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Molecular cloning and functional expression of the guinea pig cardiac Na^+–Ca^{2+} exchanger

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Abstract

The cDNA of the guinea pig cardiac Na^+–Ca^{2+} exchanger was cloned from a λZAP cDNA library. The deduced sequence of the protein corresponds to 970 amino acids and is 98% identical to the canine cardiac exchanger. The leader peptide region shows substantial variation among species. The cloned cDNA can induce Na^+–Ca^{2+} exchange activity when in vitro transcribed cRNA is injected into *Xenopus laevis* oocytes.

Keywords: Sodium–calcium ion exchanger; cDNA cloning ; (Guinea pig); (*X. laevis* oocyte)

The Na^+–Ca^{2+} exchanger is a highly active counter-transporter in the plasma membrane that uses the electrochemical energy of the Na^+ gradient to remove Ca^{2+} ions from cells [1,2]. The stoichiometry is 3 Na^+ to 1 Ca^{2+} [3,4]. In myocardial cells, the sarcolemmal Na^+–Ca^{2+} exchanger is the dominant system to extrude Ca^{2+} which enters the cell during the cardiac action potential [2,5,6]. The Na^+–Ca^{2+} exchanger is an important regulator of excitation-contraction coupling in cardiac muscle [7,8].

Nicoll et al. [9] cloned the Na^+–Ca^{2+} exchanger from a canine heart cDNA library by antibody screening. Recently, the Na^+–Ca^{2+} exchangers from human heart [10,11], bovine heart [12], rabbit kidney [13], rat heart [14], and rat brain [15] have been cloned and sequenced. The open reading frames of the cloned dog, rat, and human heart Na^+–Ca^{2+} exchangers code for proteins of 970, 971, and 973 amino acids, respectively.

Ventricular myocytes from the guinea pig are often used in physiological studies of excitation-contraction coupling. It is of interest to see if the Na^+–Ca^{2+} exchanger from this species is similar to the canine cardiac exchanger, for which the most structural and functional information is available. We report here the cloning, sequencing, and functional expression in *Xenopus laevis* oocytes of the guinea pig cardiac Na^+–Ca^{2+} exchanger.

A λZAP cDNA library (Dr. A. McDonough, USC School of Medicine, Los Angeles, CA) was screened with inserts from pA4 [9] as probes as described previously [9]. Approximately $5 \cdot 10^5$ plaques were probed to find the full length cDNA clone described here. Nucleotide sequencing was performed by the method of dideoxynucleotide termination [16] using the Sequenase system (US Biochemical, USA). Restriction fragments generated from the cDNA insert by digesting with restriction enzymes (*Eco*RI, *Kpn*I, *Apa*I, *Pst*I) were subcloned into Bluescript II SK^+ plasmid (Stratagene, USA) and were sequenced from both directions. The sequence of the cDNA was assembled and analyzed by MacVector and AssemblyLign program (Kodak, USA). A single open reading frame of 2910 nucleotides encodes a protein of 970 amino acids (Fig. 1). The first ATG is 23-bp from the 5′-end of the clone. The nucleotide sequence around this putative translation start site, CAACATG, is similar to the Kozak consensus initiation site [17]. In the open reading frame, the nucleic acid sequence of the guinea pig clone is 91%, 93%, and 89% identical to canine, human, and rat cardiac exchangers,

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respectively. The deduced amino acid sequence is about 98%, 98%, and 95% identical to the canine, human, and rat heart exchangers, respectively.

To demonstrate the function of the cloned Na^+–Ca^{2+} exchanger, cRNA was prepared using T7 polymerase, and the RNA was injected into *Xenopus laevis* oocytes, which were incubated at 18°C. Three days later, the oocytes were loaded with Na^+ using nystatin and assayed for Na^+–Ca^{2+} exchange activity by measuring Na^+-gradient dependent $^{45}Ca^{2+}$ uptake in either 100 mM KCl or 100 mM NaCl as

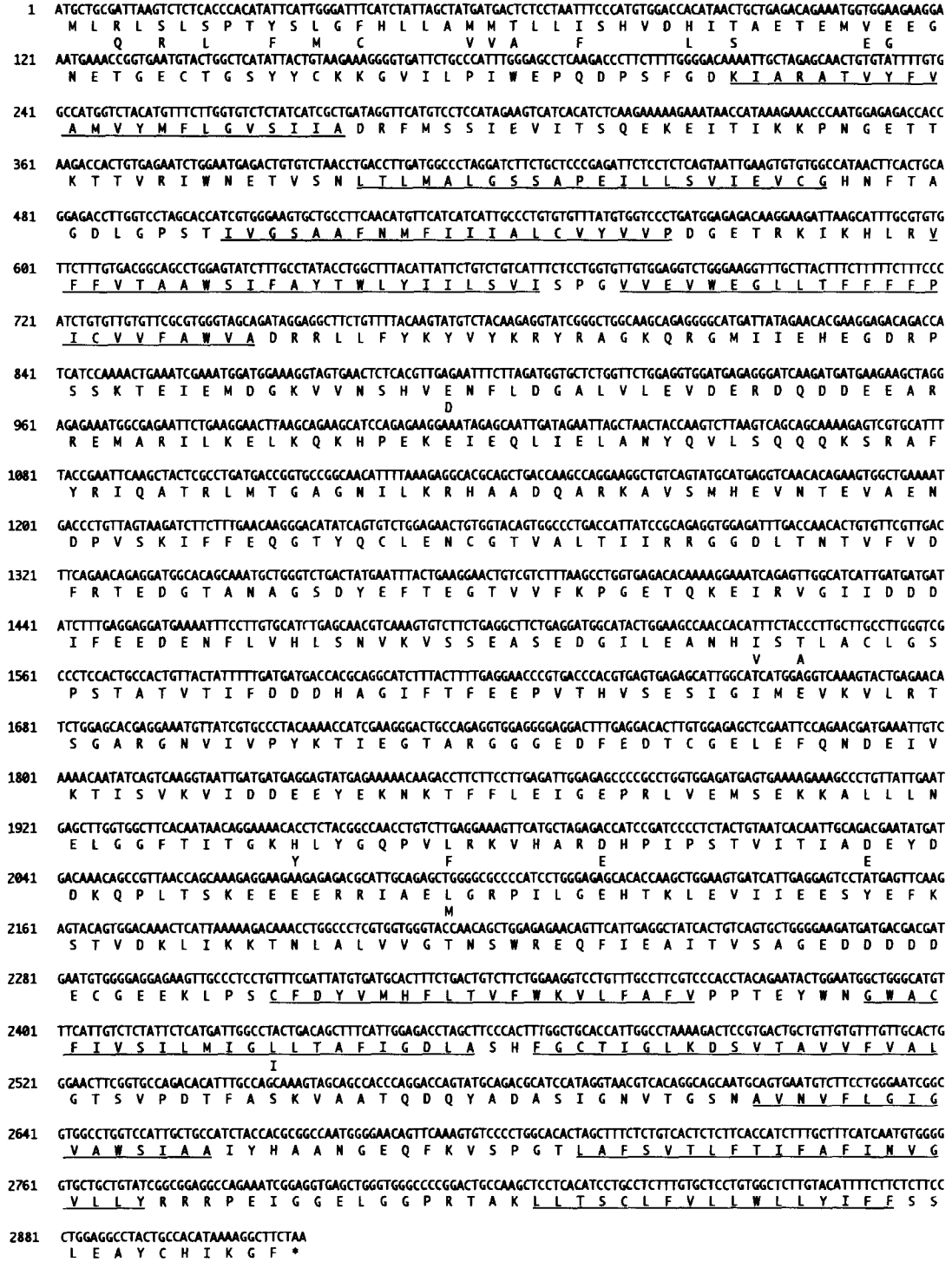

Fig. 1. Nucleotide and amino acid sequences of the guinea pig Na^+–Ca^{2+} exchanger. A third line, when present, indicates differences in the amino acid sequence from that of the canine Na^+–Ca^{2+} exchanger [9]. The putative membrane-spanning regions [9] are underlined. The guinea pig cardiac exchanger nucleotide and amino acid sequence data are available through GenBank, accession number U04955.

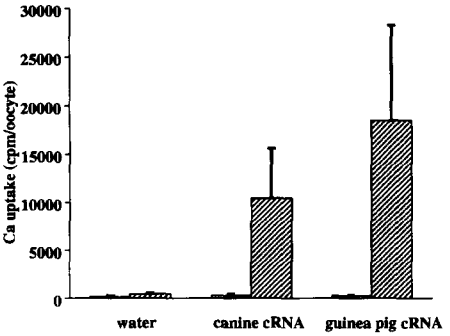

Fig. 2. Expression of Na^+–Ca^{2+} exchange activity in *Xenopus laevis* oocytes. Na^+ gradient-dependent uptake of $^{45}Ca^{2+}$ (hatched bars) was measured by placing the oocytes in K^+ medium. The background, Na^+-gradient independent $^{45}Ca^{2+}$ uptake (open bars), was determined by placing the oocytes into high-Na^+ medium. Oocytes were injected with 50 nl of water or cRNA (50 ng) synthesized from cDNA for the canine or guinea-pig cardiac Na^+–Ca^{2+} exchanger. Bars represent means ± S.D. for $n = 8$.

described previously [9,18]. The results demonstrate that the cDNA clone encodes a functionally active Na^+–Ca^{2+} exchanger (Fig. 2). The magnitude of Ca^{2+} uptake in the oocytes injected with the cRNA from the guinea pig cardiac exchanger clone was comparable to that seen with canine cardiac exchanger cRNA. No Na^+ gradient-dependent Ca^{2+} uptake was seen in control water-injected oocytes. There was no significant Ca^{2+} uptake without Na^+ loading (not shown) or when Na^+ was present in the external solution.

In the 970 amino acids of the exchanger open reading frame, there are 23 differences between the canine and guinea pig cardiac Na^+–Ca^{2+} exchangers. Twelve of these differences are located in the initial 32 amino acids which comprise a cleaved leader peptide region [19–21]. Of the other eleven differences, only one (Leu 778) is modeled to be intramembrane and most are very conservative changes. Therefore, the mature canine and guinea pig cardiac Na^+–Ca^{2+} exchangers appear to be structurally very similar. Large variations in the leader peptide region have been noted previously, and the sequences of this region for six species are shown in Fig. 3. Only nine of 32 amino acids are conserved for all species.

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                 .   . .   . .       .    ..   .
guinea pig       MLRLSLSPTY SLGFHLLAMM TLLISHVDHI TA
canine           MLQLRLLPTF SMGCHLLAVV ALLFSHVDLI SA
rat              MLRLSLPPNV SMGFRLVTLV ALLFTHVDHI TA
bovine           MLQFSLSPTL SMGFHVIAMV ALLFSHVDHI SA
rabbit           MPRFSLSPPF SMGFHLLAIV ALFFFRVDHV SA
human            MRRLSLSPTF SMGFHLLVTV SLLFSHVDHV IA
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Fig. 3. Comparison of amino acid sequences of the cleaved leader peptide region of guinea pig, canine [9], rat [14,15], bovine [12], and human [10,11] cardiac and rabbit kidney [13] Na^+–Ca^{2+} exchangers. Amino acids conserved in all species are indicated by the dots at top.

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