SEMINARS IN
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Structure-Function Relationships in the Na^+,K^+-Pump

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The Na,K-pump was discovered about 50 years ago. Since then there has been a methodic investigation of its structure and functional characteristics. The development of the Albers-Post model for the transport cycle was a milestone that provided the framework for detailed understanding of the transport process. The pump is composed of 2 subunits that exist in the membrane as an $\alpha\beta$ heterodimer. All known enzymatic functions of the pump occur through the α subunit. Although necessary for activity, the complete role of the β subunit is not understood fully. Numerous studies have established that the $\alpha\beta$ protomer is the minimal functional unit needed to perform the Albers-Post reaction cycle. However, higher orders of aggregation $[(\alpha\beta)_n]$ are commonly detected. There is little evidence that oligomerization has functional consequence for ion transport. The Na^+,K^+-adenosine triphosphatase (ATPase) is a member of the P-type ATPase family of transporters. Proteins within this family have common amino acid sequence motifs that share functional characteristics and structure. Low-resolution 3-dimensional reconstruction of 2-dimensional crystal diffractions provide evidence for the similarity in tertiary structure of the α subunit and the Ca^{2+}ATPase (a closely related P-type ATPase). The spatial location of the β subunit also is obvious in these reconstructions. Recent high-resolution reconstructions from 3-dimensional crystals of the Ca^{2+}ATPase provide structural details at the atomic level. It now is possible to interpret structurally some of the key steps in the Albers-Post reaction. Some of these high-resolution interpretations are translatable to the Na^+,K^+-ATPase, but a high-resolution structure of the Na,K-pump is needed for the necessary details of those aspects that are unique to this transporter.
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Essentially all animal cells maintain a cytoplasmic environment that is relatively high in potassium and low in sodium compared with extracellular space. The mystery of how this ionic gradient is maintained began to be unraveled in the early 1950s as the red cell developed into a valuable system for studying sodium and potassium transport.[1] In 1957, Skou[2] discovered a membrane-associated enzyme in nerve tissue that hydrolyzes adenosine triphosphate (ATP) in the presence of Na and K. Subsequent studies established that the Na^+- and K^+-dependent ATPase and the Na,K-pump studied in red cells were the same enzyme.[3] (In 1997, Skou[4,5] shared the Nobel Prize in Chemistry for his classic work in the discovery and characterization of the Na^+,K^+-ATPase.)

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Since its initial identification, a tremendous scientific effort has been devoted to understanding the details of the structure and function of this enzyme. The need for this understanding is made clear when one considers the important physiologic roles of the Na^+,K^+-ATPase (also known as *Na^+,K^+pump*, *Na^+ pump*). The ionic gradient created by the Na^+,K^+-ATPase is responsible for maintaining the membrane potential in vertebrate cells that is essential in excitable cells, making action potentials and neuronal transmission possible.[6] A major role of the Na^+,K^+-ATPase is that of an energy transducer converting the chemical energy from the hydrolysis of ATP to the chemical potential energy created by an ionic concentration gradient. This gradient is coupled to and provides the energy for other transport processes that are responsible for cell volume regulation; for secretory processes in epithelia; for intracellular transport of vital solutes such as glucose, amino acids, and neurotransmitters within the organism; and for the absorption of metabolites from the intestine.[3,7,8] The electrochemical gradient generated by the

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Na^+,K^+pump provides the driving force for reabsorption of sodium and many other vital solutes in the kidney where the pump exists in its greatest concentration in the outer renal medulla.[9] In a resting animal, greater than 20% of cellular ATP is consumed by the Na^+,K^+-ATPase to maintain these and other functions.[3] One easily could characterize the Na^+,K^+-ATPase as a sine qua non of animal life. This review provides an overview of what is known currently about the structure-function relationship for the Na^+,K^+-ATPase. I have tried to avoid most speculative issues although certainly some aspects remain open-ended. There is a vast pool of biochemical data relating to the issues discussed later and most of the details of that data are beyond the limits of this review. However, for those interested in more biochemical and molecular biological details, I refer the reader to these reviews and the references found therein for classic background[3] and more recent developments.[10,11]

The Albers-Post Reaction Model

Before structural details about the pump were obtained, there was an accumulating body of data concerning the functional aspects and mechanistic requirements for the active transport process. In the mid-1950s, evidence collected, in the main, from studies using red blood cells established some fundamental properties of the transport process. Ouabain, a plant-derived steroid, was shown to be a potent inhibitor of the transporter and proved to be a valuable tool in the dissection of Na^+,K^+-ATPase function.[12] Pioneering work by Post and Jolly[13] established that the transport stoichiometry was 3 Na^+ transported out of the cell for 2 K^+ transported into the cell. The 3 Na/2 K–exchange ratio of the Na^+,K^+-pump remains a unique feature of this ATPase. Another characteristic feature of the Na^+,K^+-ATPase is that during the course of ion transport the pump goes through a cycle that involves, in the presence of Na^+, the transfer of the high-energy phosphate of ATP to form a covalently bound phosphorylated intermediate of the transport enzyme.[14] This phosphorylated intermediate represents a key step in the transfer of the energy needed for active transport. Further along the reaction cycle, the phosphate group is released from the enzyme (promoted by K^+) as a free phosphate (P_i), and the pump returns to a state ready to bind another ATP. It was observed that the pump reaction cycle is reversible and under the proper conditions the ATPase generates ATP from adenosine diphosphate and P_i.[15-22] These observations prompted proposals in the mid- to late 1960s of a reaction that now is referred to commonly as the Albers-Post model.

The Albers-Post model has provided a framework for the investigation of the mechanism of the Na^+,K^+-ATPase for more than 30 years and has been shown to be applicable to other transport proteins that belong to the general class of P-type ATPases discussed later. As more information has been obtained the model has been revised with greater detail. A simplified version of it appears in Fig. 1. A key feature of the reaction model is that the ATPase must exist in at least 2 major conformational states, characterized as E_1 and E_2. Simply stated, in the E_1 conformation the ion binding sites face the cytosol and have a high affinity for Na^+ and a low affinity for K^+, in the E_2 state the binding sites face the extracellular space and have a lower affinity for Na^+ ($K_D > 0.3$ mol/L) and a higher affinity for K^+ ($K_D < 1.3$ mmol/L). Another feature of the reaction model is that at particular stages of the transport cycle the ions are occluded and presumably inaccessible to either the cytoplasmic or extracellular surfaces of the cell. Additionally, 1 of the 3 Na^+ ions is bound with a lower affinity than the other 2 and comes off the transporter in a distinct step.[23-25] There has been extensive kinetic investigation of this reaction scheme and controversy still exists about the details of some steps. ATP can bind to either the E_1 or E_2 conformation of the ATPase. In the E_1 conformation, ATP binds with high affinity ($K_D \sim 50$ nmol/L) whereas it binds with a reduced affinity to the E_2 state ($K_D \sim 300$ μmol/L). The binding of ATP to the E_2(2K) form accelerates the conformational transition from E_2 to E_1 and the release of K^+ into the cytosol. Under optimal conditions the overall reaction can proceed at a rate approaching 10,000 cycles/min.[26]

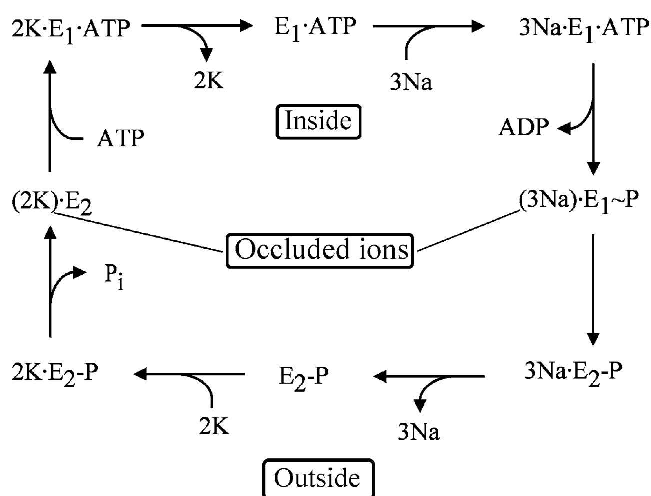

Figure 1 The Albers-Post reaction cycle. This is a simplified version of the reaction cycle as it is currently understood. Na release to the outside, which is shown as a single step in this figure, actually occurs in 2 steps with 1 ion being released as a step separate from the release of the other 2 ions. The arrows indicate the normal direction of the reaction cycle, however, all steps are reversible. Adapted from Glynn.[129]

Na^+,K^+-ATPase Structure

Subunits

The Na^+,K^+-ATPase is composed of 2 protein subunits termed α and β. The α subunit has a molecular weight of approximately 112,000, based on amino acid composition. The β subunit has an amino acid mass of approximately 35,000, but it is highly glycosylated. On a typical electrophoretic gel the α subunit migrates as a well-defined band with a M_r of approximately 100,000 whereas the β subunit presents itself as a diffuse band that ranges from an M_r of approximately 45,000 to 58,000. Virtually all that is known about the function of the Na^+,K^+-ATPase pertains to opera-

tions and aspects of the α subunit. However, efforts to separate the α and β subunits result in loss of function.[27-31] Cloning and expression studies have indicated that the β subunit may be involved in the trafficking and delivery of the ATPase to the cell membrane.[32] Other studies have suggested that it also may influence K^+ interactions and Na^+,K^+-ATPase stability.[33-37] A third subunit, characterized as the γ subunit, also has been identified in kidney tissue.[38-41] This is a small protein of approximately 6,500 to 7,500 molecular weight and appears to belong to the FXYD family of regulatory peptides,[42,43] which is covered more fully in an accompanying article, by Garty and Karlish, in this issue. Multiple isoforms of the α and β subunits have been identified. The α and β subunits exist ubiquitously in most tissue as the α_1 and β_1 isoform and this is the predominant isoform found in kidney and organs involved in ion homeostasis[26,41,44,45] and is the isoform used in essentially all structural studies. Other isoforms (α_2, β_2, α_3, β_3, and α_4) are found in many tissues.[46-49] The isoforms have some minor sequence differences and functionally have altered affinities for Na^+, K^+ and ouabain and display different reaction kinetics compared with the α_1 form.[50-54] The functional significance of isoforms are dealt with in more detail in an accompanying article, by Blanco, in this issue.

The Oligomer Question: $\alpha\beta$, $(\alpha\beta)_2$, $(\alpha\beta)_n$?

It is clear that α and β units exist in equimolar amount in the membrane and that the pump is an $\alpha\beta$ heterodimer.[3] However, for the past 3 decades, there has been considerable controversy as to whether the protomeric unit of the pump exists in a functional state as an $\alpha\beta$ heterodimer or some higher-order oligomer. Early surveys of membrane transport proteins in membrane-bound and detergent-soluble forms suggested that perhaps all transport proteins must exist as oligomers.[55,56] It was attractive to imagine that a transport protein would use the oligomeric structure to form a transport channel (this is often the structure drawn in textbook cartoons of the Na^+,K^+-ATPase[57]) and indeed many ion channel proteins have been shown to form their functional channel through oligomerization.[58] In dealing with the Na^+,K^+-ATPase specifically, there has been an array of data that were interpreted as indicating the functional unit was an $(\alpha\beta)_2$ or higher-order oligomer or was explained most easily by an oligomeric structure.[59-61]

Highly purified forms of membrane-bound Na^+,K^+-ATPase display functional stoichiometries, indicating that all $\alpha\beta$ protomers are active simultaneously.[26,62-66] However, there is little doubt that when α subunits are found in membranes at high density they tend to be associated closely. For example, chemical cross-linking studies with purified membrane fragments from mammalian kidney (in which pump density is $>$ 10,000 units/μ^2) showed that the Na^+,K^+-ATPase units were close enough to be cross-linked by chemical reagents.[67] But when the same techniques were applied to human red cell membranes, which have about 400 pumps/cell, α subunits were found to cross-link with the band-3 cation exchanger of which there are approximately 3×10^6 copies per cell and there was no evidence of α-α cross-links.[68] By using fluorescent resonance energy transfer, which can measure the distances between fluorescent probes bound to the Na^+,K^+-ATPase, it was shown that in preparations that had high densities of pumps, α subunits had an average separation consistent with an oligomeric (dimer) structure.[69-71] However, when fluorescent resonance energy transfer measurements were performed on samples that had a lower pump density the average separation between the pumps increased, showing that the distances between α subunits was not constant, as would have been expected had the α subunits existed in fixed oligomeric structures. The data indicated that about half of the α subunits were much further apart and in a monomeric form, yet the activity per $\alpha\beta$ protomer was unchanged, showing that individual $\alpha\beta$ protomers were fully active in natural membranes.[71] The Na^+,K^+-ATPase can be solubilized out of the membrane to monomeric form with detergents and still maintain ATPase activity.[72] Hydrodynamic analysis of this activity (by using a technique called *active enzyme centrifugation*[73]) revealed that the activity originated from a monomeric ($\alpha\beta$) species.[74] Taken together, these and other studies[75-77] have shown the functionality of the $\alpha\beta$ protomer in both detergent-solubilized forms and in the native membrane, and no clearly defined function has been identified that requires an oligomeric structure.

Although evidence argues that the $\alpha\beta$ protomer is the minimal functional unit, as cited earlier, aggregation of protomeric units is a common observation. A series of coprecipitation studies have shown that α subunits can associate when Na^+,K^+-ATPase is overexpressed in insect cells.[78-80] These studies concluded that the association occurs between the TM4-TM5 loop (see later) of the Na^+,K^+-ATPase cytoplasmic domain and that loop association required magnesium ATP (MgATP).[80] A recent study further showed that α subunits with truncated loops that only contain the phosphate binding motifs can associate with wild-type Na^+,K^+-ATPase.[81] Earlier studies showed that mutants lacking this region did not associate with wild-type Na^+,K^+-ATPase.[78] Taken together, the data suggest that in this expression system α subunits associate through specific interactions that create aggregates that are detectable in the endoplasmic reticulum and plasma membrane. These associations may be mediated through the linkage of nucleotide and phosphate binding domains in which ATP serves as the cross-linking agent.[80] The strong tendency of α subunits to aggregate continues to support speculation that oligomerization may serve some unidentified regulatory or functional role and it appears this issue will continue to be a topic of interest for some time.

The α Subunit

The primary sequence of the α subunit has been obtained from a variety of sources. Comparisons reveal a tremendous conservation of sequence throughout the animal kingdom. For example, the amino acid sequence of the Na^+,K^+-ATPase from duck nasal salt gland shows 95.5% similarity and 93.5% identity to that from human kidney. This suggests a very restricted range of divergence in sequence before loss of

transport function. (In contrast, a similar comparison for the β subunits reveals 81% similarity and 69% identity). Within the α sequence there are key regions that help define structural and functional classifications of the Na^+,K^+-ATPase. Of perhaps paramount importance is a 5 amino acid sequence motif of aspartic acid-lysine-theronine-glycine-threonine (DKTGT). The aspartate (D) in this sequence is the site of phosphorylation discussed in the Albers-Post model earlier. We now know that the Na^+,K^+-ATPase belongs to the P-type ATPase family, all of which contain this important sequence motif[82-85] and are part of the haloacid dehalogenase superfamily.[86] This family of ATPases contains more than 300 varieties of enzymes identified in the animal and plant kingdoms. More familiar members include the Ca^{2+}ATPases found in muscle and blood cells and the gastric H^+,K^+-ATPase. Of all the members in the P-type ATPase family, only the Na^+,K^+-ATPase and H^+,K^+-ATPase require a β subunit for activity and, accordingly, they make up a distinct subfamily. It is worth noting that both of these enzymes counter transport K^+, and there are indications that the β subunit may be needed or involved in the K^+ interaction.[33,35,36,87] To the extent that the reaction mechanisms of the other members of the P-type ATPases are understood, it appears that the fundamental steps of the Albers-Post model are applicable to all members.

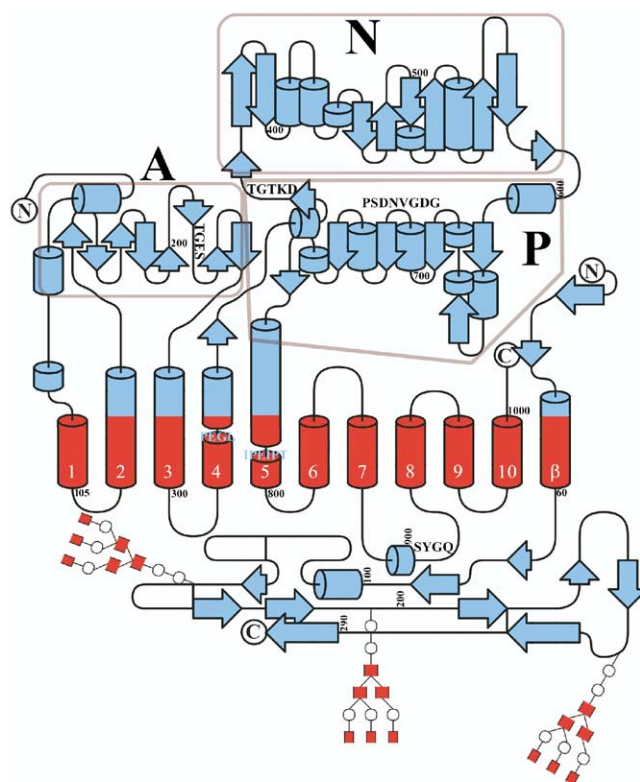

Figure 2 Topology model of the Na^+,K^+-ATPase structure that was constructed by using the available structural data for the Na^+,K^+-ATPase and incorporating known high-resolution structural data from the highly homologous Ca^{2+}ATPase.[94] The A and P domains are based on the Ca^{2+}ATPase structure of these domains. The N domain is based on high-resolution structures of the isolated Na^+,K^+-ATPase N domain.[124,125] The dark-toned (red in the online version) helices represent hydrophobic transmembrane segments. The lighter tones (blue in the online version) are more hydrophilic regions. Amino acid sequence motifs discussed in the text are shown at their approximate locations. The motifs in the P domain read right to left, consistent with the direction of the amino acid sequence. The small black numbers indicate their approximate numeric location in sequence. The PEGL and IPEIPT motifs cause breaks in TM4 and TM5, respectively (the motifs are visible more clearly in the color version of this figure that is available online). The secondary structure of the β subunit was obtained by using a predictive algorithm[130] folded to reflect known and suggested interactions with the α subunit. The branched N-linked oligosaccharides (unknown specific structure) are positioned at their approximate location in the sequence, as are the disulfide bridges. The figure initially was drawn using the TopDraw (Edmonton, Alberta) algorithm[131] with subsequent modification using Corel PhotoPaint 8 (Ottawa, Ontario). (Color version of figure is available online.)

Fig. 2 shows a 2-dimensional topology cartoon of the structure of Na^+,K^+-ATPase based on secondary structure predictions modified with the incorporation of low- and high-resolution structure information discussed later. After extensive biochemical and molecular biological investigations, it has been established that the α subunit has 10 transmembrane segments with both the N and C terminals exposed to the cytoplasm.[88,89] On the cytoplasmic side of the membrane there is a major peptide loop between transmembrane regions 4 and 5 (TM4 and TM5). This loop alone contains approximately 430 amino acids, or approximately 42% of the mass of the α subunit. Another approximately 14% of the protein mass is located in the loop between TM2 and TM3, and approximately 9% of the mass is on the N terminal side of TM1. In fact, if one treats membrane fragments enriched in Na^+,K^+-ATPase with proteolytic enzymes the resulting product, which is comprised of the transmembrane segments, has an M_r of only 19 kd. Amazingly, this 19-kd fragment still is capable of binding transport ions and has been shown to occlude kinetically those ions from access to either surface of the membrane.[90-92] Biochemical and molecular biological investigations of this occlusion process have helped to focus on the transmembrane regions most likely involved in the transport process.[93] Although the ion binding sites may be located in the transmembrane region, the machinery of energy transduction and transport resides in the massive cytoplasmic regions of the α subunit.

The cytoplasmic segments of the Na^+,K^+-ATPase can be divided into several structural and functional regions. Immediately adjacent to the membrane surface is an area termed the *stalk region*, which is comprised mainly of extensions of transmembrane α helices (TM2-TM5), which in general are less hydrophobic than the transmembrane regions and contain a greater proportion of charged amino acids. (Structure predictions suggested that TM1 also would be involved in the stalk region, however, high-resolution structures of Ca^{2+}ATPase indicate that TM1 does not contribute to the stalk and this is likely to be true for Na^+,K^+-ATPase). Structurally the stalk region serves to extend the rest of the cytoplasmic segment farther away from the membrane surface. The cytoplasmic extension of TM1 contains the N-terminal segment and (based on the Ca^{2+}ATPase structure) portions

of this chain are associated closely with the TM2-TM3 loop to form a structural element termed the A (*anchor* or *activator*) domain. The A domain contains the threonine-glycine-glutamic acid-serine (TGES) motif found in most P-type ATPases. This motif may be important in stabilizing the E_2P form of the enzyme.[94] The N-terminal chain of the Na^+,K^+-ATPase has an additional approximately 26 amino acids compared with the Ca^{2+}ATPase and there is evidence that this may have regulatory or functional significance.[19,95-98]

The portion of the TM4-TM5 loop that extends beyond the stalk region can be subdivided into areas defined as *P* (*phosphorylation*) and *N* (*nucleotide binding*) domains. Within the P domain are amino acid motifs identified as being involved in the phosphorylation of the α subunit, including the DKTGT signatory region of the P-type ATPases and the glycine-aspartic acid-glycine-valine-asparagine-aspartic acid-serine-proline (GDGVNDSP) motif that is associated closely with the phosphorylation site and stabilization of phosphoenzyme intermediates.[99,100] The P-domain contains segments that are attached directly to TM4 and TM5. The segment attached to TM5 has a structure of alternating parallel β sheets and α helices termed a *Rossman fold*, which is a structure common to many nucleotide-binding proteins. The N-domain contains motifs that form the hydrophobic pocket necessary for nucleotide binding (ATP, adenosine diphosphate, and so forth). The N-domain also contains segments identified as isoform-specific regions[101] and an adaptor protein-2 (AP-2) binding site that has been associated with the regulation of Na^+,K^+-ATPase endocytosis.[102]

The transmembrane segments contain the ion-binding sites that transfer ions between cytosol and extracytoplasmic space. TM4 contains the proline-glutamic acid-glycine-leucine (PEGL) signature motif of P-type ATPases[83] and this region in conjunction with interactions with other transmembrane segments (TM5, TM6, and TM8 in the Ca^{2+}ATPase) make up the central core region in which the ions bind and are transported by the ATPase. The PEGL sequence is associated with an unwound section in the helical structure of TM4 in the Ca^{2+}ATPase and there is speculation that a similar unwound section containing the sequence isoleucine-proline-glutamic acid-isoleucine-proline-threonine (IPEIPT) also may occur in TM5 in the Na^+,K^+-ATPase.[103]

The extracellular loops on the Na^+,K^+-ATPase in total contain a minor fraction of the mass of the transporter (~65 residues, ~6%). Greater than half of the extracellular mass can be found in the loop between TM7 and TM8. Mutational modification of the extracellular loops associated with TM5-TM6 and TM7-TM8 results in altered ion binding.[104-107] In addition to ion binding, the extracellular face of the Na^+,K^+-ATPase is also the site for ouabain interaction. Ouabain interaction with Na^+,K^+-ATPase is dependent on the conformation and phosphorylation state of the protein, with the affinity being highest in the E_2 conformation.[3] Mutational studies indicate that high-affinity ouabain binding is dependent on residues in or near the extracellular loops of TM1-TM2, TM5-TM6, and TM7-TM8. Only the TM1-TM2 loop is involved in the lower-affinity interaction of ouabain with nonphosphorylated forms of the Na^+,K^+-ATPase. The TM1-TM2 loop is also the site of residue variations between species that appear related to ouabain sensitivity and as such this region often is termed the *ouabain binding domain*.[104] The relatively large extracellular TM7-TM8 loop is involved in the α-β–subunit interaction. A segment within this loop, serine-tyrosine-glycine-glutamine (SYGQ), has been identified as being very important for this interaction.[108,109] This sequence does not exist in the Ca^{2+}ATPase, which does not have a β subunit but does exist in the H,K ATPase, which also has a β subunit.[33]

The β Subunit

The β subunit contains a single transmembrane helix. In contrast to the α subunit, most of the mass (~244 residues, 80%) is exposed to the extracellular surface, as is the C-terminal. The N-terminal segment on the cytoplasmic side of the membrane is about 11% (~34 residues) of total mass. The large extracellular domain is characterized by 3 S-S bridges between cysteines and 3 sites of N-glycosylation. Although there is considerable interspecies β-sequence variation, the S-S bridges and N-glycosylation sites are highly conserved. The peptide segment that runs between the extracellular end of the transmembrane helix and the location of the first S-S bridge (~60 amino acids) contains the site(s) of interaction with the TM7-TM8 loop of the α subunit. This interaction is established early on in the protein assembly process and does not require S-S formation or glycosylation.[108] There are some indications that the β subunit also may interact with the α subunit transmembrane and cytoplasmic regions although the specifics of those interactions are not established clearly.[32]

Three-Dimensional Low- and High-Resolution Structures

Most of the earlier-described structural information has been obtained through the use of clever biochemical approaches that included proteolytic, chemical and mutational modification, molecular biological and expression techniques. Structure prediction algorithms and homologies to the known structure of Ca^{2+}ATPase have been used to fill in the gaps. Direct structural data for the Na^+,K^+-ATPase has been more difficult to obtain. The highest resolution structures for the Na^+,K^+-ATPase have subatomic resolutions of 9 to 11 Å.[110,111] At these resolutions we can see the outline of structural elements of the pump

Under the proper conditions some membrane proteins, when present in high concentration in the membrane, can organize into ordered 2-dimensional crystalline arrays that are visible by using electron microscopy. This order, when examined under the electron microscope, can produce a diffraction pattern that can be analyzed to give a structure of the diffracting particles. Usually the 2-dimensional arrays of proteins are in a planar sheet and a 3-dimensional structure can be reconstructed by diffracting the sheet as it is tilted at varied angles.[112,113] In some instances it is possible to have a helical array form a tubular structure and the cylindric structure of the tube provides multiple angles for the incident electron

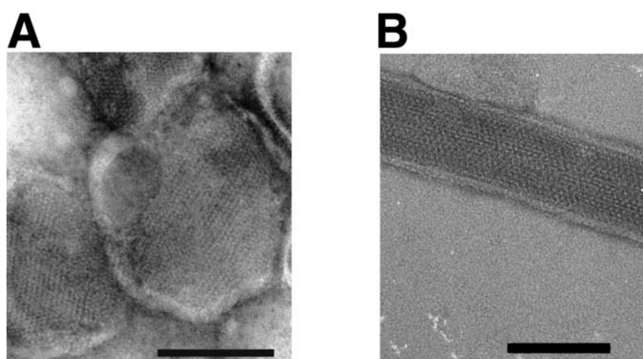

Figure 3 Ordered crystalline arrays of Na^+,K^+-ATPase from duck nasal salt glands. The crystals were generated by fixing the enzyme in an E_2 conformation. (A) Planar crystals obtained by incubating purified Na^+,K^+-ATPase in the presence of 1 mmol/L Na_3VO_4, 5 mmol/L $MgCl_2$, 5 mmol/L $CaCl_2$, and 10 mmol/L Tris-HCl (pH 7.5).[26] (B) Tubular crystals obtained by incubating purified Na^+,K^+-ATPase in the presence of 0.5 mmol/L Na_3VO_4, 10 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 0.5 mmol/L Ethylene glycol-bis(â-Aminoethylether)-N,N,N?,N?-Tetraacetic acid (EGTA), and 50 mmol/L imidazole pH 7.5.[110] The samples were negatively stained with uranyl acetate. The bars are 0.1 μm.

beam and thereby allows for the reconstruction of a 3-dimensional structure.[114] Fig. 3 shows crystalline arrays of the Na^+,K^+-ATPase fixed in the E_2 conformation produced from duck nasal salt glands (an organ enriched in α_1 Na^+,K^+-ATPase[26,115]) in both planar (Fig. 3A) and tubular (Fig. 3B) form. Three-dimensional reconstruction from the diffraction pattern produced by many such tubular crystals results in the structure shown in Fig. 4A.[110] From this structure, which has an 11 Å resolution, it is fairly easy to identify the cytoplasmic regions and domains discussed in the predicted topology model shown in Fig. 2. A similar analysis has been performed on the Ca^{2+}ATPase from sarcoplasmic reticulum[116,117] (Fig. 4B) and it is instructive to compare the structures. In Fig. 4C the Ca^{2+}ATPase and Na^+,K^+-ATPase structures are overlaid. The cytoplasmic regions for both pumps have very similar structures and this is in agreement with all of the predictive biochemical data. The regions where the overlap is the poorest can be explained by slightly different tilts in the N domains and sequence differences between the 2 ATPases. The Na^+,K^+-ATPase crystals had p_1 symmetry (1 $\alpha\beta$ protomer per unit cell) and crystallization contacts occurred between the N and P domains of adjacent protomers. There is a clear difference in the protein mass on the extracellular side of the membrane. The extra mass in the Na^+,K^+-ATPase undoubtedly can be attributed to the β subunit. It is not difficult to imagine that such a mass lying across the extracellular region of the α subunit transmembrane segments could affect transport function. Higher-density cut-off analysis of the data were consistent with the β subunit having a strong interaction with the TM7-TM8 loop and suggested a potential second site of interaction with the TM3-TM4 loop. An extra mass on the cytoplasmic side suggested that the transmembrane region of the β subunit passes close to the TM7 and TM10 segments of the α subunit. A recent 9 Å reconstruction from planar 2-dimensional crystals of Na^+,K^+-ATPase from kidney membranes gave a somewhat better resolution of the transmembrane region. This reconstruction also was consistent with a location of the β transmembrane region near TM7 and TM10 of the α subunit and suggested a possible location for the γ subunit transmembrane region near TM2 and TM9.[111] These crystals had p_2 symmetry and the α-α contacts were different from those observed in the tubular p_1 crystals.

Atomic Resolution Structure and Mechanism

Over the past few years great strides have been made in determining the atomic resolution structure of the Ca^{2+}ATPase. Earlier work of Toyoshima et al[118] produced 2 crystalline structures under conditions favoring the E_1 and E_2[119] conformations, which diffracted to resolutions of 2.6 and 3.1 Å, respectively. These structures revealed extremely large domain movements in the transition between E_1 and E_2 (for reviews see Toyoshima and Inesi[94] and Toyoshima et al[120]). More recently, a third crystal structure of the Ca^{2+}ATPase at 2.9 Å resolution gave details of the structure of the transition state of phosphoryl transfer between bound nucleotide and the P domain.[121,122] With this third structure it now is possible to visualize structurally and understand part of the transport reaction cycle described by the Albers-Post model as applied to Ca^{2+}ATPase. The 3 structures are shown in sequence in Fig. 5. The structures are rotated such that the TM7-TM10 helices are in approximately the same position. These helices essentially are fixed during this stage of the cycle. The figure on the left shows the structure obtained from the E_1Ca_2 crystal.[118] This structure has been described as the open conformation where the N and A domains are separated widely from the P domain and each other. The center structure was obtained using ATP analogs AMPPCP or adenosine diphosphate and AlF_x to fix conformation.[121,122] Both analogs give essentially the structure that is believed to be the conformation that exists just before the transfer of the γ phosphate of ATP to the aspartate residue of

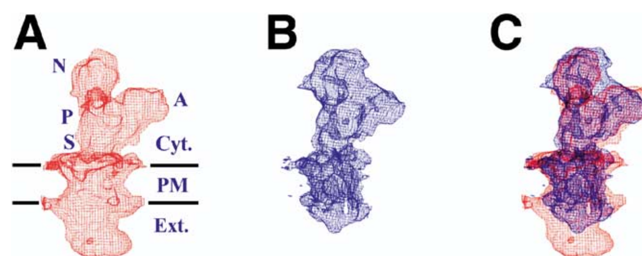

Figure 4 Comparison of 3-dimensional reconstructions of the Na^+,K^+-ATPase and Ca^{2+}ATPase obtained by cyroelectron microscopy. The reconstruction was performed on diffraction data obtained from tubular crystals. (A) Na^+,K^+-ATPase at approximately 11 Å resolution. The locations of the N, P, and A domains and the Stalk region (S) are indicated. Cyt, cytoplasmic space; pm, plasma membrane; Ext., extracellular space. (B) Ca^{2+}ATPase at approximately 8 Å resolution. (C) Structural alignment and overlay of the 2 ATPases. The data are adapted from Rice et al.[110] (Color version of figure is available online.)

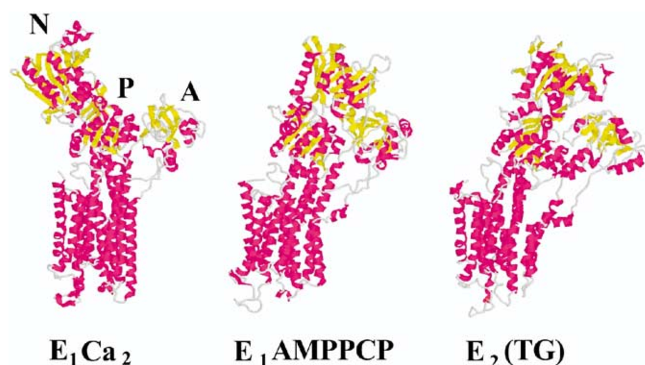

Figure 5 Comparison of high-resolution structures of the Ca^{2+}ATPase in varied conformations. E_1Ca_2 structure at 2.6 Å resolution.[118] The location of the N, P, and A domains are indicated. E_1AMPPCP at 2.6 Å resolution.[121,122] E_2(TG) at 3.1 Å resolution.[119] The structures are rotated so that each TM10 is roughly in the same position on the left side of the transmembrane helical bundle and is perpendicular to the plane of the membrane. The Protein Data Bank files were viewed and manipulated using Protein Explorer.[132] The figure was generated using high-resolution copies of screen images as described in the Protein Explorer protocols. (Color version of figure is available online.)

the DKTGT motif of the P domain. Comparison of E_1Ca_2 and E_1AMPPCP structures shows that on binding ATP, the N domain makes a large (~90°) inclination to the P domain. The N domain makes this transition essentially as a rigid body with only slight internal changes in the domain structure.[119] Concomitant with these movements, the A domain rotates approximately 30° on an axis approximately parallel to the membrane to a point where it actually makes contact with the N domain. The end result is a very compact or closed structure (E_1AMPPCP). The A domain is linked directly to the TM1-TM3 helices (see Fig.2), and its movement causes stress and rearrangement of these segments. TM3 shows a small movement with an approximately 20° bend in the stalk region. TM2 shows a substantial and complex movement whereby it is pulled out and tilted into the cytoplasm by a distance of approximately 1 helical turn. M1 shows a large movement as it is pulled out into the cytoplasm by nearly 2 helical turns and a small helical segment bends over and lies parallel on the membrane surface. This large movement and rearrangement of M1 and M2 essentially form a plug or close the access gate by locking in a conformation that prevents bound Ca^{2+} from diffusing back into the cytoplasm. The ions now are occluded from the cytoplasm. On the right side of Fig. 5 we see the E_2(TG) conformation. This structure was generated by using the Ca^{2+}ATPase inhibitor thapsigargin, which locks the enzyme in the E_2 conformation. In this conformation the P domain has an approximately 30° inclination compared with the E_1 conformation and this results in a large downward movement on TM4, which results in a disruption of the coordination of the ion binding sites, thereby lowering their affinity. The A domain in E_2 undergoes an additional rotation so that it is now approximately 110° rotated around an axis perpendicular to the membrane compared with its position in E_1Ca. This rotation blocks access to trypsin-sensitive proteolytic sites present in the E_1 conformation. The N domain in E_2 is not closed so tightly on the A domain as in E_1AMPPCP, where the ATP serves as a cleavable cross-linker between the N domain and the P domain. Concomitant with these domain movements are additional complex movements and tilts of TM1-TM6. For a clearer 3-dimensional animation of the structural changes the reader is encouraged to look at the online supplemental materials sections of each of these references.[118,119,121,122] Additionally, David Stokes has constructed a short movie that depicts the structural transitions in the context of steps in the Albers-Post model (http://saturn.med.nyu.edu/research/sb/stokeslab/research/E1-E2-confchange/).

With data from Fig. 5 we now have a good idea of how Ca^{2+} binds to its high-affinity site, is occluded, and how the site changes to low affinity for release on the lumenal side of the membrane. The close structural relationship between the Na^+,K^+-ATPase α subunit and the Ca^{2+}ATPase leads us to believe that similar domain movements occur in the analogous steps of the Na^+,K^+-ATPase reaction cycle.

Although there are no atomic resolution structures of the entire Na^+,K^+-ATPase, recently atomic resolution structures of recombinant Na^+,K^+-ATPase N domains have been obtained from diffraction of 3-dimensional crystals[123,124] and high-resolution nuclear magnetic resonance spectroscopy (NMR).[125] In general, the structures confirm the tertiary structural similarities between the N domains of Na^+,K^+-ATPase and Ca^{2+}ATPase, however, they also reveal some substantial secondary structural differences of undetermined significance. As mentioned earlier, under optimal conditions the Na^+,K^+-ATPase runs through its reaction cycle at speeds greater than 150 cycles per second. If the earlier-described high-resolution Ca^{2+}ATPase structures represent direct homologies of the Na^+,K^+-ATPase structure, the kinetic data suggest that the domain movements not only are enormous but also are very rapid. A recent analysis of the Ca^{2+}ATPase crystal structure proposes that the N domain is a highly mobile segment that moves on flexible-hinge regions largely as the result of random Brownian motion, which gets direction during ATP hydrolysis.[126] The recent NMR study of the isolated Na^+,K^+-ATPase N domain[125] concludes that N domain movement results from ATP-binding–induced changes in the bond angles of amino acids in the short hinge regions that normally connect the N and P domains but that, in this isolated preparation, are not connected to the P domain.

It seems likely that the mechanism of N-domain movement will be similar for both ATPases. However, there are significant differences between these transporters. The most obvious is the ion specificity and the 3 Na/2K stoichiometry of the Na^+,K^+-ATPase. Recent homology models of the Na^+,K^+-ATPase using the Ca^{2+}ATPase crystal structures as models estimate structural resolutions to explain Na^+ and K^+ binding specificity.[127,128] The other major difference, for which there is no homology, concerns the β and γ subunits. Fig. 4A shows the position of the β subunit over the extracellular surface of the Na^+,K^+-ATPase. Only a high-resolution structure of the entire protomer will permit clear de-

tailed insight into how this arrangement and that of the γ subunit potentially can affect function.

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References

1. Glynn IM: Sodium and potassium movements in human red cells. J Physiol 134:278-310, 1956
2. Skou JC: The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim Biophys Acta 23:394-401, 1957
3. Glynn IM: The Na^+, K^+ -transporting adenosine triphosphatase, in Martonosi A (ed): The Enzymes of Biological Membranes, Membrane Transport (vol 3, ed 2). New York, Plenum Press, 1985, pp 35-114
4. Skou JC: The identification of the sodium-potassium pump, in Grenthe I (ed): Nobel Lectures in Chemistry 1996-2000. Singapore, World Scientific Publishing Co., 2003, pp 179-194
5. Skou JC: Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. Physiol Rev 45:596-617, 1965
6. Hodgkin AL, Huxley AF: Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J Physiol 116:449-472, 1952
7. Crane RK: Comments and experiments on the kinetics of Na+ gradient-coupled glucose transport as found in rabbit jejunal brush-border membrane vesicles. Ann N Y Acad Sci 456:36-46, 1985
8. Hoffmann EK, Simonsen LO: Membrane mechanisms in volume and pH regulation in vertebrate cells. Physiol Rev 69:315-382, 1989
9. Jorgensen PL, Skou JC: Preparation of highly active (Na^+ + K^+)-ATPase from the outer medulla of rabbit kidney. Biochem Biophys Res Commun 37:39-46, 1969
10. Kaplan JH: Biochemistry of Na,K-ATPase. Annu Rev Biochem 71:511-535, 2002
11. Jorgensen PL, Hakansson KO, Karlish SJ: Structure and mechanism of Na,K-ATPase: Functional sites and their interactions. Annu Rev Physiol 65:817-849, 2003
12. Schatzmann HJ: [Cardiac glycosides as inhibitors of active potassium and sodium transport by erythrocyte membrane]. Helv Physiol Pharmacol Acta 11:346-354, 1953
13. Post RL, Jolly PC: The linkage of sodium, potassium, and ammonium active transport across the human erythrocyte membrane. Biochim Biophys Acta 25:118-128, 1957
14. Post RL, Hegyvary C, Kume S: Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. J Biol Chem 247:6530-6540, 1972
15. Albers RW, Fahn S, Koval GJ: The role of sodium ions in the activation of electrophorus electric organ adenosine triphosphatase. Proc Natl Acad Sci U S A 50:474-481, 1963
16. Siegel GJ, Koval GJ, Albers RW: Sodium-potassium-activated adenosine triphosphatase. IV. Characterization of the phosphoprotein formed from orthophosphate in the presence of ouabain. J Biol Chem 244:3264-3269, 1969
17. Albers RW, Koval GJ, Siegel GJ: Studies on the interaction of ouabain and other cardio-active steroids with sodium-potassium-activated adenosine triphosphatase. Mol Pharmacol 4:324-336, 1968
18. Fahn S, Koval GJ, Albers RW: Sodium-potassium-activated adenosine triphosphatase of Electrophorus electric organ. V. Phosphorylation by adenosine triphosphate-32P. J Biol Chem 243:1993-2002, 1968
19. Post RL, Kume S: Evidence for an aspartyl phosphate residue at the active site of sodium and potassium ion transport adenosine triphosphatase. J Biol Chem 248:6993-7000, 1973
20. Post RL, Sen AK, Rosenthal AS: A phosphorylated intermediate in adenosine triphosphate dependent sodium and potassium transport across kidney membranes. J Biol Chem 240:1437-1445, 1965
21. Post RL, Kume S, Tobin T, et al: Flexibility of an active center in sodium-plus-potassium adenosine triphosphate. J Gen Physiol 54: 306S-326S, 1969
22. Albers RW: Biochemical aspects of active transport. Annu Rev Biochem 36:727-756, 1967
23. Schneeberger A, Apell HJ: Ion selectivity of the cytoplasmic binding sites of the Na,K-ATPase: I. Sodium binding is associated with a conformational rearrangement. J Membr Biol 168:221-228, 1999
24. Rakowski RF, Bezanilla F, De Weer P, et al: Charge translocation by the Na/K pump. Ann N Y Acad Sci 834:231-243, 1997
25. Jorgensen PL: Conformational transitions in the alpha-subunit and ion occlusion. Soc Gen Physiol Ser 46:189-200, 1991
26. Martin DW, Sachs JR: Preparation of Na+,K+-ATPase with near maximal specific activity and phosphorylation capacity: Evidence that the reaction mechanism involves all of the sites. Biochemistry 38:7485-7497, 1999
27. DeTomaso AW, Jian Xie Z, Liu G, et al: Expression, targeting, and assembly of functional Na,K-ATPase polypeptides in baculovirus-infected insect cells. J Biol Chem 268:1470-1478, 1993
28. Xie Z, Wang Y, Liu G, et al: Similarities and differences between the properties of native and recombinant Na+/K+-ATPases. Arch Biochem Biophys 330:153-162, 1996
29. Gatto C, McLoud SM, Kaplan JH: Heterologous expression of Na(+)-K(+)-ATPase in insect cells: Intracellular distribution of pump subunits. Am J Physiol 281:C982-C992, 2001
30. Noguchi S, Mishina M, Kawamura M, et al: Expression of functional (Na+ + K+)-ATPase from cloned cDNAs. FEBS Lett 225:27-32, 1987
31. Horowitz B, Eakle KA, Scheiner-Bobis G, et al: Synthesis and assembly of functional mammalian Na,K-ATPase in yeast. J Biol Chem 265: 4189-4192, 1990
32. Geering K: The functional role of beta subunits in oligomeric P-type ATPases. J Bioenerg Biomembr 33:425-438, 2001
33. Koenderink JB, Swarts HG, Hermsen HP, et al: The beta-subunits of Na+,K+-ATPase and gastric H+,K+-ATPase have a high preference for their own alpha-subunit and affect the K+ affinity of these enzymes. J Biol Chem 274:11604-11610, 1999
34. Ackermann U, Geering K: Mutual dependence of Na,K-ATPase α- and β-subunits for correct posttranslational processing and intracellular transport. FEBS Lett 269:105-108, 1990
35. Lutsenko S, Kaplan JH: An essential role for the extracellular domain of the Na, K-ATPase β-subunit in cation occlusion. Biochemistry 32: 6737-6743, 1993
36. Eakle KA, Kabalin MA, Wang S-G, et al: The influence of β subunit structure on the stability of Na^+/K^+-ATPase complexes and interaction with K^+. J Biol Chem 269:6550-6557, 1994
37. Muller-Ehmsen J, Juvvadi P, Thompson CB, et al: Ouabain and substrate affinities of human Na(+)-K(+)-ATPase alpha(1)beta(1), alpha(2)beta(1), and alpha(3)beta(1) when expressed separately in yeast cells. Am J Physiol 281:C1355-C1364, 2001
38. Forbush B III, Kaplan JH, Hoffman JF: Characterization of a new photoaffinity derivative of ouabain: Labeling of the large polypeptide and of a proteolipid component of the Na, K- ATPase. Biochemistry 17:3667-3676, 1978
39. Collins JH, Forbush B III, Lane LK, et al: Purification and characterization of an (Na^++K^+)-ATPase proteolipid labeled with a photoaffinity derivative of ouabain. Biochim Biophys Acta 686:7-12, 1982
40. Collins JH, Leszyk J: The "gamma subunit" of Na,K-ATPase: A small, amphiphilic protein with a unique amino acid sequence. Biochemistry 26:8665-8668, 1987
41. Reeves AS, Collins JH, Schwartz A: Isolation and characterization of (Na,K)-ATPase proteolipid. Biochem Biophys Res Commun 95:1591-1598, 1980
42. Therien AG, Pu HX, Karlish SJ, et al: Molecular and functional studies of the gamma subunit of the sodium pump. J Bioenerg Biomembr 33:407-414, 2001

43. Therien AG, Blostein R: Mechanisms of sodium pump regulation. Am J Physiol 279:C541-C566, 2000
44. Farman N, Corthesy-Theulaz I, Bonvalet JP, et al: Localization of α-isoforms of Na^+-K^+-ATPase in rat kidney by in situ hybridization. Am J Physiol 260:C468-C474, 1991
45. Herrera VLM, Cova T, Sassoon D, et al: Developmental cell-specific regulation of Na^+-K^+-ATPase α_1-, α_2-, and α_3-isoform gene expression. Am J Physiol 266:C1301-C1312, 1994
46. Lingrel JB, Orlowski J, Shull MM, et al: Molecular genetics of Na,K-ATPase. Prog Nucleic Acid Res Mol Biol 38:37-89, 1990
47. Sweadner KJ: Isozymes of the Na+/K+-ATPase. Biochim Biophys Acta 988:185-220, 1989
48. Shull GE, Greeb J, Lingrel JB: Molecular cloning of three distinct forms of the Na+,K+-ATPase alpha-subunit from rat brain. Biochemistry 25:8125-8132, 1986
49. Woo AL, James PF, Lingrel JB: Characterization of the fourth alpha isoform of the Na,K-ATPase. J Membr Biol 169:39-44, 1999
50. Segall L, Javaid ZZ, Carl SL, et al: Structural basis for alpha1 versus alpha2 isoform-distinct behavior of the Na,K-ATPase. J Biol Chem 278:9027-9034, 2003
51. Segall L, Daly SE, Blostein R: Mechanistic basis for kinetic differences between the rat alpha 1, alpha 2, and alpha 3 isoforms of the Na,K-ATPase. J Biol Chem 276:31535-31541, 2001
52. Munzer JS, Daly SE, Jewell-Motz EA, et al: Tissue- and isoform-specific kinetic behavior of the Na, K-ATPase. J Biol Chem 269:16668-16676, 1994
53. Blanco G, Xie ZJ, Mercer RW: Functional expression of the α2 and α3 isoforms of the Na, K-ATPase in baculovirus-infected insect cells. Proc Natl Acad Sci U S A 90:1824-1828, 1993
54. Blanco G, Mercer RW: Isozymes of the Na-K-ATPase: Heterogeneity in structure, diversity in function. Am J Physiol 275:F633-F650, 1998
55. Klingenberg M: Membrane protein oligomeric structure and transport function. Nature 290:449-454, 1981
56. Kyte J: Molecular considerations relevant to the mechanism of active transport. Nature 292:201-204, 1981
57. Stryer L: Membrane transport, in Biochemistry (ed 4). New York, W.H. Freeman, 1995, pp 291-311
58. MacKinnon R: Potassium channels. FEBS Lett 555:62-65, 2003
59. Taniguchi K, Kaya S, Abe K, et al: The oligomeric nature of Na/K-transport ATPase. J Biochem (Tokyo) 129:335-342, 2001
60. Froehlich JP, Taniguchi K, Fendler K, et al: Complex kinetic behavior in the Na,K- and Ca-ATPases. Evidence for subunit-subunit interactions and energy conservation during catalysis. Ann N Y Acad Sci 834:280-296, 1997
61. Schoner W, Thönges D, Hamer E, et al: Is the sodium pump a functional dimer, in Bamberg E, Schoner W (eds): The Sodium Pump: Structure Mechanism, Hormonal Control and its Role in Disease. New York, Springer, 1994, pp 332-341
62. Moczydlowski EG, Fortes PA: Inhibition of sodium and potassium adenosine triphosphatase by 2′,3′-O-(2,4,6-trinitrocyclohexadienylidene) adenine nucleotides. Implications for the structure and mechanism of the Na:K pump. J Biol Chem 256:2357-2366, 1981
63. Moczydlowski EG, Fortes PA: Characterization of 2′,3′-O-(2,4,6- trinitrocyclohexadienylidine)adenosine 5′-triphosphate as a fluorescent probe of the ATP site of sodium and potassium transport adenosine triphosphatase. Determination of nucleotide binding stoichiometry and ion-induced changes in affinity for ATP. J Biol Chem 256:2346-2356, 1981
64. Martin DW, Sachs JR: Ligands presumed to label high affinity and low affinity ATP binding sites do not interact in an (alpha beta)2 diprotomer in duck nasal gland Na+,K+-ATPase, nor do the sites coexist in native enzyme. J Biol Chem 275:24512-24517, 2000
65. Jorgensen PL: Purification of Na+,K+-ATPase: Enzyme sources, preparative problems, and preparation from mammalian kidney. Methods Enzymol 156:29-43, 1988
66. Jorgensen PL: Purified renal Na+/K+-ATPase; subunit structure and structure-function relationships of the N-Terminus of the alpha1 subunit, in Bamberg E, Schoner W (eds): The Sodium Pump: Structure Mechanism, Hormonal Control and its Role in Disease. New York, Springer, 1994, pp 297-308
67. Periyasamy SM, Huang W-H, Askari A: Subunit associations of $(Na^+ + K^+)$-dependent adenosine triphosphatase: Chemical cross-linking studies. J Biol Chem 258:9878-9885, 1983
68. Martin DW, Sachs JR: Cross-linking of the erythrocyte (Na^+,K^+)-ATPase. Chemical cross-linkers induce Bamberg E, Schoner W (eds), -subunit-band 3 heterodimers and do not induce α-subunit homodimers. J Biol Chem 267:23922-23929, 1992
69. Amler E, Abbott A, Ball WJ Jr: Structural dynamics and oligomeric interactions of Na^+, K^+-ATPase as monitored using fluorescence energy transfer. Biophys J 61:553-568, 1992
70. Linnertz H, Urbanova P, Obsil T, et al: Molecular distance measurements reveal an (alpha beta)2 dimeric structure of Na+/K+-ATPase. High affinity ATP binding site and K+- activated phosphatase reside on different alpha-subunits. J Biol Chem 273:28813-28821, 1998
71. Martin DW, Marecek J, Scarlata S, et al: Alphabeta protomers of Na+,K+-ATPase from microsomes of duck salt gland are mostly monomeric: Formation of higher oligomers does not modify molecular activity. Proc Natl Acad Sci U S A 97:3195-3200, 2000
72. Brotherus JR, Jacobsen L, Jorgensen PL: Soluble and enzymatically stable $(Na^+ + K^+)$-ATPase from mammalian kidney consisting predominantly of protomer $\alpha\beta$-units. Biochim Biophys Acta 731:290-303, 1983
73. Martin DW: Active unit of solubilized sarcoplasmic reticulum calcium adenosine triphosphatase: An active enzyme centrifugation analysis. Biochemistry 22:2276-2282, 1983
74. Ward DG, Cavieres JD: Solubilized $\alpha\beta$ Na,K-ATPase remains protomeric during turnover yet shows apparent negative cooperativity toward ATP. Proc Natl Acad Sci U S A 90:5332-5336, 1993
75. Craig WS: Determination of the distribution of sodium and potassium ion activated adenosine triphosphatase among the various oligomers formed in solutions of nonionic detergents. Biochemistry 21:2667-2674, 1982
76. Craig WS: Monomer of sodium and potassium ion activated adenosine triphosphatase displays complete enzymatic function. Biochemistry 21:5707-5717, 1982
77. Sachs JR: The role of $(\alpha\beta)$ protomer interaction in determining functional characteristics of red cell Na,K-ATPase. Biochim Biophys Acta 1193:199-211, 1994
78. Blanco G, Koster JC, Mercer RW: The α subunit of the Na,K-ATPase specifically and stably associates into oligomers. Proc Natl Acad Sci U S A 91:8542-8546, 1994
79. Koster JC, Blanco G, Mercer RW: A cytoplasmic region of the Na,K-ATPase α-subunit is necessary for specific α/α association. J Biol Chem 270:14332-14339, 1995
80. Costa CJ, Gatto C, Kaplan JH: Interactions between Na,K-ATPase alpha-subunit ATP-binding domains. J Biol Chem 278:9176-9184, 2003
81. Laughery M, Todd M, Kaplan JH: Oligomerization of the Na,K-ATPase in cell membranes. J Biol Chem 279:36339-36348, 2004
82. Lutsenko S, Kaplan JH: Organization of P-type ATPases: Significance of structural diversity. Biochemistry 34:15607-15613, 1995
83. Moller JV, Juul B, Le Maire M: Structural organization, ion transport, and energy transduction of P-type ATPases. Biochim Biophys Acta 1286:1-51, 1996
84. Axelsen KB, Palmgren MG: Evolution of substrate specificities in the P-type ATPase superfamily. J Mol Evol 46:84-101, 1998
85. Palmgren MG, Axelsen KB: Evolution of P-type ATPases. Biochim Biophys Acta 1365:37-45, 1998
86. Aravind L, Galperin MY, Koonin EV: The catalytic domain of the P-type ATPase has the haloacid dehalogenase fold. Trends Biochem Sci 23:127-129, 1998
87. Blanco G, Koster JC, Sánchez G, et al: Kinetic properties of the $\alpha2\beta1$ and $\alpha2\beta2$ isozymes of the Na,K-ATPase. Biochemistry 34:319-325, 1995
88. Karlish SJD, Goldshleger R, Jorgensen PL: Location of Asn^{831} of the α chain of Na/K-ATPase at the cytoplasmic surface. Implication for topological models. J Biol Chem 268:3471-3478, 1993

89. Hu YK, Kaplan JH: Site-directed chemical labeling of extracellular loops in a membrane protein. The topology of the Na,K-ATPase alpha-subunit. J Biol Chem 275:19185-19191, 2000
90. Karlish SJD, Goldshleger R, Stein WD: A 19-kDa C-terminal tryptic fragment of the α chain of Na/K-ATPase is essential for occlusion and transport of cations. Proc Natl Acad Sci U S A 87:4566-4570, 1990
91. Capasso JM, Hoving S, Tal DM, et al: Extensive digestion of Na^{+},K^{+}-ATPase by specific and nonspecific proteases with preservation of cation occlusion sites. J Biol Chem 267:1150-1158, 1992
92. Schwappach B, Stürmer W, Apell H-J, et al: Binding of sodium ions and cardiotonic steroids to native and selectively trypsinized Na,K pump, detected by charge movements. J Biol Chem 269:21620-21626, 1994
93. Shainskaya A, Schneeberger A, Apell HJ, et al: Entrance port for Na(+) and K(+) ions on Na(+),K(+)-ATPase in the cytoplasmic loop between trans-membrane segments M6 and M7 of the alpha subunit. Proximity of the cytoplasmic segment of the beta subunit. J Biol Chem 275:2019-2028, 2000
94. Toyoshima C, Inesi G: Structural basis of ion pumping by Ca^{2+}ATPase of the sarcoplasmic reticulum. Annu Rev Biochem 73: 269-292, 2004
95. Chibalin AV, Pedemonte CH, Katz AI, et al: Phosphorylation of the catalytic alpha-subunit constitutes a triggering signal for Na+,K+-ATPase endocytosis. J Biol Chem 273:8814-8819, 1998
96. Chibalin AV, Ogimoto G, Pedemonte CH, et al: Dopamine-induced endocytosis of Na+,K+-ATPase is initiated by phosphorylation of Ser-18 in the rat alpha subunit and is responsible for the decreased activity in epithelial cells. J Biol Chem 274:1920-1927, 1999
97. Daly SE, Lane LK, Blostein R: Functional consequences of amino-terminal diversity of the catalytic subunit of the Na,K-ATPase. J Biol Chem 269:23944-23948, 1994
98. Segall L, Lane LK, Blostein R: New insights into the role of the N terminus in conformational transitions of the Na,K-ATPase. J Biol Chem 277:35202-35209, 2002
99. Jorgensen PL, Jorgensen JR, Pedersen PA: Role of conserved TGDGVND-loop in Mg2+ binding, phosphorylation, and energy transfer in Na,K-ATPase. J Bioenerg Biomembr 33:367-377, 2001
100. Pedersen PA, Jorgensen JR, Jorgensen PL: Importance of conserved alpha -subunit segment 709GDGVND for Mg2+ binding, phosphorylation, and energy transduction in Na,K-ATPase. J Biol Chem 275: 37588-37595, 2000
101. Pierre SV, Duran MJ, Carr DL, et al: Structure/function analysis of Na(+)-K(+)-ATPase central isoform-specific region: Involvement in PKC regulation. Am J Physiol 283:F1066-F1074, 2002
102. Done SC, Leibiger IB, Efendiev R, et al: Tyrosine 537 within the Na+,K+-ATPase alpha-subunit is essential for AP-2 binding and clathrin-dependent endocytosis. J Biol Chem 277:17108-17111, 2002
103. Pedersen PA, Nielsen JM, Rasmussen JH, et al: Contribution to Tl+, K+, and Na+ binding of Asn776, Ser775, Thr774, Thr772, and Tyr771 in cytoplasmic part of fifth transmembrane segment in alpha-subunit of renal Na,K-ATPase. Biochemistry 37:17818-17827, 1998
104. Lingrel JB, Arguello JM, Van Huysse J, et al: Cation and cardiac glycoside binding sites of the Na,K-ATPase. Ann N Y Acad Sci 834:194-206, 1997
105. Argüello JM, Lingrel JB: Substitutions of serine 775 in the α subunit of the Na,K- ATPase selectively disrupt K^{+} high affinity activation without affecting Na^{+} interaction. J Biol Chem 270:22764-22771, 1995
106. Palasis M, Kuntzweiler TA, Arguello JM, et al: Ouabain interactions with the H5-H6 hairpin of the Na,K-ATPase reveal a possible inhibition mechanism via the cation binding domain. J Biol Chem 271: 14176-14182, 1996
107. Kuntzweiler TA, Arguello JM, Lingrel JB: Asp804 and Asp808 in the transmembrane domain of the Na,K-ATPase alpha subunit are cation coordinating residues. J Biol Chem 271:29682-29687, 1996
108. Colonna T, Kostich M, Hamrick M, et al: Subunit interactions in the sodium pump. Ann N Y Acad Sci 834:498-513, 1997
109. Colonna TE, Huynh L, Fambrough DM: Subunit interactions in the Na,K-ATPase explored with the yeast two-hybrid system. J Biol Chem 272:12366-12372, 1997
110. Rice WJ, Young HS, Martin DW, et al: Structure of Na+,K+-ATPase at 11-A resolution: comparison with Ca2+-ATPase in E1 and E2 states. Biophys J 80:2187-2197, 2001
111. Hebert H, Purhonen P, Vorum H, et al: Three-dimensional structure of renal Na,K-ATPase from cryo-electron microscopy of two-dimensional crystals. J Mol Biol 314:479-494, 2001
112. DeRosier DJ, Klug A: Reconstruction of three-dimensional structures from electron micrographs. Nature 217:130-134, 1968
113. Amos LA, Henderson R, Unwin PN: Three-dimensional structure determination by electron microscopy of two-dimensional crystals. Prog Biophys Mol Biol 39:183-231, 1982
114. DeRosier DJ, Moore PB: Reconstruction of three-dimensional images from electron micrographs of structures with helical symmetry. J Mol Biol 52:355-369, 1970
115. Boldyrev AA, Lopina OD, Kenney M, et al: Characterization of the subunit isoforms of duck salt gland Na/K adenosine triphosphatase. Biochem Biophys Res Commun 216:1048-1053, 1995
116. Toyoshima C, Sasabe H, Stokes DL: Three-dimensional cryo-electron microscopy of the calcium ion pump in the sarcoplasmic reticulum membrane. Nature 362:469-471, 1993
117. Zhang P, Toyoshima C, Yonekura K, et al: Structure of the calcium pump from sarcoplasmic reticulum at 8-A resolution. Nature 392: 835-839, 1998
118. Toyoshima C, Nakasako M, Nomura H, et al: Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 A resolution. Nature 405:647-655, 2000
119. Toyoshima C, Nomura H: Structural changes in the calcium pump accompanying the dissociation of calcium. Nature 418:605-611, 2002
120. Toyoshima C, Nomura H, Sugita Y: Structural basis of ion pumping by Ca(2+)-ATPase of sarcoplasmic reticulum. FEBS Lett 555:106-110, 2003
121. Sorensen TL, Moller JV, Nissen P: Phosphoryl transfer and calcium ion occlusion in the calcium pump. Science 304:1672-1675, 2004
122. Toyoshima C, Mizutani T: Crystal structure of the calcium pump with a bound ATP analogue. Nature 430:529-535, 2004
123. Haue L, Pedersen PA, Jorgensen PL, et al: Cloning, expression, purification and crystallization of the N-domain from the alpha(2) subunit of the membrane-spanning Na,K-ATPase protein. Acta Crystallogr D Biol Crystallogr 59:1259-1261, 2003
124. Hakansson KO: The crystallographic structure of Na,K-ATPase N-domain at 2.6A resolution. J Mol Biol 332:1175-1182, 2003
125. Hilge M, Siegal G, Vuister GW, et al: ATP-induced conformational changes of the nucleotide-binding domain of Na,K-ATPase. Nat Struct Biol 10:468-474, 2003
126. Xu C, Rice WJ, He W, et al: A structural model for the catalytic cycle of Ca(2+)-ATPase. J Mol Biol 316:201-211, 2002
127. Ogawa H, Toyoshima C: Homology modeling of the cation binding sites of Na+K+-ATPase. Proc Natl Acad Sci U S A 99:15977-15982, 2002
128. Hakansson KO, Jorgensen PL: Homology modeling of Na,K-ATPase: A putative third sodium binding site suggests a relay mechanism compatible with the electrogenic profile of Na+ translocation. Ann N Y Acad Sci 986:163-167, 2003
129. Glynn IM: All hands to the sodium pump. J Physiol (Lond) 462:1-30, 1993
130. Cuff JA, Barton GJ: Application of multiple sequence alignment profiles to improve protein secondary structure prediction. Proteins 40: 502-511, 2000
131. Bond CS: TopDraw: A sketchpad for protein structure topology cartoons. Bioinformatics 19:311-312, 2003
132. Martz E: Protein Explorer: Easy yet powerful macromolecular visualization. Trends Biochem Sci 27:107-109, 2002