Topology of a Functionally Important Region of the Cardiac Na\(^+\)/Ca\(^{2+}\) Exchanger*

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The cardiac Na\(^+\)/Ca\(^{2+}\) exchanger, NCX1, has been modeled to consist of 11 transmembrane segments and a large cytoplasmic loop (loop f). Cysteine mutagenesis and sulfhydryl modification experiments demonstrate that the loop connecting transmembrane segments 1 and 2 (loop b) is located on the cytoplasmic side of the membrane, as previously modeled. A mutation in loop b, asparagine 101 to cysteine (N101C), renders the exchanger insensitive to regulation by cytoplasmic Na\(^+\) and Ca\(^{2+}\). Nearby mutations at residue threonine 103 (T103C or T103V) increase the apparent affinity of the Na\(^+\) transmembrane segment 2 is important in Na\(^+\) transport and also in secondary regulation. Thus, this region may form part of the link between the ion translocation pathway formed by the transmembrane segments and regulatory sites that have previously been localized to loop f.

The Na\(^+\)/Ca\(^{2+}\) exchanger transports one Ca\(^{2+}\) across the cell membrane in exchange for three Na\(^+\) and is important in maintaining Ca\(^{2+}\) homeostasis in many cell types, including muscle, nerve, and epithelium. In cardiac muscle, Na\(^+\)/Ca\(^{2+}\) exchange has been well characterized as the primary mechanism for extrusion of the Ca\(^{2+}\) that enters the cell to initiate contraction. Na\(^+\)/Ca\(^{2+}\) exchange is therefore essential to cardiac relaxation, and an understanding of the function and regulation of this protein is essential to understanding the basis of cardiac excitation-contraction coupling.

Na\(^+\)/Ca\(^{2+}\) exchange activity can be readily observed using inside-out giant excised patches in which “reverse” exchange is initiated by applying Na\(^+\) to the intracellular surface. This generates an outward current as intracellular Na\(^+\) is exchanged for extracellular Ca\(^{2+}\). This protocol, and other studies, have demonstrated that the exchanger is subject to modulation by several cytoplasmic factors, including Na\(^+\) and Ca\(^{2+}\).

Secondary Na\(^+\) regulation is demonstrated by rapidly raising the intracellular Na\(^+\) concentration to activate outward Na\(^+\)/Ca\(^{2+}\) exchange current. Current peaks and then declines over several seconds to a reduced steady-state level. The extent and rate of this partial inactivation are dependent on the concentration of intracellular Na\(^+\). This regulatory process has been referred to as Na\(^+\)-dependent inactivation (1, 2).

Regulation of the Na\(^+\)/Ca\(^{2+}\) exchanger by intracellular Ca\(^{2+}\) has been observed in several preparations. For example, squid axon and mammalian cardiac Na\(^+\)/Ca\(^{2+}\) exchangers have an absolute requirement for intracellular Ca\(^{2+}\). The stimulatory effect of intracellular Ca\(^{2+}\) is most easily demonstrated by measuring reverse Na\(^+\)/Ca\(^{2+}\) exchange. Under these conditions, the nontransported regulatory Ca\(^{2+}\) is on the opposite side of the membrane from the transported extracellular Ca\(^{2+}\).

When NCX1 is expressed in oocytes, a reduction in the intracellular Ca\(^{2+}\) markedly decreases subsequent Ca\(^{2+}\) influx via reverse exchange (3). These and other regulatory effects can be abolished by partial proteolysis with chymotrypsin (2).

Based on hydrophathy analysis, the exchanger is modeled to consist of 11 transmembrane segments with a large intracellular loop (loop f) between transmembrane segments 5 and 6 (Fig. 1). The extracellular location of the amino terminus (4) and the intracellular location of loop f (6, 7) have been experimentally verified. Also, loop e, between transmembrane segments 4 and 5, appears to be extracellular, based on the glycosylation of an exchanger truncated at that site (5). The transmembrane segments are essential for ion transport, whereas loop f is involved in regulation (6).

Through mutagenesis studies, it has been determined that the endogenous XIP\(^1\) region in loop f (8) is involved in Na\(^+\)-dependent inactivation (9) and that another portion of loop f is involved in the binding of regulatory Ca\(^{2+}\) (Fig. 1) (3, 10). The two regulatory processes, however, are not independent. Binding of regulatory Ca\(^{2+}\) decreases Na\(^+\)-dependent inactivation (11), and mutations in the endogenous XIP region alter the kinetics of Ca\(^{2+}\) regulation (9).

Other mutagenesis studies have implicated the involvement of two highly conserved and internally homologous regions (the ω-repeats) in ion transport (12). However, the molecular details of ion transport are unknown, as are the mechanisms by which loop f interacts with the transmembrane segments to effect regulation.

We used substituted cysteine mutagenesis and sulphydryl modification reagents to determine the membrane topology and explore the function of residues modelled to be at the interface of loop b and transmembrane segment 2. Wild-type NCX1 mutants were expressed in Xenopus oocytes, and Na\(^+\)/Ca\(^{2+}\) exchange activity was measured as 45Ca\(^{2+}\) uptake into intact oocytes or as Na\(^+\)-activated outward current in giant patches excised from oocyte membranes. We show that this region is

* The abbreviations used are: XIP, exchanger inhibitory peptide; MTS, methanethiosulfonate; MTSET, (2-trimethylammonium)ethyl methanethiosulfonate; MTSES, (2-sulfonatoethyl) methanethiosulfonate; NEM, N-ethylmaleimide; TEAOH, tetraethylammonium hydroxide.

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located on the cytoplasmic side of the membrane and may form part of the link between transport and regulation.

EXPERIMENTAL PROCEDURES

Mutations in the wild-type exchanger were generated using the Sculptor in vitro mutagenesis kit (Amersham Corp.). cRNA was synthesized with mMessage mMachine (Ambion) and injected into Xenopus laevis oocytes. Na+/Ca2+-exchange activities were measured after incubation of the oocytes for 3–6 days at 17–19 °C.

To directly measure 45Ca2+ fluxes, oocytes were first loaded with Na+ by incubation in ice-cold Barth’s solution containing 20 mM nystatin, 0.2% dimethyl sulfoxide for 10 min. Oocytes were then washed four times in 1 ml of 90 mM NaCl, 5 mM HEPES, pH 7.5, and placed into a 45Ca2+ uptake medium containing either NaCl (90 mM) (to measure nonspecific Ca2+ uptake) or KCl (90 mM) (to measure Na+ gradient-dependent Ca2+ uptake) and 10 μM CaCl2. 6.4 mM 4-morpholinepropanesulfonic acid, pH 7.4, and 6 μCi/ml 45CaCl2 for 10 min, at which time Ca2+ uptake was still in a linear phase. Unincorporated 45Ca2+ was removed by washing the oocytes four times in 1 ml of ice-cold Barth’s solution containing 20 mM ouabain, 0.05 mM niflumic acid, 30 mM HEPES, pH 7.5, and placed into an uptake medium containing either NaCl (90 mM) (to measure nonspecific Ca2+ uptake) or KCl (90 mM) (to measure Na+ gradient-dependent Ca2+ uptake) and 10 μM CaCl2. 6.4 mM 4-morpholinepropanesulfonic acid, pH 7.4, and 6 μCi/ml 45CaCl2 for 10 min, at which time Ca2+ uptake was measured by liquid scintillation counting. The small nonspecific Ca2+ uptake was subtracted from the Na+ gradient-dependent Ca2+ uptake as a measure of Na+/Ca2+ exchange activity. We have previously used this technique extensively to quantitate Na+/Ca2+ exchange activity (6, 12, 13).

To investigate the effects of extracellular sulfhydryl reagents NEM, MTSES, and MTSET, oocytes were preincubated with reagents (10 mM) for 10 min and then washed four times. To determine the effects of intracellular sulfhydryl reagents, 100 mM MTSES or MTSET was injected into oocytes 10 min before measuring 45Ca2+ uptake to produce an estimated cytoplasmic concentration of 10 mM (assuming an intracellular oocyte volume of 0.5 μl). MTS reagents were purchased from Toronto Research Chemicals.

Na+/Ca2+ exchange current was measured under voltage clamp in inside-out membrane patches using the giant excised patch technique (2, 3, 6, 11). Pipettes were filled with the following external solution: 120 mM N-methyl-D-glucamine-2-(N-morpholino) ethanesulfonic acid, 30 mM HEPES, 8 mM CaCl2, 2 mM Ba(OH)2, 2 mM Mg(OH)2, 0.025 mM ouabain, 0.05 mM niflumic acid, 30 mM TEAOH. The bath, or cytoplasm, solution contained 100 mM 2-(N-morpholino) ethanesulfonic acid, 20 mM CsOH, 20 mM HEPES, 10 mM EGTA, 1 mM Mg(OH)2, 20 mM TEAOH, and 100 mM CsOH, NaOH, or LiOH. CaCO3 was added to give the indicated free Ca2+ concentration. In experiments involving deregulation of the exchange by partial proteolysis, 1 mg/ml chymotrypsin was added to a cytoplasmic solution containing 0 Na+ and 1 μM free Ca2+. All solutions were pH 7.0, membrane holding potential was 0 mV, and experiments were conducted at 34–35 °C.

Data summaries are presented as an average of n experiments ± S.E. Results from 6–8 oocytes were averaged for each experiment measuring 45Ca2+ flux.

RESULTS

Fig. 1 shows a model for the Na+/Ca2+-exchange protein, based primarily on hydropathy analysis. Loop b consists of amino acids 63–101 and is modeled to be on the cytoplasmic side of the membrane. To determine the orientation of loop b with respect to the membrane, we used substituted cysteine mutagenesis. Specifically, residues modeled to be near the surface of the membrane were mutated to cysteine, and the mutants were exposed to sulfhydryl modifying reagents that form covalent bonds with accessible cysteines. We then examined the effects on exchange activity of membrane-permeable reagents and of impermeable reagents applied either extracellularly or intracellularly.

The wild-type and mutated canine NCX1 exchangers were expressed in Xenopus oocytes. Amino acids Ser-100, Asn-101, Leu-102, Thr-103, and Ala-106 were each individually mutated to cysteine. The mutant T103V (12) was also examined in this study. Fig. 2 shows the activity of each of the mutants relative to the wild-type exchanger. Except for mutant N101C, which had a wild-type level of activity, all of the mutants displayed approximately half the activity of the wild-type exchanger. This may reflect either a reduction in the level of protein expression or a direct effect of the mutation on exchange activity.

The membrane-permeable reagent NEM had no significant effect on the wild-type exchanger but inhibited each of the cysteine mutants except A106C (Fig. 3). T103C was inhibited least. T103C and A106C were modeled to be within the membrane, and may be less accessible, even to membrane-permeable sulfhydryl reagents, than the other cysteine mutants.

The effects of intracellular or extracellular application of MTSET or MTSES are illustrated in Fig. 4. MTS reagents have been used extensively in sulfhydryl modification experiments (14–16). These relatively large, charged molecules (MTSET is cationic, and MTSES is anionic) are membrane-impermeable. The MTS reagents were either applied extracellularly, by incubating oocytes in medium containing 10 mM reagent, or applied intracellularly, by injecting 100 mM MTS solution into the oocyte for an estimated cytoplasmic concentration of 10 mM. The wild-type exchanger is relatively insensitive to either MTSET or MTSES, although intracellular MTSET caused a modest stimulation (Fig. 4).

Extracellular applications of MTSET or MTSES had no substantial effects on any of the cysteine mutants. On the other hand, intracellular application of MTSET or MTSES inhibited...
There was no decay in current amplitude after current was activated by raising intracellular Na\(^+\) and Ca\(^2+\), which suggests that loop b is involved in secondary regulation by these factors. Fig. 5 shows effects of mutants on specific aspects of Na/Ca exchange function. Mutant N101C showed altered sensitivity to intracellular Na\(^+\) and Ca\(^2+\), which suggests that loop b is involved in secondary regulation by these factors. Fig. 5A illustrates the regulatory effects of intracellular Na\(^+\) and Ca\(^2+\) on wild-type Na\(^+\)/Ca\(^2+\) exchange current. Current is activated by application of intracellular Na\(^+\); the slow decay is due to the secondary inhibitory effect of Na\(^+\) (2). When intracellular Ca\(^2+\) is removed, the current is further inhibited, demonstrating the Ca\(^2+\) regulatory effect. These regulatory effects are also characteristic of cardiac Na\(^+\)/Ca\(^2+\) exchange activity measured in situ (1). Both of these effects are abolished following partial proteolysis of the exchanger by chymotrypsin.

Fig. 5B shows that N101C has altered regulatory properties. There was no decay in current amplitude after current was activated by raising Na\(^+\), and when Ca\(^2+\) was lowered to 0,

there was only a small reduction in current amplitude. In a series of experiments, the wild-type Na\(^+\)/Ca\(^2+\) exchange current decayed to 43 ± 4% of peak after Na\(^+\)-dependent inactivation (n = 5), but decay of N101C current was insignificant (n = 8). Removal of regulatory Ca\(^2+\) reduced the wild-type current by 55 ± 5% even after Na\(^+\)-dependent inactivation, but it reduced the N101C current by only 24 ± 4%. N101C appeared to function like the wild-type exchanger after deregulation by chymotrypsin. In preliminary experiments, exchangers S100C and L102C displayed normal regulatory properties.

Although mutant N101C resembles the chymotrypsin-treated wild-type exchanger in terms of a lack of sensitivity to regulatory ions, chymotrypsin digestion still stimulates the current of N101C. This stimulation was small in the experiment shown in Fig. 5B, but in other experiments, the stimulation was more substantial (97 ± 14%; n = 5). Interestingly, chymotrypsin also abolished NEM inhibition of N101C, as shown in Fig. 6. In the experiment shown in Fig. 6A, outward current activated by raising cytoplasmic Na\(^+\) was inhibited by NEM applied in the cytoplasmic solution, and this inhibitory effect was irreversible. The results indicate that NEM modified the cysteine introduced at residue 101 in such a way as to interfere with exchange, consistent with the data shown above (Fig. 3). When the exchanger was partially proteolyzed by cytoplasmic chymotrypsin and then exposed to NEM, no inhibitory effect was seen (Fig. 6B).

The preceding results indicate that mutation N101C alters the response of the Na\(^+\)/Ca\(^2+\) exchanger to regulatory ions. We also find that mutations in putative transmembrane segment 2, near loop b, affect ion translocation properties. Mutation of threonine 103 modifies the dependence of the Na\(^+\)/Ca\(^2+\) exchanger on cytoplasmic monovalent cations. Fig. 7 shows the dependence of outward Na\(^+\)/Ca\(^2+\) exchange current on cytoplasmic Na\(^+\) concentration. The excised patches were first exposed to chymotrypsin to remove complicating regulatory effects in these experiments. The wild-type Na\(^+\) dependence was sigmoidal, with half-maximal activation at 16 mm Na\(^+\). The Na\(^+\) dependence for mutant T103V was shifted to the left, with half-maximal activation at lower Na\(^+\) (8 mm) and with a decreased Hill coefficient. Mutant T103V thus has a higher apparent affinity for cytoplasmic Na\(^+\) and an apparent decreased cooperativity of Na\(^+\)-binding.

The observed increase in apparent affinity for cytoplasmic Na\(^+\) raised the possibility that mutant T103V might also have an altered selectivity for monovalent cations. We tested this...
possibility using $^{45}\text{Ca}^{2+}$ flux measurements. Oocytes were loaded with Li$^+$ in place of Na$^+$, and $^{45}\text{Ca}^{2+}$ uptake was measured. The T103V mutant is capable of a greater Li$^+$-dependent $^{45}\text{Ca}^{2+}$ uptake than the wild-type exchanger (Fig. 8). Similar results were seen after loading with K$^+$ or Cs$^+$ (data not shown). Mutant T103V therefore appears to have a reduced selectivity for Na$^+$ over other monovalent cations and may be capable of translocating other monovalent cations besides Na$^+$ in exchange for Ca$^{2+}$.

An alternative possibility is that T103V could support a higher level of Ca$^{2+}$/Ca$^{2+}$ exchange than does the wild-type exchanger, so that increased extracellular $^{45}\text{Ca}^{2+}$ would be taken up in exchange for endogenous intracellular Ca$^{2+}$, irrespective of the internal monovalent cation. In this case, there would be no associated membrane current, because Ca$^{2+}$/Ca$^{2+}$ exchange is not electrogenic. However, in voltage clamp experiments (Fig. 9), Li$^+$ activates a significant, concentration-dependent, outward current in oocytes expressing T103V. The wild-type exchanger shows no change in current when Li$^+$ is applied. Neither Na$^+$ nor Li$^+$ activated significant outward current in control water-injected oocytes (data not shown). The ability of K$^+$ to activate currents was not tested, although Ca$^{2+}$
did not appear to do so (Fig. 9). Ca$^{2+}$/Ca$^{2+}$ exchange may have been the prominent mode of $^{45}$Ca$^{2+}$ uptake for T103V when oocytes were loaded with these monovalent cations (see "Discussion").

A summary of the dependence of outward membrane current on [Na$^+$], or [Li$^+$], for mutant T103V is shown in Fig. 10. The estimated $K_{1/2}$ for Na$^+$ is 8 mM, and for Li$^+$, it is 100 mM. Thus, in addition to an apparent increase in affinity for Na$^+$ (Fig. 7), T103V displays a significant Li$^+$-dependent current. Mutant T103C has similar properties (data not shown).

**DISCUSSION**

In this study, we have investigated a previously unexamined region of the Na$^+$/Ca$^{2+}$ exchange protein. Mutations in loop b and the cytoplasmic end of transmembrane segment 2 verify that this region is located at the cytoplasmic face and suggest that it is involved in ion translocation and regulation by cytoplasmic factors.

The transmembrane segments of the Na$^+$/Ca$^{2+}$ exchange protein have been assigned based on hydropathy analysis of the amino acid sequence (13). Previous results support the extracellular assignment of the amino terminus (4) and loop e, between transmembrane segments 4 and 5 (5), and the intracellular assignment of loop f, between transmembrane segments 5 and 6 (6, 7). In this study, mutants S100C and L102C, located in loop b, were sensitive only to intracellular application of membrane-impermeable reagents MTSET and MTSES, thus assigning these residues to the intracellular surface. These results extend our understanding of the structure of the exchanger by assigning loop b, between transmembrane segments 2 and 3, to the inside of the cell.

These results were dependent upon the fact that none of the sulfhydryl modifying reagents we used inhibited the wild-type exchanger. It appears that the endogenous cysteines are inaccessible to modifying reagents or that the accessible cysteines are not intimately involved in exchanger function. An alternative, though unlikely, interpretation is that the mutations in loop b induce a conformational change that exposes a native cysteine to cytoplasmic MTS reagent. We are in the process of constructing a cysteine-less mutant to investigate this possibility.

There are two interesting points in the effect of the membrane-permeable reagent NEM on the loop b mutants. First, the effect of NEM was reduced as the mutations were introduced further into transmembrane segment 2 (Fig. 3). This may be an indication that the mutated residues with reduced NEM sensitivity are in the transmembrane segment as modeled. Second, mutants N101C and T103C are sensitive to inhibition by NEM but not by the MTS reagents. Thus, these two residues are solvent-accessible but are more restricted to chemical reaction than residues Ser-100 or Leu-102. There may perhaps be local hydrophobic or steric constraints preventing reaction with the MTS reagents.

Secondary regulation by cytoplasmic Na$^+$ and Ca$^{2+}$ has previously been localized to functional sites in loop f, but no other regions of the exchanger were implicated in the regulatory process. Mutations in the endogenous XIP region (Fig. 1) can remove Na$^+$-dependent inactivation but not Ca$^{2+}$ stimulation (9), whereas deletion of amino acids 562–685 has the opposite effect (6), removing Ca$^{2+}$ stimulation but not Na$^+$-dependent inactivation. A functionally important binding site for regulatory Ca$^{2+}$ has been identified at amino acids 371–508 (10). Some mutations in loop f alter both Na$^+$ inhibition and Ca$^{2+}$ stimulation, indicating that the two processes interact (9).

Loop f must exert regulatory effects on transport by influencing the transmembrane segments that catalyze ion translocation. We show here that a mutation in loop b (N101C)
interferes with secondary regulation by Na" and Ca"++. Two explanations are considered. First, the residue at position 101 may interact with loop f. Mutating residue Asn-101 to cysteine may then alter interaction between loops b and f and block regulation. Second, the mutation may alter the protein conformation so that loop f is no longer able to appropriately influence, and regulate, the transmembrane segments. We are unable to distinguish these possibilities. Nevertheless, in both cases, the interface of loop b and transmembrane segment 2 is identified as a key site in the coupling of regulation to translocation.

We explored the possibility that the cysteine introduced in N101C interrupts regulatory mechanisms by forming an internal disulfide bond with a native cysteine. We found that the reducing agent dithiothreitol does not restore regulation to the mutant as would be expected under these circumstances (data not shown). Thus, it does not appear that disulfide bond formation is the cause of loss of regulation in N101C.

Partial proteolysis by cytoplasmic chymotrypsin, which removes secondary regulatory effects of intracellular Na" and Ca"++ in the wild-type Na"/Ca"++ exchanger, also prevents the inhibitory effect of the sulphydryl reagent NEM on N101C. Proteolysis may remove secondary regulation in the wild-type exchanger by disrupting the interaction between regulatory sites in loop f and the translocation pathway. It is thought that the major site for proteolysis of the exchanger is within loop f (17). The fact that proteolysis removes the NEM effect on mutant N101C suggests a functional relationship between loops b and f. Again, the relationship could be either a direct or an indirect interaction.

The cytoplasmic end of transmembrane segment 2 appears to play a role in the translocation of Na" ions. Mutant T103V shows an increased apparent affinity for cytoplasmic Na" and also seems to support Li"/Ca"++ exchange, T103C has identical characteristics, but mutant N101C does not show any Li"-activated current (data not shown). The Li"-activated current of mutant T103V is relatively small. At 100 mM activating ion, the Li"-activated exchange current is about 20% of Na"-activated exchange current. Because mutant T103V has a higher affinity for Na" than the wild-type exchanger, the possibility arises that contaminating Na" in the cytoplasmic Li" solution could activate significant current in the mutant but not the wild-type exchanger. This is unlikely because we used 99.95% pure LiOH, and 2-3 mM contaminating Na" would have to be present in the 100 mM Li" solution to produce a contamination artifact.

The 45Ca"++ flux measurements show that mutant T103V transports Ca"++ ions across the membrane without requiring a Na" gradient (Fig. 8), and the Li"-activated outward current indicates that an electrogenic process accompanies this Ca"++ transport (Fig. 9). This suggests that mutant T103V can directly catalyze Li"/Ca"++ exchange. Other Na" transporters, such as the Na"/H" exchanger (18), the Na"/K" ATPase (19), and the mitochondrial Na"/Ca"++ exchanger (20), can substitute Li" for Na" to varying degrees. However, this is the first report of a plasma membrane Na"/Ca"++ exchanger capable of Li" transport.

The relative level of Ca"++-Ca"++ exchange may also be higher for mutant T103V than for the wild-type exchanger. When 45Ca"++ uptake was measured in the presence of a Li" gradient (Fig. 8), 45Ca"++ flux is due to a combination of Li"/Ca"++ exchange and Ca"++-Ca"++ exchange (external 45Ca"++ exchanging for internal endogenous Ca"++). On the other hand, Li"-induced currents (Fig. 9) are due only to Li"/Ca"++ exchange (Ca"++-Ca"++ exchange is not electrogenic). For mutant exchanger T103V, the 45Ca"++ flux into Li"-loaded oocytes was about 80% of that into Na"-loaded oocytes, but the Li"-induced current was only about 20% of the Na"-induced current. Thus, Ca"++-Ca"++ exchange activity of T103V appeared to be much more robust than that of the wild-type exchanger.

In parallel experiments (not shown; n = 2), Li"-loaded oocytes were diluted into 45Ca"++ uptake medium containing K" (Li" gradient present) or Li" (no Li" gradient). 45Ca"++ uptake levels for the wild-type exchanger were 33 and 27%, respectively, of that obtained in the presence of a Na" gradient. For mutant T103V, these values were 107 and 173%, respectively. These results are consistent with the idea that the level of Ca"++-Ca"++ exchange is greater in mutant T103V than in the wild-type exchanger.

The data in Fig. 10, showing the relative transport activity of Na"/Ca"++ exchange and Li"/Ca"++ exchange for T103V, were fit well with a simple model. Using a sequential Na"/Ca"++ exchange cycle to represent the wild-type exchanger, we added low-affinity Li" binding and Li"/Ca"++ exchange. In this model, the best fit is produced when Li" binds with a K" of 100 mM and the Li" translocation rate is 40% of the Na" translocation rate. It is possible that residue Thr-103 is part of a Na" binding site and that the mutation increases the affinity of the site for Na" and also for Li". It is striking to note the proximity of the residue to α-repeat 1 (Fig. 1), which begins at alanine 106. The α-repeats are highly conserved among different isoforms of the Na"/Ca"++ exchanger and play an important role in function (12).

The functional map of the Na"/Ca"++ exchange protein is augmented by the data presented here. Loop b has been localized to the cytoplasmic side of the membrane and assigned a potential role in secondary regulation of the exchanger by cytoplasmic factors. The cytoplasmic end of transmembrane segment 2 may have an important functional role in the transport of Na". The loop b-transmembrane segment 2 interface could be an important structural link between ion translocation and regulation.

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