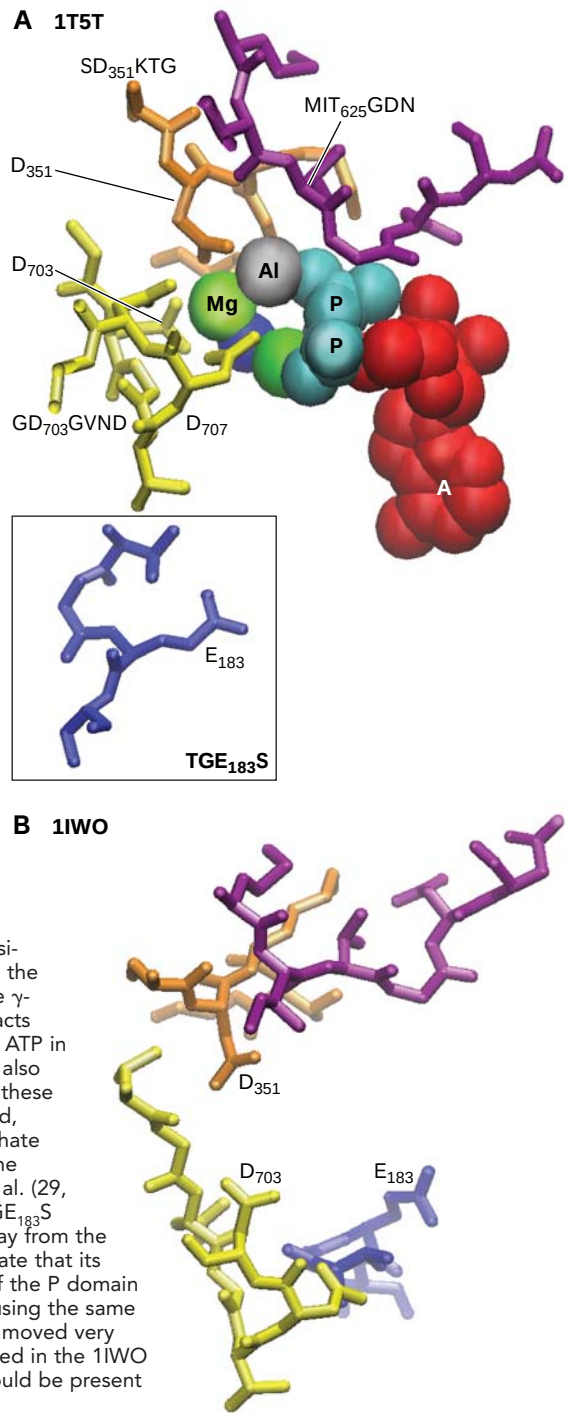


FIGURE 4. **Structure of the phosphorylation site**
Structure of some of the main elements of the phosphorylation site as they appear in the 1T5T (SERCA with ADP + Al F_4 = transition state of the phosphorylation step in E1) and 1IWO (SERCA with thapsigargin in E2) are represented in *A* and *B*, respectively. Only a few short segments of the P domain are represented: $SD_{351}KTG$ (corresponding to $SD_{369}KTG$ in the Na-K-ATPase), $TGE_{183}S$ (corresponding to $TGE_{214}S$ in the Na-K-ATPase), $IT_{625}GDN$ (corresponding to $MVT_{610}GDH$ in the Na-K-ATPase), and $GD_{703}GVND$ (corresponding to $GD_{710}GVND$ in the Na-K-ATPase). The nucleotide moiety of ADP is shown in red. The two phosphate groups are labeled P. The AlF_4 group (Al) takes the position of the γ-phosphate of ATP. Two Mg^{2+} ions are present in the 1T5T structure. One coordinates the D351 side chain and the γ-phosphate of ATP (AlF_4 in this case), and the other one interacts with the α- and β-phosphates of ATP, apparently maintaining ATP in the appropriate conformation (38). One molecule of water is also shown (dark blue). This image shows the close interaction of these three conserved sequences with the phosphorylation site [and, notably, the position of D351 and T625 close to the γ-phosphate (AlF_4 in this case)] and the role of T625, D703, and D707 in the coordination of the Mg^{2+} ions, as predicted by Patchornik et al. (29, 30) for the homologous residues in the Na-K-ATPase. The $TGE_{183}S$ sequence that belongs to the A domain is located ~30 Å away from the phosphorylation site and is represented inside a box to indicate that its position is not exact in this scheme. *B*: the same segments of the P domain in the 1IWO structure, an E2-type conformation, are shown using the same color coding. In this conformation, the $TGE_{183}S$ segment has moved very close to the phosphorylation site. No Mg^{2+} ions were identified in the 1IWO structure, but according to Pachornik et al. one Mg^{2+} ion should be present and coordinated by E183, D703, and D707.

ninth transmembrane helices are clearly illustrated in Refs. 27 and 41.

In contrast with the Na-K-ATPase, the two forms of the H-K-ATPase (gastric and nongastric) appear to carry out electroneutral cation exchange (7). Remarkably, a positively charged residue is present in the middle part of the fifth transmembrane segment of all known sequences of H-K-ATPases, whereas a neutral serine occupies the corresponding position in all isoforms of the Na-K-ATPase. Replacement of this serine by a positively charged arginine in the Na-K-ATPase abolished the electrogenic nature of its activity, whereas removal of the charged lysine and replacement by alanine in the nongastric H-K-ATPase of *Bufo marinus* resulted in electrogenic ion pumping similar to that observed with the Na-K-ATPase (6). Assuming that the nongastric H-K-ATPase exchanges two extracellular K^+ against two intracellular cations (H^+ and/or Na^+), we can hypothesize that the positive charge provided by the side chain of the lysine in the H-K-ATPases acts like a tethered cation at a position occupied by a Na^+ ion in the Na-K-ATPase. Structure modeling indicates that the lysine side chain is close to the position of the third sodium proposed by Ogawa and Toyoshima (27).

Interestingly, a very recent analysis of the gastric H-K-ATPase (22) proposes the existence of a single cation site in the proton pump, a site that would be occupied alternatively by a K^+ or a hydronium ion. This single site would be homologous to site II of SERCA. The gastric proton pump is not electrogenic, and thus it must transport a symmetrical number of H^+ and K^+ ions. The exact stoichiometry, however, has been debated, with proposals of 2 H^+/2 K^+/1 ATP and 1 H^+/1 K^+/1 ATP (22, 33, 36). The single-site model of Koenderink et al. (22) favors the hypothesis of the exchange of single cations per ATP, which seems more compatible with the thermodynamic requirement of proton pumping against a ~10^6-fold activity gradient (from an intracellular pH of 7.4 to a luminal pH of ~1.4), but these conclusions rest only on a model, and more data will be needed to solve this issue.

A Novel Aspect of Functional Properties: The Sodium Pump Transformed by Palytoxin

Palytoxin is a large organic nonpeptidic toxin (mol wt ~2,700) produced by a marine coelenterate that has been shown to bind to Na-K-ATPase (15) and induce a large, nonselective cation conductance. It has been clearly demonstrated that the induction of the conductance requires the direct association of the toxin with the Na-K-ATPase to produce a channel with a ~7-pS unitary conductance (2, 35, 46). Mutagenesis experiments have shown that the pathway opened by palytoxin is constituted, at least in part, by the same elements that make the cation-binding site in the normal Na/K pump cycle (13, 14). Artigas and Gadsby (2) have studied the kinetics of the palytoxin-transformed Na/K pump at the level of the single-channel current and as macroscopic currents under various conditions of Na^+ and K^+ and nucleotide concentrations. In a second recent publication (3), they have determined the influence of extracellular K^+, intracellular Na^+, and nucleotide concentrations on the apparent affinity of palytoxin. Together, these results allow them to draw a model of the interactions between the palytoxin and the Na/K pump as well as propose an interesting hypothesis concerning the modifications induced by palytoxin and their relation with the normal function of the Na/K pump. Briefly summarized, their main conclusions are that palytoxin acts by altering the control of one or both of the two gates of the ion-translocation mechanism while preserving many aspects of this control by intracellular or extracellular ligands. These findings will be discussed in detail with the integrated model in the next section, with the help of FIGURE 5.

In addition, Artigas and Gadsby (3) showed that cardiotonic steroids and palytoxin must be able to bind simultaneously to the Na/K pump, because palytoxin is able to accelerate the dissociation of previously bound strophanthidin. They also determined a minimal pore diameter of ~7.5 Å for the channel opened in the Na/K pump by palytoxin. The possibility of reaching cysteine residues all across the membrane width with a sulfhydryl reagent of comparable size also indicated the presence of a large-diameter pore in the palytoxin-transformed Na/K pump (14). The rather small unitary conductance (~7 pS) of this channel is rather surprising considering this large diameter, but it can be understood if one assumes that the current observed with the palytoxin-treated Na/K pump is due to cations that interact strongly with their rather-high-affinity binding sites in the middle of the transmembrane part of the Na/K pump (3).

Binding Sites for Extracellular Inhibitors

A last point is the definition of the binding site for extracellular inhibitors. Some of these inhibitors are largely used in clinical medicine: the cardiac steroid inhibitors of the Na-K-ATPase are used for the treatment of heart failure (the pharmacological target is most probably the α2-isoform of the Na-K-ATPase), and the gastric proton pump inhibitors are used for the treatment of peptic ulcers. It has long been known that the substituted benzimidazole compounds that are used for

proton pump inhibition act by forming a covalent bond, a disulfide bridge, with one (or two) specific cysteine residues located in the sixth transmembrane segment of the α-subunit (23). The binding site of the cardiac steroid on the Na/K pump has been more difficult to define. Comparison of ouabain-sensitive and -resistant isoforms of the Na/K pump and subsequent mutagenesis work (summarized in Ref. 26) initially pointed to the outer part of the first hairpin (the transmembrane segments 1 and 2 and the short extracellular loop between them), but extensive mutagenesis work disclosed the important contribution of the fifth, sixth, and seventh transmembrane segments (28), and recent work with Na-K-ATPase and H-K-ATPase chimerical constructs demonstrated that the third hairpin (transmembrane segments 5 and 6) provides the structure of the binding site itself (21, 32). The very strong influence of the extracellular part of the first and second transmembrane segments can be understood by the hypothesis that this domain may modulate the binding on or off rate by acting on the access to the ouabain-binding site, as suggested by recent work comparing the kinetics of ouabain binding in human α-isoforms (10).

The State-of-the-Art Model

Comparison with the other types of ion-motive ATPases

With these pieces of the puzzle, we can now build a general picture of the Na/K pump "engine," and this picture can be compared with the two other main types of ion-motive ATPases: the F- and V-ATPases. In all cases, there is *1*) a cytoplasmic domain containing the structure that is responsible for binding and hydrolyzing ATP (or synthesizing ATP for the F-ATPases), *2*) a membrane part of the protein that allows the vectorial transport of cations, and *3*) an energy-transducing mechanism that links the first two parts and allows changes in one part to drive changes in the other part. The case of the V-ATPases, which like the P-ATPases function as ATP-driven ion pumps, will be considered here. In the V-ATPases, the large cytoplasmic part provides three ATP-binding sites in a six-subunit assembly, and the substrate binding, hydrolysis, and product release is tightly linked to the rotation of a central shaft (the D subunit) with regards to the ATP-binding subunits. In the P-ATPases, there is a single ATP site per unit (the question of protomer assembly in a higher-order complex will not be discussed here), and in the binding of ATP its hydrolysis (with transfer the γ-subunit of the phosphate onto the aspartic residue) and the release of ADP are linked to a folding movement of the N domain and a complex rotation of the A domain (41, 44). In the F- and V- ATPases, the energy-transducing mechanism is very simply the transmission of the rotation of the central "shaft" to the membrane-associated part of the protein. This rotation positions a proton-acceptor site successively in contact with the cytoplasmic side (allowing the loading of one proton) and then the extracytoplasmic side (for proton release). The energy-transducing mechanism of the P-ATPases is more difficult to describe and not yet fully understood, but it is clear that the large movements of the A, N, and P domains result in translation movements and rotations of several transmembrane helices, resulting in the opening and closing of the intracellular and extracellular access pathway to the cation-binding site(s) and reconfiguration of the binding site(s) resulting in large changes in their affinity. The movements of the A, P, and N domains are only schematically illustrated in FIGURE 5. For a more precise description and analysis of these movements in three-dimensional space and a complete description of the complex movements of the transmembrane helices, the reader should refer to the original data (44, 45) and the recent review by Toyoshima and Inesi (41).

An integrated model of ion pumping by the Na-K-ATPase

FIGURE 5 puts together in a very schematic way the main points of our structural and functional understanding of the cation transport cycle by the Na-K-ATPase. The A, N, and P domains form what we could call the "core engine" of the P-type ATPases. Because this hydrolyzing "core engine" has the same function in all P-ATPases and because of the very high degree of sequence conservation in these domains, it is expected that the mechanism existing in this part of the protein will be very similar in all P-ATPases, and we are confident that the results of the observations made with SERCA can be applied to the Na-K-ATPase. In contrast, the fine steric changes that define the precise selectivity of the cation sites are obviously specific to each P-ATPase.

Starting at *step 1* in FIGURE 5 (E1 state) with ATP present in its binding site, the entry of three Na^+ ions through the open internal gate and the binding of these ions in their high-affinity binding sites produces a conformational change (red arrow). First, the N domain follows a large rotation that positions the γ-phosphate of ATP close to the phosphorylation site. In this position, ATP appears to be a "crosslinker" between the P and N domains (42). This "crosslinker" can then be cleaved, allowing the transfer of the γ-phosphate to D376 (*step 2*). Second, the A domain also rotates by ~30° around a

horizontal axis (42), producing a large translation of the first transmembrane segment toward the intracellular side and a 90° kink in the internal third of this α-helix. Both Sørensen et al. (38) and Toyoshima and Mizutani (42) propose that this movement of the first TM is responsible for the closing of the internal gate (*step 3*), a step also accompanied by the release of ADP, resulting in the E1~P state. During the physiological cycle, this high-energy E1~P state rapidly relaxes to the E2-P conformation with opening of the outside gate (*step 4*) and reconfiguration of the cation-binding sites. The subsequent release of Na^+ ions to the extracellular side (*step 5*) is in fact a rather complex phenomenon that could be further analyzed thanks to high-time-resolution measurements (17, 18). After opening of the outside gate, a first Na^+ is released, carrying with it a large charge movement through the membrane; this is followed by a reconfiguration of the external access channel, followed in turn by release of the two other Na^+ ions. The cation-empty E2 conformation is now ready for the entry of K^+ ions (*step 6*). Occupancy of the cation sites of the E2-P state by two extracellular K^+ ions results in two major changes (two red arrows): *1*) dephosphorylation of D376 and *2*) K^+ occlusion by closing of the extracellular gate (*step 7*).

Concerning the effect of palytoxin, as depicted at right in FIGURE 5, interference with this step appears as a major mechanism. In the native Na/K pump, closure of the outside gate occurs with a fast time course [rate faster than 1,000/s (16)], but it seems to be slowed down by about four orders of magnitude to a time constant of ~9 s when palytoxin is bound (2) (red arrow at *left* in FIGURE 5). Because K^+ occlusion and dephosphorylation are tightly associated, palytoxin also considerably slows down dephosphorylation. Thus palytoxin appears to stall the cycle at the E2-P conformation by preventing the closure of the extracellular gate and thereby the occlusion of K^+. In the absence of extracellular K^+, palytoxin is bound with an extreme stability (a half-life of about a day). In the presence of extracellular K^+, occlusion does slowly occur, leading to dephosphorylation and eventually to release of palytoxin.

The N domain now presents a site accessible to intracellular nucleotide. During the normal transport cycle, ATP (or other nucleotides such as ADP or nonhydrolyzable analogs of ATP), binding to this low-affinity (on the order of 10–100 μM) intracellular site (*step 8*), promotes the change from the E2K to the E1 conformation, which includes the opening of the intracellular gate (*step 9*) and the subsequent release of K^+ to the cytoplasmic side (*step 10*). These steps may also occur in the absence of nucleotide but at a much lower rate.

The observation by Artigas and Gadsby (3) that nucleotides activate the palytoxin-induced conductance indicates that the effect on intracellular nucleotide binding is preserved in the palytoxin-bound pump: opening the intracellular gate corresponds then to the opening of a channel, because the extracellular gate is also maintained open (encircled states at *right* in FIGURE 5). The importance of these data resides in the fact that it is now possible to study the control opening and closing of the individual gates, the "nuts and bolts" of the ion-pumping mechanism, in real time and at the level of a single protein molecule in mutant as well as in wild-type Na-K-ATPase.

Future Directions

Even if a clear picture of the Na/K pump structure and function has emerged from the recently published results, a number of important points remain to be elucidated and several hypotheses need to be confirmed before we have a full mechanistic understanding of active cation transport.

First, many pieces of structural information are only derived through homology to the Na/K pump and are in great need of experimental confirmation. This could be obtained by a high-resolution structure of the Na/K pump itself, but improvement of the modeling procedures and confirmation by analysis of the biochemical and functional consequences of site-directed mutagenesis may also bring significant confirmation. For instance, a hypothesis about the structural basis for cation selectivity has been proposed by analogy modeling of these cation sites, but experimental verification is really needed.

The key determinants of the energy-transduction mechanism between the ion-binding sites in the transmembrane segments and nucleotide-binding and phosphorylation sites in the cytoplasm are still largely ignored. High-resolution structures of additional conformations [which are soon to be available for SERCA (C. Toyoshima, personal communication)] and various modes of molecular simulation will be very helpful in testing the hypothesis about these mechanisms, but the possibility of direct testing of the command of individual gate function in the palytoxin-transformed pump seems very promising.

Another unresolved question is the cation pathway from their binding sites to both the extracellular and the intracellular sides of the membrane. Concerning the pathway from the intracellular side in SERCA, Toyoshima et al. (43) has proposed that Ca^{2+} enters through a space between the transmembrane segments TM1 and TM4 on one side and TM2 and TM6 on the other side. This hypothesis is now supported by the structural data on the

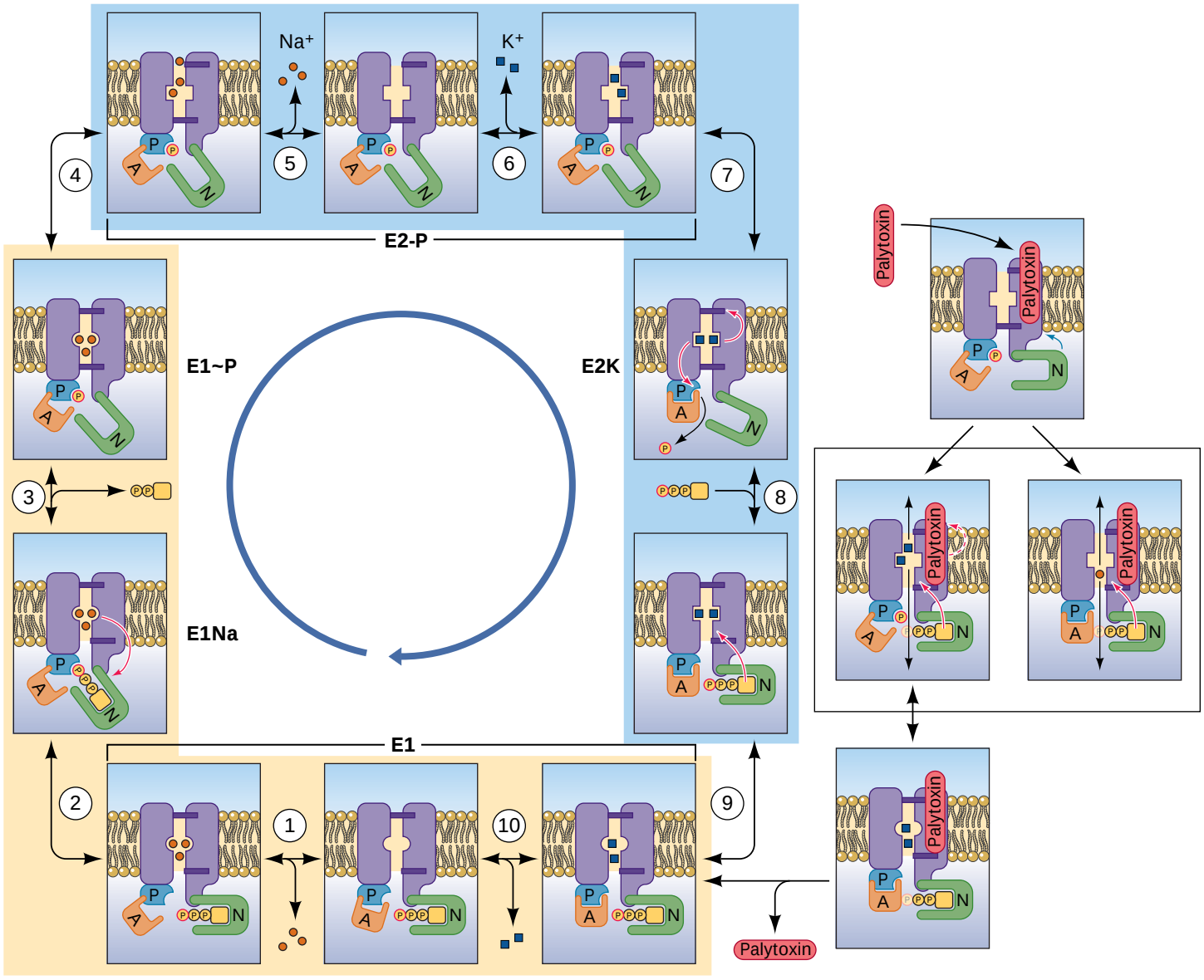


FIGURE 5. **Integrated scheme of the Na-K-ATPase functional cycle**
Left: the main steps of the cation-transport cycle. The circular arrow in the center shows the direction of the physiological cycle driven by a high ATP/ADP ratio, but all of the steps are reversible, and under appropriate conditions the cycle can run in the reverse direction (11). Each step is described in the main text. The E1Na state corresponds to the state of SERCA crystallized by Sørensen et al. (38) and by Toyoshima and Mizutani (11). *Right*: hypothetical mechanism for the effect of palytoxin. According to this hypothesis, the cation conductance is due to the stabilization by palytoxin of a conformation of the Na/K pump in which both gates are allowed to be open simultaneously (confirmations enclosed in a rectangle), a state that is nonexistent or extremely short-lived in the native Na/K pump cycle. This stabilization may be related to a major slowing down of the closure of the external gate upon binding of K^+, a very fast step in the normal Na/K pump.

E1~P state (38, 42), in which formation of a cluster of hydrophobic residues (belonging to TM1 and TM2) is interpreted as the closure of the cytoplasmic access pathway. However, the effects of various intracellular cations on the proteolytic cleavage of the intracellular loop between the sixth and seventh transmembrane segments has been interpreted as indicating that this loop participates in the entrance port for intracellular Na^+, and this view was supported by the detection of a site with negative electrostatic valence in this region in the modeling work by Rakowski and Sagar (34). Concerning the external access pathway, on the one hand no obvious widely open channel can be seen in the 1IWO structure that is thought to represent the E2 conformation (44). On the other hand, cation permeability measurements in the presence of palytoxin indicate that a pore with a narrowest section of ~7.5 Å must exist through the Na/K pump in the (palytoxin-bound) E2 conformation (3). The results of cysteine scanning experiments in the presence of palytoxin have demonstrated the involvement of

the fourth, fifth, and sixth transmembrane helices (13, 14, 19). Here again a high-resolution structure with an opened extracellular pathway would greatly help to create a full picture of the cation translocation mechanism.

This review has not addressed the question of the structure-function relationship of the β- and γ-subunits (or other FXYD proteins). Although it is now well demonstrated that both subunits can significantly influence the physiological properties, the absence of high-resolution structural data for these subunits makes it very difficult to draw any conclusions concerning the mechanism of these effects. Recent data suggest that the transmembrane segments of the FXYD7 protein are closely associated with the ninth transmembrane segment, probably lying in a groove between the second and ninth transmembrane segments (25). Confirmation of this interaction and definition of the position of the intracellular, transmembrane, and extracellular parts of the β- and γ-subunits with regards to the α-subunit will be a most important step in the near future and could be brought about by a high-resolution structure of the αβ or αβγ complexes.

We are at a very exciting point in the developing understanding of the working mechanism of such a wonderful molecular machine, and this makes research in this field very interesting, despite the fact that a Nobel Prize has already been awarded (in 1997 to Jens C. Skou) for investigations in the Na/K pump field. ■

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