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 αβ 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 αβ protomer is the minimal functional unit needed to perform the Albers-Post reaction cycle. However, higher orders of aggregation [(αβ)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 Ca²⁺-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 Ca²⁺-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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KEYWORDS Na⁺,K⁺-ATPase, Na, K pump, structure, Na(+)-K(+)-exchanging ATPase
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\(^5\) 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.\(^23,24\) 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\(_D\) ~ 50 mmol/L) whereas it binds with a reduced affinity to the E\(_2\) state (K\(_D\) ~ 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.\(^26\)

**Na\(^+\),K\(^+\)-ATPase Structure**

**Subunits**

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

![Figure 1](Image)
tions and aspects of the α subunit. However, efforts to separate the α and β subunits result in loss of function. 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. Other studies have suggested that it also may influence K⁺ interactions and Na⁺,K⁺-ATPase stability. A third subunit, characterized as the γ subunit, also has been identified in kidney tissue. This is a small protein of approximately 6,500 to 7,500 molecular weight and appears to belong to the FYXD family of regulatory peptides, 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 α₁ and β₁ isoform and this is the predominant isoform found in kidney 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 × 10⁶ copies per cell and there was no evidence of α-α cross-links. 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. 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 αβ protomer was unchanged, showing that individual αβ protomers were fully active in natural membranes. The Na⁺,K⁺-ATPase can be solubilized out of the membrane to monomeric form with detergents and still maintain ATPase activity. Hydrodynamic analysis of this activity (by using a technique called active enzyme centrifugation) revealed that the activity originated from a monomeric (αβ) species. Taken together, these and other studies have shown the functionality of the αβ 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 αβ 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. 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). 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. Earlier studies showed that mutants lacking this region did not associate with wild-type Na⁺,K⁺-ATPase. 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. 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 Oligomer Question: αβ, (αβ)₂, (αβ)ₙ?

It is clear that α and β units exist in equimolar amount in the membrane and that the pump is an αβ heterodimer. 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 αβ 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. 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) and indeed many ion channel proteins have been shown to form their functional channel through oligomerization. In dealing with the Na⁺,K⁺-ATPase specifically, there has been an array of data that were interpreted as indicating that the functional unit was an (αβ)₂; or higher-order oligomer or was explained most easily by an oligomeric structure.

Highly purified forms of membrane-bound Na⁺,K⁺-ATPase display functional stoichiometries, indicating that all αβ protomers are active simultaneously. 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/μ²) showed that the Na⁺,K⁺-ATPase units were close enough to be cross-linked by chemical reagents. 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 × 10⁶ copies per cell and...
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-arginine-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 motif82-85 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 Ca²⁺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.

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 Mₐ of only 19 kDa. Amazingly, this 19-kDa 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 Ca²⁺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 Ca²⁺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 E1P form of the enzyme.94 The N-terminal chain of the Na+/K+-ATPase has an additional approximately 26 amino acids compared with the Ca2+-ATPase and there is evidence that this may have regulatory or functional significance.99,100

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 ATPases83 and this region in conjunction with interactions with other transmembrane segments (TM5, TM6, and TM8 in the Ca2+-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 Ca2+-ATPase and there is speculation that a similar unwound section containing the sequence iso-leucine-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 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 E2 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 Ca2+-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.12

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 Ca2+-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
membrane. The extra mass in the Na

Figure 3 Ordered crystalline arrays of Na⁺,K⁺-ATPase from duck nasal salt glands. The crystals were generated by fixing the enzyme in an E₂ conformation. (A) Planar crystals obtained by incubating purified Na⁺,K⁺-ATPase in the presence of 1 mmol/L Na₃VO₄, 5 mmol/L MgCl₂, 5 mmol/L CaCl₂, and 10 mmol/L Tris-HCl (pH 7.5).⁴⁶ (B) Tubular crystals obtained by incubating purified Na⁺,K⁺-ATPase in the presence of 0.5 mmol/L Na₃VO₄, 10 mmol/L KCl, 2.5 mmol/L MgCl₂, 0.5 mmol/L Ethylene glycol-bis(α-Aminoethyl ether)-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.

Atomic Resolution
Structure and Mechanism

Over the past few years great strides have been made in determining the atomic resolution structure of the Ca²⁺-ATPase. Earlier work of Toyoshima et al¹¹⁸ produced 2 crystalline structures under conditions favoring the E₁ and E₂¹¹⁰ conformations, which diffracted to resolutions of 2.6 and 3.1 Å, respectively. These structures revealed extremely large domain movements in the transition between E₁ and E₂ (for reviews see Toyoshima and Inesi⁹⁴ and Toyoshima et al¹²⁰). More recently, a third crystal structure of the Ca²⁺-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 is now possible to visualize structurally and understand part of the transport reaction cycle described by the Albers-Post model as applied to Ca²⁺-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 E₁Ca₂ 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 AlFx 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 Ca²⁺-ATPase obtained by cryoelectron microscopy. The reconstruction was performed on diffraction data obtained from planar 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) Ca²⁺-ATPase at approximately 8 Å resolution. (C) Structural alignment and overlay of the 2 ATPases. The data are adapted from Rice et al.¹²² (Color version of figure is available online.)
the DKTGT motif of the P domain. Comparison of E1Ca2 and E1AMPPCP 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.110 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 (E1AMPPCP). 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 the Na+/H+ 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 crystals123,124 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 Ca2+-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 Ca2+-ATPase structures represent direct homologies of the Na+,K+-ATPase structure, the kinetic data suggest that the domain movements not only are numerous but also are very rapid. A recent analysis of the Ca2+-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 domain125 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 Na2K stoichiometry of the Na+,K+-ATPase. Recent homology models of the Na+,K+-ATPase using the Ca2+-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.

Acknowledgments
The author thanks Dr. John Sachs for his critical review of the manuscript and for many useful discussions. The author also thanks Drs. William Rice and David Stokes for the electron micrograph of tubular crystal, files of 3-dimensional reconstructions, and advice on figure preparation.

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