Minireview

Structure–function relationships of cation translocation by Ca^{2+}- and Na^+,K^+-ATPases studied by site-directed mutagenesis

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Abstract Site-directed mutagenesis studies of the sarcoplasmic reticulum Ca^{2+}-ATPase have pinpointed five amino acid residues that are essential to Ca^{2+} occlusion, and these residues have been assigned to different parts of a Ca^{2+} binding pocket with channel-like structure. Three of the homologous Na^+,K^+-ATPase residues have been shown to be important for binding of cytoplasmic Na^+ at transport sites. In addition, three of the above mentioned Ca^{2+}-ATPase residues appear to participate in the countertransport of H^+, and two of the Na^+,K^+-ATPase residues to participate in the countertransport of K^+. Residues involved in energy transducing conformational changes have also been identified by mutagenesis. In the Ca^{2+}-ATPase, ATP hydrolysis is uncoupled from Ca^{2+} transport following mutation of a tyrosine residue located at the top of transmembrane segment M5. This tyrosine, present also in the Na^+,K^+-ATPase, may play a critical role in closing the gate to a transmembrane channel.

Key words: P-type ATPase; Calcium; Sodium; Potassium; Ion pump; Ion channel

1. Introduction

Ca^{2+}-ATPases and Na^+,K^+-ATPases of P-type mediate active ion transport coupled to ATP hydrolysis through formation and breakdown of a phosphoryl aspartyl enzyme intermediate existing in at least two major conformational states E1P and E2P (Fig. 1). The Ca^{2+}-ATPase of sarcoplasmic reticulum** is composed of a single 110 kDa peptide chain [1] which is homologous to the α-subunit of the $\alpha\beta$-protomer of Na^+,K^+-ATPase. A common structural model has been proposed for the Ca^{2+}-ATPase peptide and the Na^+,K^+-ATPase α-subunit, in which the cytoplasmic subdomains are joined through a 'stalk' region to a membrane buried part consisting of 8 or 10 transmembrane segments ([2,3] and Fig. 2).

Modern molecular biology has made it feasible to examine the roles of individual amino acid residues by site-directed mutagenesis. To date, more than 200 different point mutants of the sarcoplasmic reticulum Ca^{2+}-ATPase have been expressed transiently in mammalian cell lines and analysed for function by a panel of assays comprising measurement of the rates of Ca^{2+} uptake in the microsomal vesicles and ATP hydrolysis, phosphorylation from ATP or P_i, as well as Ca^{2+} occlusion stabilized with CrATP [4–31]. The mutagenesis experiments have pinpointed 40–50 functionally important amino acid residues of the Ca^{2+}-ATPase, and it has been possible to assign the functions of these residues to specific partial reaction steps as indicated in Fig. 2.

Similar site-directed mutagenesis studies of the Na^+,K^+-ATPase are rapidly progressing [32–41]. Mammalian cell lines can also be used for expression of the Na^+,K^+-ATPase cDNA, albeit with the complication that such cells contain considerable amounts of endogenous Na^+,K^+-ATPase. To circumvent this problem, non-rodent cell lines are transfected with cDNA encoding the ouabain-resistant rat Na^+,K^+-ATPase α1-isoform, so that stable transfectants can be selected in the presence of ouabain which inhibits only the endogenous non-rodent Na^+,K^+-ATPase [32,34].

The mutagenesis studies have made it clear that the cation binding structure is located in the transmembrane sector of the protein well separated from the catalytic ATP binding and phosphorylation site in the cytoplasmic portion. The energy coupling between scalar and vectorial reactions must therefore require long-distance communication between the cytoplasmic and transmembrane protein segments. The present review summarizes information on the residues involved in Ca^{2+} binding in the Ca^{2+}-ATPase, and discusses this in relation to the recent work on Na^+,K^+-ATPase mutants. In addition we will focus on residues involved in the coupling of ATP hydrolysis to ion transport. The mutagenesis data add to the growing evidence that ion pumps can be regarded as channel-like structures equipped with energy dependent locks and gate controls.

2. The Ca^{2+} binding structure of the Ca^{2+}-ATPase

The two calcium ions to be translocated are initially recognized and bound at cytoplasmically facing enzyme sites displaying high apparent affinity with a $K_{0.5}$ near 1 μM. Simultaneously with the binding, or immediately following this process, the two calcium ions are transferred into an occluded state with no access to the aqueous medium on either side of the membrane [42–44]. When both calcium ions are bound, the enzyme can be phosphorylated from ATP [45] and is thereby activated for transport of the bound Ca^{2+} (Fig. 1). As indicated in Fig. 2 (open circles), a number of mutations to residues at or near

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Abbreviations: CrATP, β,γ-bidentate chromium(III) complex of ATP; $K_{0.5}$, concentration giving half maximal activation; M1-M10, putative transmembrane segments numbered from the NH_2-terminal end of the peptide; wild type, the non-mutated form of the exogenous enzyme expressed following transfection.

**The Ca^{2+}-ATPases treated in this article are those of sarco(endo)-plasmic reticulum, *not* the plasma membrane Ca^{2+}-ATPases.

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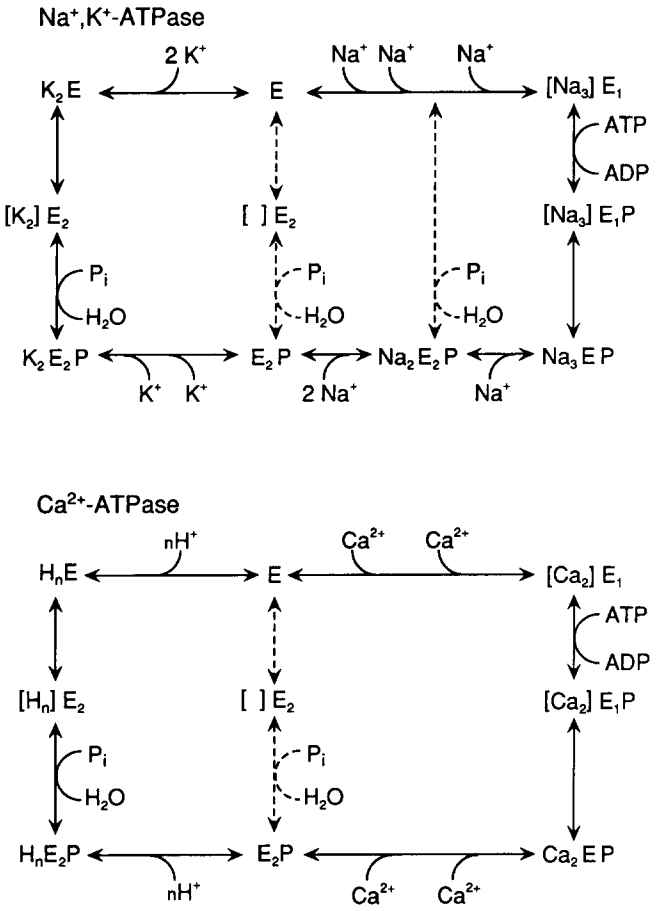

Fig. 1. Simplified reaction cycles of the Ca^{2+}- and Na^+,K^+-ATPases showing binding and dissociation steps for the cations and the phosphoryl group, as well as transitions between different conformational states of the enzymes. The cytoplasmic side of the membrane is upward and the extracytoplasmic side downward. Brackets indicate occluded states of the cation binding sites. E1 indicates the form with chemical specificity for reaction with ATP/ADP. E2 indicates the form with chemical specificity for reaction with P_i/H_2O. Unlabelled forms are those that are difficult to stabilize and therefore not straightforward to define experimentally. A tentative H^+-countertransport limb is shown for the Ca^{2+}-ATPase according to recent findings ($n = 2$ or 3). Dashed lines indicate alternative reaction pathways that may be used under certain conditions.

the transmembrane sector reduced the apparent affinity for Ca^{2+} measured by Ca^{2+} titration of Ca^{2+} transport or phosphorylation from ATP. These residues must in some way be important for the binding of cytoplasmic Ca^{2+} at the high-affinity sites. A more direct measurement of the function of the Ca^{2+} sites is obtained by studying Ca^{2+} occlusion in the presence of the non-phosphorylating substrate analog CrATP, which stabilizes the occluded state of the wild-type enzyme [18,44]. Five residues with oxygen containing side chains, Glu^{309}, Glu^{771}, Asn^{796}, Thr^{799}, and Asp^{800}, located in transmembrane segments M4, M5, and M6, appear to have a unique importance, since it was impossible to alter one of these residues without a complete loss of the ability to occlude Ca^{2+} as measured at Ca^{2+} concentrations up to 10 mM [18,27,29]. The side chain oxygens of these residues are thus strong candidates for Ca^{2+} ligands in the occluded state.

An important property of the Ca^{2+} binding sites in the state with access from the cytoplasmic side is that the two calcium ions bind and dissociate in an ordered (consecutive) manner [42–46]. The structural correlate is thought to be a single-file arrangement of the sites, one on top of the other [43], as shown in Fig. 3. It has been of major interest to try by mutagenesis to distinguish between the respective amino acid residues providing side chains for coordination of Ca^{2+} at the two sites. To this end, it has been very useful to compare the different mutants with respect to their Ca^{2+} dependencies of phosphorylation from ATP and P_i [16,17,27,29]. The studies of Ca^{2+} inhibition of 'backdoor' phosphorylation from P_i complement the studies of Ca^{2+} activation of phosphorylation from ATP because the binding of only the first calcium ion in the sequence is sufficient to prevent phosphorylation from P_i [46], whereas phosphorylation from ATP requires that both calcium sites are occupied [45]. Three classes of 'Ca^{2+} site mutants' were distinguished. In mutants $Glu^{309} \rightarrow Gln$, $Glu^{309} \rightarrow Lys$, and $Asn^{796} \rightarrow Ala$, Ca^{2+} inhibited phosphorylation from P_i with almost the same apparent affinity as in the wild type [16,17,27], indicating that the particular site binding the first calcium ion in the sequence (the 'deeper' site) was functioning normally despite the inability of these mutants to occlude Ca^{2+} and phosphorylate from ATP at Ca^{2+} concentrations up to 10 mM. By contrast, mutations to residues Glu^{771} and Thr^{799} reduced the apparent Ca^{2+} affinities measured in the two phosphorylation assays to the same extent, suggesting that the deeper site was disturbed [17,27,29]. Finally, the $Asp^{800} \rightarrow Asn$ mutation appeared to hit both Ca^{2+} sites since the apparent Ca^{2+} affinity was reduced in either phosphorylation assay but to different extents [17,27]. The phosphorylation data have led us to assign the residues with oxygen containing side chains to the two Ca^{2+} sites in the E1 form as shown in Fig. 3 (left side). The fact that at least one calcium ion was bound with normal affinity but not occluded in mutants with alterations to Glu^{309} and Asn^{796} adds further evidence to the single-file arrangement of the binding sites since it appears that the superficial Ca^{2+} site must be occupied and its gate closed for occlusion of the deeper site to occur.

3. Cytoplasmically facing Na^+ sites in Na^+,K^+-ATPase

The residues Glu^{309}, Glu^{771}, Asn^{796}, Thr^{799}, and Asp^{800} shown to be important for Ca^{2+} occlusion in the Ca^{2+}-ATPase have homologous counterparts Glu^{329}, Glu^{781}, Asp^{806}, Thr^{809}, and Asp^{810} in the Na^+,K^+-ATPase [47] (numbering referring to the α1-isoform of rat). Mutants with alterations to these residues have been examined using ouabain selection methodology. While mutants $Glu^{329} \rightarrow Ala$, $Glu^{329} \rightarrow Asp$, $Glu^{781} \rightarrow Leu$, $Asp^{806} \rightarrow Leu$, $Asp^{806} \rightarrow Asn$, $Asp^{806} \rightarrow Glu$, $Asp^{810} \rightarrow Asn$, and $Asp^{810} \rightarrow Leu$ were unable to confer ouabain resistance to the cells and therefore inactive [39,40], mutation $Glu^{329} \rightarrow Leu$ [39] as well as mutations $Glu^{329} \rightarrow Gln$, $Glu^{781} \rightarrow Ala$, and $Thr^{809} \rightarrow Ala$ [38,40] were compatible with cell growth. The functional analysis of the latter mutants summarized in Table 1 demonstrated a reduction of the apparent affinity for cytoplasmic Na^+ in accordance with a role for these residues in Na^+ binding/occlusion similar to the role played by their homologous counterparts in the Ca^{2+}-ATPase [38,40]. Oligomycin, known to promote occlusion of Na^+, was moreover shown to be required to obtain complete phosphorylation of the $Glu^{781} \rightarrow Ala$ mutant from ATP [40].

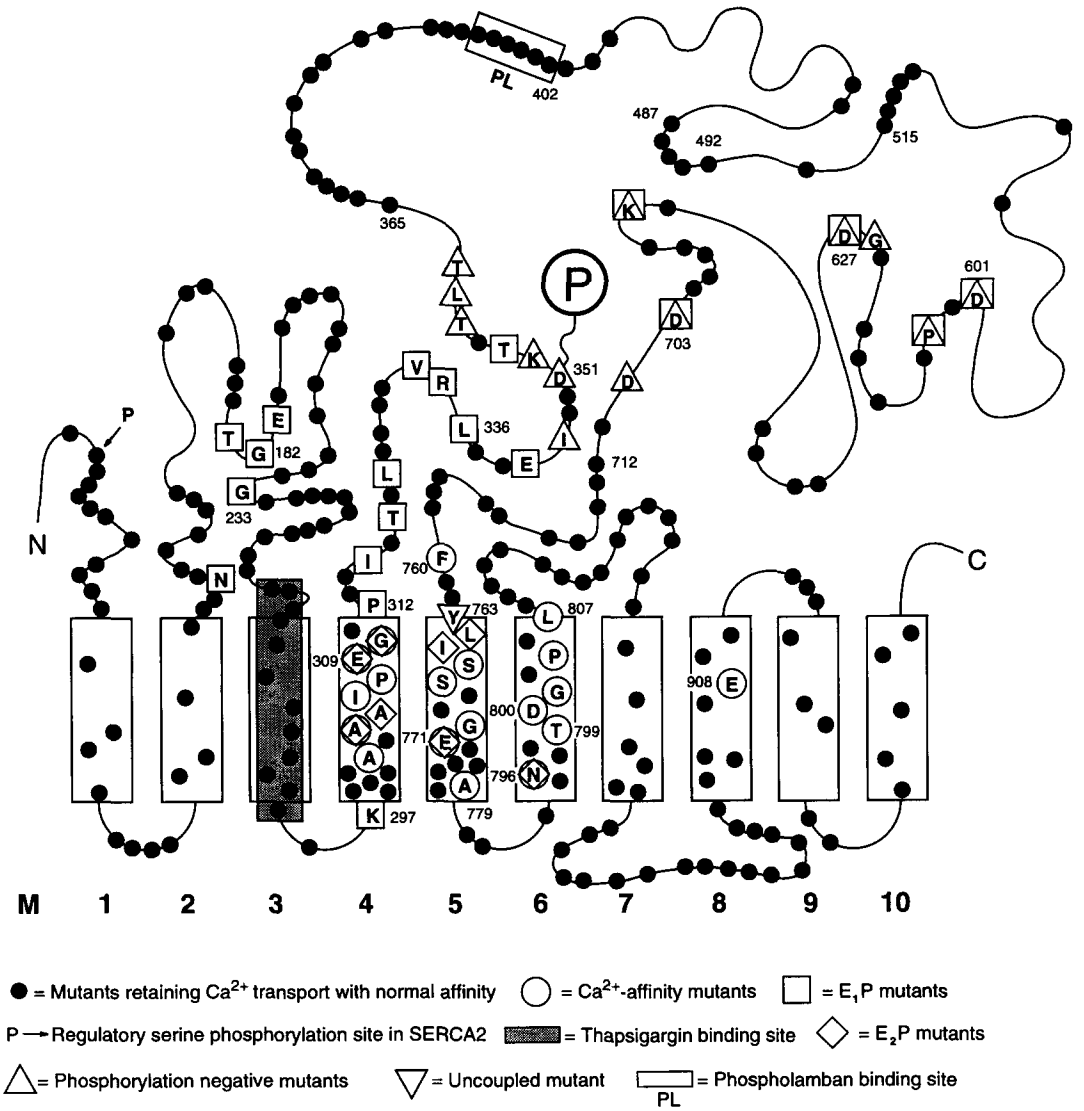

Fig. 2. Model of the topology of the sarcoplasmic reticulum Ca^{2+}-ATPase with indication of the residues that have been studied by site-directed mutagenesis. Ten putative transmembrane segments (M1–M10) are shown [2,3]. The functionally important amino acid residues are indicated according to the single-letter code inside a symbol showing the functional class to which the mutants with replacement of the indicated residue belong. This assignment is based on studies in [4–31] (and unpublished). Open circles, mutants unable to occlude Ca^{2+} or displaying at least 3-fold reduction in apparent Ca^{2+} affinity in transport or phosphorylation assay; triangles pointing upwards, mutants that are unable to phosphorylate with ATP as well as with P_i as substrate; squares, mutants in which the transition from E1P to E2P is blocked; diamonds, mutants in which the dephosphorylation of E2P is blocked; triangle pointing downwards, the $Tyr^{763} \rightarrow Gly$ mutant in which ATP hydrolysis is uncoupled from Ca^{2+} transport. The filled circles indicate mutants displaying normal Ca^{2+} affinity and a maximum turnover rate above 20% that of the wild type. Double labelling of residues indicate either that two partial reaction steps are affected by the same mutation or that different substituents elicit different effects. Sites for binding of phospholamban [26] and for regulatory serine phosphorylation of the SERCA2 isoform [30] are also indicated, as is the M3 region shown to be essential to thapsigargin binding [51].

It is noteworthy that substitution of Glu^{329} in Na^+,K^+-ATPase with either glutamine or leucine led to functional pumps, whereas substitution with either alanine or aspartic acid, both of which have smaller side chains, led to inactive pumps [38–40]. The requirement for a bulky side chain would be explained if Glu^{329} were part of the gate at the entrance to the occlusion pocket as depicted for the homologous residue Glu^{309} of the Ca^{2+}-ATPase in Fig. 3. Such a role for Glu^{329} seems to be supported by studies of the ATP and pH dependencies of Na^+,K^+-ATPase activity of the $Glu^{329} \rightarrow Gln$ mutant, which have indicated that partial reaction steps leading to deocclusion at the cytoplasmic surface are accelerated in this mutant relative to the wild type [38].

Contrary to the situation with Glu^{329}, replacement of Glu^{781} in Na^+,K^+-ATPase with leucine led to inactivation [39], while the $Glu^{781} \rightarrow Ala$ mutant was compatible with cell growth [40]. This seems to be consistent with a model for the occlusion pocket of the Na^+,K^+-ATPase bearing resemblance to that shown for Ca^{2+}-ATPase in Fig. 3, since Glu^{781} is homologous to Glu^{771} in Ca^{2+}-ATPase. With Glu^{781} contributing its side chain to the deeper part of the occlusion pocket in Na^+,K^+-ATPase it would be unlikely that a large substitutent such as

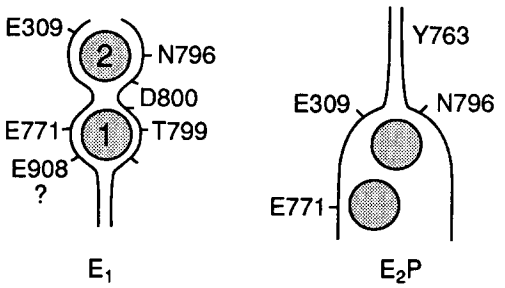

Fig. 3. Hypothesis for the assignment of residues to Ca^{2+} occlusion sites and H^+ binding sites in the E1 (left) and E2P (right) forms, respectively. The cytoplasmic surface is at top. The question mark indicates that Glu^{908} is less crucial to Ca^{2+} occlusion compared with the other residues with oxygen containing side chains. Glu^{309} and Tyr^{763} may be part of different gating mechanisms. See text for further explanation. Note also that it would be impossible for the three residues Asn^{796}, Thr^{799}, and Asp^{800}, all belonging to the M6 segment, to donate ligands to the two Ca^{2+} sites in the order suggested for the E1 form and at the same time be part of an α-helix [27].

leucine could be accommodated with preservation of cation occlusion, while it seems plausible that the smaller alanine substituent would be compatible with ion binding, albeit with the reduced affinity apparent from Table 1.

4. Counterions

The Na^+,K^+-ATPase exchanges 2 K^+ for 3 Na^+. It has recently been demonstrated that the Ca^{2+}-ATPase countertransports H^+ (or H_3O^+) with a stoichiometry of 2 or 3 H^+ exchanged for 2 Ca^{2+} [48,49]. Assuming that the latter reaction occurs by the same mechanism as K^+ transport by the Na^+,K^+-ATPase, the protons or hydroxonium ions would enter the reaction cycle by binding to extracytoplasmically facing sites on the E2P intermediate (Fig. 1). We have found several Ca^{2+}-ATPase mutants in which the dephosphorylation of the E2P intermediate is blocked at neutral pH ('E2P mutants' in Fig. 2), and some of these are identical to the mutants in which Ca^{2+} occlusion is defective. Hence, at pH 7.0 the rate of dephosphorylation of E2P formed by the mutants $Glu^{309}\rightarrow Gln$, $Glu^{771}\rightarrow Gln$, $Glu^{771}\rightarrow Ala$, and $Asn^{796}\rightarrow Ala$ is at least 10-fold lower than that corresponding to wild-type Ca^{2+}-ATPase [16,17,27,29]. The residues Glu^{309}, Glu^{771}, and Asn^{796} are, therefore, candidate donators of oxygens for the binding of H^+ (or H_3O^+) to be countertransported (Fig. 3, right side). To test the hypothesis that the side chain of Glu^{771} participates in proton countertransport, Glu^{771} was replaced with lysine, expecting that this substitution would mimick the binding of positive charge corresponding to two protons near the free carboxylate group of Glu^{771}. Indeed, it was found that the dephosphorylation of E2P occurred rapidly in the $Glu^{771}\rightarrow Lys$ mutant [29].

The studies of Na^+,K^+-ATPase mutants have indicated that the residues Glu^{329} and Glu^{781} homologous to Glu^{309} and Glu^{771} in Ca^{2+}-ATPase are quite important determinants of apparent K^+ affinity at the extracellularly facing sites, whereas this seems not to be the case for Thr^{809}, which appears to be involved predominantly in determination of Na^+ affinity (Table 1). Correspondingly, the homologous Ca^{2+}-ATPase residue Thr^{799}, critical to Ca^{2+} occlusion, has been shown not to be important for the dephosphorylation of E2P [27]. The results are consistent with a consecutive mechanism of ion pumping in which the ions taken up from the cytoplasm (Ca^{2+} or Na^+) are released on the extracytoplasmic side prior to the binding of the counterions (H^+ or K^+) at sites composed of some of the same residues (including Glu^{309}/Glu^{329} and Glu^{771}/Glu^{781}, but not Thr^{799}/Thr^{809}) that bind the cytoplasmic ions. The Na^+,K^+-ATPase residue Glu^{781} may play a crucial role in the discrimination between Na^+ and K^+ at the extracellularly facing sites, since in the $Glu^{781}\rightarrow Ala$ mutant Na^+ was able to activate dephosphorylation and support high ATPase activity in the absence of K^+ [40].

5. Other residues of importance for cation affinity

Because the above discussed residues with oxygen containing side chains in transmembrane segments M4, M5, and M6 are conserved between the Ca^{2+}-ATPase and the Na^+,K^+-ATPase, these residues are not the ones determining the preference of the Ca^{2+}-ATPase for Ca^{2+} over Na^+.

The Ca^{2+}-ATPase residue Glu^{908} located in the putative transmembrane segment M8 is unique to the Ca^{2+}-ATPase, a valine occupying the homologous position in Na^+,K^+-ATPase [47]. Substitution of Glu^{908} with alanine reduced the apparent affinity for Ca^{2+} more than 100-fold as measured in the phosphorylation assays [5,27], but Ca^{2+} occlusion was not disturbed even at a Ca^{2+} concentration as low as 10 μM [27], and at high Ca^{2+} concentrations in the millimolar range the $Glu^{908}\rightarrow Ala$ mutant transports Ca^{2+} at a significant rate (J.P. Andersen, unpublished). Glu^{908} might be involved in the initial recognition of Ca^{2+} without participating in the coordination of Ca^{2+} in the occluded state.

Steric factors owing to the constraints imposed by hydrophobic side chains are likely to be important for the specific cation selectivity. Mutagenesis studies of Phe^{760} and Gly^{770} of the Ca^{2+}-ATPase have indicated that these residues contribute more than 100-fold to the apparent affinities of the superficial and deep Ca^{2+} sites, respectively [15,28,31]. The respective residues at the homologous positions in Na^+,K^+-ATPase are serine and proline [47].

Experiments with chimeric fusion proteins between Na^+,K^+-ATPase and Ca^{2+}-ATPase have demonstrated that the large cytoplasmic loop between M4 and M5 of the Na^+,K^+-ATPase can be substituted for that of the Ca^{2+}-ATPase without any effect on apparent Ca^{2+} affinity [50]. On the other hand, when the transmembrane segment M3 of Na^+,K^+-ATPase was substi-

Table 1
Apparent cation affinities of Na^+,K^+-ATPase mutants

Mutants	Na^+,K^+-ATPase[a]		Phosphorylation[b]
	Na^+	K^+	Na^+
		$K_{0.5}$ (mM)	
Wild type	7.1	1.0	0.8
$Glu^{329}\rightarrow Gln$	13.8	4.8	2.6[c]
$Glu^{781}\rightarrow Ala$	35.6	2.8	6.5
$Thr^{809}\rightarrow Ala$	29.3	1.4	5.0

[a] $K_{0.5}$ values determined by titration of Na^+,K^+-ATPase activity with Na^+ and K^+, respectively.
[b] $K_{0.5}$ values determined by Na^+ titration of phosphorylation from ATP in the absence of K^+.
[c] B. Vilsen, unpublished data; all other data were reproduced from [38,40].

tuted for that of Ca^{2+}-ATPase, the apparent affinity for Ca^{2+} as activator of the phosphorylation reaction with ATP was found to be reduced more than 100-fold [51]. M3 is not well conserved among P-type ATPases of different cation specificity, and in studies of several Ca^{2+}-ATPase/Na^+,K^+-ATPase chimeras it was demonstrated that M3 is essential to the specific inhibition of the Ca^{2+}-ATPase by thapsigargin, which behaves like a Ca^{2+} antagonist [51]. Other studies of chimeras have implicated the NH_2-terminal 69 amino acid residues of the Na^+,K^+-ATPase as a 'Na^+-sensor' [52].

6. Coupling of ATP hydrolysis to ion translocation

The conversion of the ADP-sensitive ('high energy') Ca^{2+}-occluded E1P phosphoenzyme intermediate into the ADP-insensitive ('low energy') E2P phosphoenzyme intermediate of the Ca^{2+}-ATPase can be prevented or slowed down by single mutations, with resulting loss of Ca^{2+} transport as well as ATP hydrolysis, but with retention of the ability to phosphorylate from ATP and form E1P in a Ca^{2+} dependent reaction [8,9,11,13,14,20,24]. Mutations giving rise to this phenotype ('E1P mutants' in Fig. 2) are located in the stalk sector connecting transmembrane segment M4 with the larger cytoplasmic domain and in the smaller cytoplasmic loop (the 'β-domain' between transmembrane segments M2 and M3), parts of which are highly conserved among the P-type ATPases. For Ca^{2+}-ATPase mutants with alteration to Pro^{312} at the top of transmembrane segment M4 or Gly^{233} in the COOH-terminal part of the β-domain, it was demonstrated that the mutant phosphoenzyme retains ADP-sensitivity for minutes following incubation with the Ca^{2+} chelator EGTA and Ca^{2+} ionophore [8,9]. As the presence of bound Ca^{2+} is required for reversal of the phosphorylation with ADP, Ca^{2+} must have been present in a stably occluded state, unable to dissociate, in the mutant phosphoenzyme, indicating that the dissociation of Ca^{2+} at the extracytoplasmic side of the membrane requires a conformational change in the phosphoenzyme which was blocked in the mutants [20].

Mutation of the Na^+,K^+-ATPase residue Leu^{332}, present at the position corresponding to that of Pro^{312} in Ca^{2+}-ATPase, resulted in a slowing of phosphoenzyme turnover consistent with an effect of the same type as that observed for Pro^{312} mutants of Ca^{2+}-ATPase [32,35].

Some conservative replacements of residues in the large cytoplasmic domain have also been shown to block the E1P→E2P transition in the Ca^{2+}-ATPase, while more drastic alterations to the same residues prevented phosphorylation from either ATP or P_i, indicating that these residues are part of the catalytic phosphorylation site (residues doubly labelled with squares and triangles in Fig. 2) [12,13]. Taken together, the data support the hypothesis that the E1P→E2P transition comprises conformational changes that originate in the phosphorylation site and are transmitted down to the cation binding structure in the transmembrane sector through rearrangements in the β-domain and in the M4 stalk connection. This may lead to a rotation or tilting of the M4 segment disrupting the Ca^{2+} binding domain and making the previously occluded ions accessible to the extracytoplasmic surface (Fig. 3 and [20]).

Very recently, it has been demonstrated that the replacement of the tyrosine Tyr^{763} at the top of transmembrane segment M5 with glycine gives rise to a Ca^{2+}-ATPase enzyme in which ATP hydrolysis is uncoupled from Ca^{2+} transport [31]. Like the ATPase activity of the wild-type Ca^{2+}-ATPase, the ATPase activity of the Tyr^{763}→Gly mutant was Ca^{2+} dependent and became fully activated below 10 μM Ca^{2+}. There was, however, no Ca^{2+} accumulation in the microsomal vesicles containing the mutant. The V_{max} of the uncoupled ATPase activity of the mutant was similar to the V_{max} measured with wild-type enzyme to which Ca^{2+} ionophore had been added to make the membrane leaky to Ca^{2+}. The ATP hydrolysis catalyzed by the mutant was found to proceed through the E1P and E2P phosphoenzyme intermediates and was inhibited by vanadate, indicating the formation of an E2 form during the enzymatic cycle [31]. Because the E1P→E2P transition normally is associated with release of Ca^{2+} at the extracytoplasmic surface (Fig. 1), it is possible that the machinery responsible for the translocation of Ca^{2+} is intact in the Tyr^{763}→Gly mutant, which would mean that the lack of net accumulation of Ca^{2+} by the mutant is due to an increased rate of efflux of transported Ca^{2+} from the vesicles through an open transmembrane channel-like structure in the ATPase protein [31]. Such an increased passive efflux of Ca^{2+} would be reminiscent of the Ca^{2+} leak through the Ca^{2+}-ATPase observed following incubation with phenothiazines and local anesthetics [53–55]. The latter Ca^{2+} leak was suggested to be mediated by reaction intermediates in the late part of the reaction cycle [53]. Because the Tyr^{763}→Gly mutant is able to occlude Ca^{2+} in the E1 form present following incubation with CrATP (J.P. Andersen, unpublished), the transmembrane channel-like structure in the mutant may likewise be open only in the late part of the cycle. The side chain of Tyr^{763} may be required to close the cytoplasmic entrance to the channel in the E2P form (Fig. 3). This can possibly be generalized to other P-type ATPases, including the Na^+,K^+-ATPase, since the aromatic nature of the residue at the position corresponding to Tyr^{763} in the Ca^{2+}-ATPase is highly conserved within the P-type family. Replacement of the homologous tyrosine in the Na^+,K^+-ATPase with alanine has resulted in a mutant that was unable to confer ouabain resistance to transfected cells and therefore inactive as a pump [41].

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