A Novel Molecular Determinant for cAMP-dependent Regulation of the Frog Heart Na\(^+\)-Ca\(^{2+}\) Exchanger*

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Na\(^+\)-Ca\(^{2+}\) exchanger is one of the major sarcolemmal Ca\(^{2+}\) transporters of cardiac myocytes. In frog ventricular myocytes the exchanger is regulated by isoproterenol via a \(\beta\)-adrenergoreceptor/adenylate-cyclase/cAMP-dependent signaling pathway providing a molecular mechanism for the relaxant effect of the hormone. Here, we report on the presence of a novel exon of 27-base pair insertion, which generates a nucleotide binding motif (P-loop) in the frog cardiac Na\(^+\)-Ca\(^{2+}\) exchanger. To examine the functional role of this motif, we constructed a full-length frog heart Na\(^+\)-Ca\(^{2+}\) exchanger cDNA (fNCX1a) containing this exon. The functional expression of fNCX1a in oocytes showed characteristic voltage dependence, divalent (Ni\(^{2+}\), Cd\(^{2+}\)) inhibition, and sensitivity to cAMP in a manner similar to that of native exchanger in frog myocytes. In oocytes expressing the dog heart NCX1 or the frog mutant (\(\Delta\)NCX1a) lacking the 9-amino acid exon, cAMP failed to regulate Na\(^+\)-dependent Ca\(^{2+}\) uptake. We suggest that this motif is responsible for the observed cAMP-dependent functional differences between the frog and the mammalian hearts.

The sarcolemmal Na\(^+\)-Ca\(^{2+}\) exchanger is one of the major Ca\(^{2+}\) extrusion pathways of the heart muscle. In nonmammalian species, the exchanger, in addition, may serve as a major Ca\(^{2+}\) influx pathway, as these hearts in general lack well developed intracellular Ca\(^{2+}\) release pools (1–3).

In mammalian species, most, if not all, tissues contain a transcript of the Na\(^+\)-Ca\(^{2+}\) exchanger (NCX1) gene (4–13) which undergoes tissue-specific alternative splicing. Two additional genes are expressed in the brain (NCX2 and NCX3) and skeletal muscle (NCX2), but the primary structure of the exchangers remains highly conserved, especially within the 11 putative transmembrane domains. Relatively greater divergence has been found in the N-terminal regions and the large intracellular loop between transmembrane domains 5 and 6, where the high affinity Ca\(^{2+}\) regulatory site is located (14). Even though a putative protein kinase A phosphorylation site has been also identified in the mammalian isoform (4), no functional evidence for the cAMP/protein kinase A-dependent phosphorylation of the exchanger has as yet been found. Recently, however, it has been shown that the Na\(^+\)-Ca\(^{2+}\) exchanger in frog but not in mammalian ventricular myocytes is regulated by isoproterenol via the activation of a \(\beta\)-adrenergoreceptor/adenylate-cyclase/cAMP-dependent pathway (15). In this report we describe the functional expression of a recombinant CAMP-sensitive frog heart Na\(^+\)-Ca\(^{2+}\) exchanger construct (fNCX1a) with a newly identified 9-amino acid exon which renders the molecule regulatable by cAMP.

**EXPERIMENTAL PROCEDURES**

**Electrophysiology**—Procedures to inject and maintain the Xenopus oocytes were identical to those described previously (16). For the recording of Na\(^+\)-Ca\(^{2+}\) exchange current the basic Cl\(^-\)-free extracellular (glass-funnel) solution contained (in mm): NaOH, 109; Ca(NO\(_3\))\(_2\), 5; MgSO\(_4\), 1; HEPES, 15; pH 7.3 (adjusted with methanesulfonic acid). Niflumic and flufenamic acids were used as Cl\(^-\) channel blockers (17). When necessary the extracellular Cl\(^-\) free solution was supplemented with either 1–5 mm Ni(NO\(_3\))\(_2\), 1 mm CdCl\(_2\), 500 \(\mu\)M CPT-cAMP, or their combinations. The intracellular Cl\(^-\) free solution contained (in mm): NaOH, 20; triethanolamine hydroxide, 100; HEPES, 10; Mg-ATP, 5; EGTA, 5; Ca(NO\(_3\))\(_2\), 3.2–4.99 (free Ca\(^{2+}\) 0.1–10 \(\mu\)M); pH 7.3 (adjusted with aspartic acid). All chemicals were purchased from Sigma, except for Ni(NO\(_3\))\(_2\) which was from Aldrich.

**Photometry**—To measure Na\(^+\)-dependent Ca\(^{2+}\) uptake both control and Ni\(^{2+}\)-free (fNCX1a)-expressing oocytes were injected with 50 ng/oocyte Ca\(^{2+}\)-sensitive photoprotein, aequorin (dissolved in 1 mm EDTA buffer), 3 to 4 h before the measurements. Aequorin-injected oocytes were loaded with Na\(^+\) by incubating them for 30 min at room temperature in K\(^+\)/Ca\(^{2+}\)-free Barth’s solution containing in mm: NaCl, 88; NaHCO\(_3\), 2.4; MgSO\(_4\), 0.82; HEPES, 15; pH 7.4 with NaOH, supplemented with 30 \(\mu\)M nystatin (Sigma, prepared as 10 mm stock in Me\(_2\)SO) and then transferred to K\(^+\)/Ca\(^{2+}\) -free Barth’s without nystatin (18). Ambient temperature in K\(^+\)/Ca\(^{2+}\)-free Barth’s was maintained at 20 °C. Photon emission was measured from Na\(^+\)-loaded oocytes placed in a scintillation vial at 6-s intervals in a liquid scintillation counter (model LS250, Beckman). First, the basal photon emission was estimated in a scintillation vial filled with 1 ml of conditioning K\(^+\)/Ca\(^{2+}\)-free Barth’s solution containing 5 mm CiCl\(_3\), 500 \(\mu\)Ci/Nco

**Screening of the Library and the Construction of the Full-length Frog Na\(^+\)-Ca\(^{2+}\) Exchanger**—The screening of Xenopus laevis heart cDNA library using a probe NcoI-Mini (nucleotides 269–2609) fragment of the dog cDNA (gift of K. D. Philipson, Los Angeles, CA) yielded two clones, H3 and H6. Further screening of the library did not produce clones which encode the N terminus. The remaining portion was retrieved from a X. laevis genomic library (Stratagene) using a PCR\(^1\)-derived

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§ The abbreviations used are: PCR, polymerase chain reaction; UTR, untranslated region; CPT-cAMP, 8-(4-chlorophenylthio)cAMP.
A Nucleotide-binding Motif in a Frog Na\(^+\)-Ca\(^{2+}\) Exchanger—The X. laevis heart cDNA library was screened with the cDNA pTB11, encoding dog heart Na\(^+\)-Ca\(^{2+}\) exchanger as a probe. Two overlapping partial cDNA clones, H3 (corresponding to amino acid residues 388–656) and H6 (residue 544–3'4TR), were isolated and sequenced (GenBank\(^\text{TM}\) accession no. X90838). Unlike H3, the H6 clone contained a 27-base pair insert located at a splicing junction between exons 7 and 8 (22) and representing a new exon (30).

The deduced amino acid sequence of H6 and H3 showed 87.8\% identity to the corresponding part of the dog sequence. However, unlike mammalian clones, the 27-base pair insertion generates an ATP/GTP binding motif (P-loop) by adding the GKS sequence to GKYLY (essential amino acids are underlined), thus forming the consensus P-loop structure (A/G)-K(G/S)-T (25). In the sequence encoded by H3 and H6, the putative protein kinase A phosphorylation site (4), and the Ca\(^{2+}\)-binding domain (26) seen in the mammalian NCX1 proteins are conserved (Fig. 1). To examine the functional role of this motif, we constructed the full-length frog Na\(^+\)-Ca\(^{2+}\) exchanger. Since the frog heart cDNA library lacked coding sequences for the N terminus of the Na\(^+\)-Ca\(^{2+}\) exchanger, the X. laevis genomic library was screened using a fragment (nucleotides 1626–1773) produced by PCR at the N terminus of the H3 clone. The stretch coding for the remainder of the region was found in X9 (GenBank\(^\text{TM}\) accession no. X90839). A small diversity was found in the region of the overlap between H3/H6 and X9 (534 nucleotides, 178 amino acids). Alignment of the amino acid sequences showed the identity of 92.1\%, and the similarity of 94.5\% (1-amino acid insertion, 13-amino acid changes, and 48 amino acid differences). The sequence for the N terminus of 90.5\% (1-amino acid insertion, 13-amino acid changes, and 48 amino acid differences).

Functional Expression of the Frog Na\(^+\)-Ca\(^{2+}\) Exchanger—Initially we were unable to express NCX1a in Xenopus oocytes. Two strategies were therefore employed to achieve functional expression. First strategy involved the replacement of the whole 3'-UTR of NCX1a with that of Na\(^+\)-glucose co-transporter clone pMJ*C424 (14), which includes a poly(A) tail (19) (gift of E. Wright, UCLA, CA). In addition, we replaced G for C in the –3 position to conform to a Kozak consensus sequence (27) and designated it pF1a/MC. In the second strategy, we replaced the 5'-UTR of NCX1a with that of alfalfa mosaic virus RNA-4 and attached a 90-nucleotide poly(A) tail to the 3'-UTR of NCX1a. This was done by subcloning the NCX1a coding and 3'-UTR into the transcription-competent vector pAGA (20).
FIG. 2. Expression of Na\(^{+}\)-Ca\(^{2+}\) exchanger current (\(I_{\text{Na-Ca}}\)) in fNCX1a-injected Xenopus oocytes. A, superimposed membrane currents obtained from fNCX1a-injected Xenopus oocyte in response to the envelope voltage-clamp protocol shown in the upper row. These as well as all other currents illustrated were recorded using the glass funnel technique (16) following oocyte superfusion with Cl\(^{-}\)-free extra- and intracellular solutions containing 109 mM Na\(^{+}\), 5 mM Ca\(^{2+}\) and 20 mM Na\(^{+}\) and 10 \(\mu\)M Ca\(^{2+}\), respectively. B, membrane currents obtained under the same experimental conditions but after external application of 3 mM Ni\(^{2+}\). C, Ni\(^{2+}\)-sensitive component of the current (\(I_{\text{Na-Ca}}\)) obtained as a result of the subtraction of current records shown in B from those in A. D, superimposed original current traces obtained in the absence and presence of 3 mM Ni\(^{2+}\) (as indicated) in response to the ramp voltage-clamp protocol shown above the records. The Ni\(^{2+}\)-sensitive \(I_{\text{Na-Ca}}\) was obtained after subtraction of the current recorded in presence of Ni\(^{2+}\) from those of control. E, current-voltage relation for Ni\(^{2+}\)-sensitive \(I_{\text{Na-Ca}}\) constructed from the ramp portion of the difference current presented in D. F, the differences in Na\(^{+}\)-dependent Ca\(^{2+}\) uptake in Na\(^{+}\)-loaded control and fNCX1a-injected oocytes in the presence and absence of 5 mM Ni\(^{2+}\) as estimated by photon emission of aequorin. Oocytes from both groups were injected with aequorin and loaded with Na\(^{+}\) by incubation for 30 min at room temperature in conditioning K\(^{+}\)/Ca\(^{2+}\)-free Barth’s solution supplemented with 30 \(\mu\)M nystatin. Photon emission was measured in both conditioning solution and following exposure of the oocytes to the test, K\(^{+}\)/Na\(^{+}\)-free Barth’s solution. The number of oocytes tested is indicated above each column. The asterisk (*) denotes significantly different values from the value in test, K\(^{+}\)/Na\(^{+}\)-free Barth’s solution at \(p < 0.05\).
(a gift of L. Birnbaumer, UCLA, CA). This plasmid was designated pF1a/AGA. We have chosen this strategy to verify: (a) if the whole 3'-UTR or only the poly(A) tail were important for translation, and (b) if the efficiency of the translation could be further increased by the presence of the 5'-UTR of alfalfa mosaic virus RNA-A. We found that the pF1a/AGA construct produced a 50% higher level of expression of the exchanger than did pF1a/MC (using \( I_{Na-Ca} \) in oocytes as a criterion). However, expression of pF1a/AGA was transient, reaching peak values within 24–48 h, and declining rapidly, while pF1a/MC produced a slow but steady level of expression for up to 6–7 days. Thus, the right Kozak consensus initiation site at the 5'-end and the presence of the poly(A) tail at the 3'-UTR are critical for the functional expression of the exchanger. Most of the experiments reported here were carried out using the pF1a/MC construct.

The frog exchanger cRNA was synthesized by transcription in vitro of the modified fNCX1a cDNA. Functional expression of the exchanger molecule was assessed 2–4 days later by direct measurements of the expressed current or monitoring of \( Na^+ \)-dependent \( Ca^{2+} \) uptake. Electrophysiological experiments were performed in Cl–free extra- and intracellular solutions, using the “glass-funnel” technique that permits both fast voltage-clamp and intracellular perfusion of devitellinated oocytes (16). \( Na^+ \)-dependent \( Ca^{2+} \) uptake was determined by measuring photon emission of \( Na^+ \)-loaded oocytes injected with the \( Ca^{2+} \)-sensitive photoprotein, aequorin, after exposure to \( Na^+ \)-free solution.

Fig. 2 illustrates the procedures used to isolate the inward and outward components of the membrane current carried by the \( Na^+ \)-Ca\(^{2+}\) exchanger in fNCX1a-injected Xenopus oocytes. The standard Cl–free experimental solutions contained 109 mM \( Na^+ \) and 5 mM \( Ca^{2+} \) on the outside and 20 mM \( Na^+ \) and 0.1–10 \( \mu M \) \( Ca^{2+} \) on the inside. Concentrations of free \( Ca^{2+} \) in the intracellular solution were estimated according to the Ca-Buf program (SPESCS) (28). In Cl–free internal and external solutions depolarization of the oocyte from a holding potential of −60 to +40 mV activated an outward current which was followed by a tail current on repolarization to −80 mV (Fig. 2A, lower panel). As the duration of the clamp pulse was prolonged, the outward current decayed slowly, and the tail currents following repolarization to −80 mV were enhanced (Fig. 2A). Exposure of the myocytes to 3 mM Ni\(^{2+}\) blocked a significant portion of both outward and accompanying inward tail currents (Fig. 2B). Subtraction of currents obtained, in the presence and absence of Ni\(^{2+}\), yielded a slowly decaying outward current followed by an expanding inward tail envelope (Fig. 2C), representing, respectively, the \( Ca^{2+} \) influx and \( Ca^{2+} \) efflux modes of the exchanger (15). 1 mM Cd\(^{2+}\) similarly suppressed the current generated by the \( Na^+ \)-Ca\(^{2+}\) exchanger in fNCX1a-injected oocytes (data not shown).

Fig. 2F documents the results of a series of experiments showing the differences in \( Na^+ \)-dependent \( Ca^{2+} \) uptake in the \( Na^+ \)-loaded control and fNCX1a-injected oocytes, and their sensitivity to Ni\(^{2+}\). Photoluminescence in oocytes (Ca\(^{2+}\) uptake) was measured in both conditioning control and K'/Na+-free Barth’s test solutions. In control oocytes no differences in photoluminescence were found between the conditioning and test solutions (Fig. 2F). On the other hand, oocytes injected with fNCX1a showed approximately 15-fold higher photoluminescence in the test compared with conditioning solutions (Fig. 2F), suggesting significant \( Na^+ \)-dependent \( Ca^{2+} \) uptake in response to the expression of fNCX1a. Photoluminescence in fNCX1a-injected oocytes was almost completely blocked by addition of 5 mM Ni\(^{2+}\) (Fig. 2F).

To measure voltage-dependence of the exchanger, the oocytes were first depolarized to +40 mV and then the voltage was linearly changed at 200 mV/s to −120 mV (ramp clamp protocol, Fig. 2D, upper panel). The lower panel of Fig. 2D shows superimposed traces of control currents, the current in the presence of 3 mM Ni\(^{2+}\), and the difference currents (Ni\(^{2+}\)-sensitive \( I_{Na-Ca} \)) activated by such a pulse protocol. The Ni\(^{2+}\)-sensitive \( I_{Na-Ca} \) had a reversal potential (\( E_{Na-Ca} \)) at +20 mV (Fig. 2E). The average experimental value for \( E_{Na-Ca} \) in 14 oocytes from different frogs injected with different samples of...
fNCX1a was +4.7 ± 2 mV, giving an $E_{\text{Ca}}$ of +62.8 mV, and suggesting an effective $[\text{Ca}^{2+}]_{i}$ of about 37 μM, (assuming a 3 Na+/1 Ca2+ stoichiometry, $[\text{Ca}^{2+}]_{o} = 5.0$ mm, $[\text{Na}^{+}]_{i} = 20$ mM, $[\text{Na}^{+}]_{o} = 109$ mM). The value of $E_{\text{Ca}}$ did not correspond to the buffered concentration of Ca2+ (0.1–10 μM) in the perfusing internal solution. This observation suggests that Ca2+ entering the oocyte via the exchanger during the conditioning depolarization (e.g. Fig. 2, A–D) might accumulate in a confined intracellular space in the vicinity of the membrane.

To verify this hypothesis we employed a pulse protocol in which the negative voltage ramp was preceded by progressively longer conditioning depolarization to +40 mV, thus increasing the entry of Ca2+ into the oocyte prior to the application of voltage ramp (Fig. 3A, upper panel). Fig. 3A (lower panel) shows that prolonging the depolarizing pulse at 40 mV shifted the reversal potential of the exchanger current to more positive values of 3.2, 15, 21.8, 26.8, and 31.8 mV, respectively (Fig. 3B). At 5 mM [Ca2+], and with external and internal Na+ of 109 and 20 mM, respectively, the measured reversal potential suggests an effective $[\text{Ca}^{2+}]_{i}$ of 35, 55.5, 72.3, 88, and 107 μM, using $E_{\text{Na-Ca}} = 3E_{\text{Na}} - 2E_{\text{Ca}}$. The experimentally measured shifts of the reversal potential, $\Delta E_{\text{Na-Ca}} = E_{\text{Na-Ca}(1)} - E_{\text{Na-Ca}(2)}$, corresponded well with the changes in the effective internal Ca2+ concentration in accordance with the relation, $\Delta E_{\text{Na-Ca}} = E_{\text{Na-Ca(1)}} - E_{\text{Na-Ca(2)}} = 59\log([\text{Ca}^{2+}]_{i(1)}/[\text{Ca}^{2+}]_{i(2)})$, supporting the idea that Ca2+ entering the oocyte via the exchanger accumulates in a confined intracellular space in the proximity of the membrane.

Digital integration of the current traces of Fig. 3A provided an estimate of the total amount of Ca2+ entering the oocyte until the exchanger reaches its reversal potential. The total amount of Ca2+ transported and the effective $[\text{Ca}^{2+}]_{i}$, determined from the measurements of the reversal potential, allows estimation of the size of the space equivalent to 27 nl, i.e. ~2.7% of the average volume of the oocyte (1 μl).

**Modulation of Exchanger Activity by cAMP—**In the frog ventricular myocytes the Na+–Ca2+ exchanger is suppressed via the β-adrenergoreceptor/adenylate-cyclase/cAMP-dependent pathway (15). Since the defolliculated and devitellinated oocytes lack β-adrenergoreceptor- and forskolin-stimulated adenylate cyclase (29), the membrane-permeable cAMP analog CPT-cAMP was used to determine the cAMP sensitivity of fNCX1a-injected oocytes. Fig. 4A shows that $I_{\text{Na-Ca}}$ at 40 mV is slowly suppressed by 50–60% ($n = 4$) on rapid (<500 ms) application of CPT-cAMP. In the presence of CPT-cAMP, 5 mM Ni2+ failed to further inhibit the current, suggesting that virtually all the Ni2+-sensitive $I_{\text{Na-Ca}}$ had been inhibited by CPT-cAMP. The CPT-cAMP-mediated suppression of the exchanger current was
CAMP-dependent Regulation of Na$^{+}$-Ca$^{2+}$ Exchanger

We have successfully expressed a functional CAMP-regulated frog Na$^{+}$-Ca$^{2+}$-exchanger (fNCX1a) in Xenopus oocytes. The most distinguishing structural feature of the construct is the presence of a consensus ATP/GTP binding 9-amino acid motif (P-loop), located between residues 636 and 646 of the main cytoplasmic linker. Deletion of this motif from the clone (ΔfNCX1a) abolished the CAMP-dependent regulation of the exchanger (Fig. 5). fNCX1a, constructed from H3/H6 and a genomic clone X9, contained a small (7.9%) divergence in the overlapping 178-amino acid sequence and only 6.4% divergence at the nucleotide level at the same region. This divergence is in the order of interspecies polymorphism for orthologous genes, since it is substantially lower than that observed between the genes of the same family in mammals (e.g. 35–40% divergence in rat) (23). Whether this divergence arises from polymorphic variation of the frog subspecies (where the structure of genus Xenopus is not well defined) or from the unlikely presence of a novel member of NCX family is not as yet clear. Irrespective of whether the clone represents a chimera of orthologous genes from related species, the presence of the putative protein kinase A phosphorylation site and Ca$^{2+}$ binding domain, the similarity in CAMP-mediated regulation between the expressed protein and the exchanger in native frog myocytes (15), as well as the abundance of fNCX1a sequence in mRNA of frog heart compared with other tissues, suggests that the clone represents a legitimate molecular model to study the functional implication of the novel motif.

Voltage clamp studies in early 1970s have shown that developed tension in frog heart has a dominant tonic and a small phasic component (1, 2, 31). In the presence of catecholamines, however, the phasic $I_{Na-Ca}$ component of tension is strongly enhanced, while the sigmoid Na$^{+}$-dependent tonic component is strongly suppressed (32). This dual effect of catecholamines was thought to result from both increased Ca$^{2+}$ influx via the Ca$^{2+}$ channels and enhanced uptake of Ca$^{2+}$ by the sarcoplasmic reticulum. In light of recent studies showing the virtual absence of SERCA II gene of Ca-ATPase and phospholamban proteins in the frog heart (33–35), the exchanger may be the molecular site that mediates the relaxant effects of catecholamines. At first the suppressive effect of CAMP on the exchanger appears contraintuitive, but considering that the Ca$^{2+}$ channel and the plateau of the action potential are significantly enhanced in the presence of catecholamines (32, 36), necessarily increasing Ca$^{2+}$ influx via the exchanger, the CAMP-dependent suppression of the exchanger may be the appropriate evolutionary solution to stem the tide of large Ca$^{2+}$ influx that would result. Thus, the suppression of the tonic Ca$^{2+}$ influx pathway may contribute to the early fall in tension observed during depolarizing pulses in the presence of isoproterenol (32).

The $\beta$-agonist/protein kinase A-induced temporal shift of the fraction of contractile Ca$^{2+}$, transported into the cell during the action potential from the exchanger to the Ca$^{2+}$ channel, may be related to the evolutionary requirement of the fight and flight reflex in almost all nonmammalian vertebrates (and possibly prenatal mammals) lacking significant SERCA II and intracellular Ca$^{2+}$ release pools. Thus, the heart of these animals may utilize the same $\beta$-adrenergic regulatory mechanism for both the control of phasic (Ca$^{2+}$ channel) and tonic (exchanger) transport of Ca$^{2+}$ and development of tension. Under sedentary conditions the exchanger would primarily deliver isofrom, consistent with the idea that the 9-amino acid domain, is critical for CAMP-mediated regulation of the exchanger.

**Discussion**

We have successfully expressed a functional CAMP-regulated frog Na$^{+}$-Ca$^{2+}$-exchanger (fNCX1a) in Xenopus oocytes. The most distinguishing structural feature of the construct is the presence of a consensus ATP/GTP binding 9-amino acid motif (P-loop), located between residues 636 and 646 of the main cytoplasmic linker. Deletion of this motif from the clone (ΔfNCX1a) abolished the CAMP-dependent regulation of the exchanger (Fig. 5). fNCX1a, constructed from H3/H6 and a genomic clone X9, contained a small (7.9%) divergence in the overlapping 178-amino acid sequence and only 6.4% divergence at the nucleotide level at the same region. This divergence is in the order of interspecies polymorphism for orthologous genes, since it is substantially lower than that observed between the genes of the same family in mammals (e.g. 35–40% divergence in rat) (23). Whether this divergence arises from polymorphic variation of the frog subspecies (where the structure of genus Xenopus is not well defined) or from the unlikely presence of a novel member of NCX family is not as yet clear. Irrespective of whether the clone represents a chimera of orthologous genes from related species, the presence of the putative protein kinase A phosphorylation site and Ca$^{2+}$ binding domain, the similarity in CAMP-mediated regulation between the expressed protein and the exchanger in native frog myocytes (15), as well as the abundance of fNCX1a sequence in mRNA of frog heart compared with other tissues, suggests that the clone represents a legitimate molecular model to study the functional implication of the novel motif.

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A. Kraev and E. Carafole, unpublished data.
and extrude the contractile Ca\(^{2+}\) into and out of the cell. Upon sympathetic stimulation, as the heart shifts to the faster Ca\(^{2+}\) delivery pathway via the phosphorylated Ca\(^{2+}\) channel, the influx of Ca\(^{2+}\) via the exchanger would be suppressed, providing the heart with faster but shorter contractions to accommodate the faster heart rate. Whether such a protein kinase A-dependent-regulatory mechanism can be made to operate by genetic manipulation of the mammalian Na\(^{+}\)-Ca\(^{2+}\) exchanger when the exchanger is overexpressed (37, 38) in heart failure remains to be tested.

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