Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 339 (2006) 443–449
BBRC
www.elsevier.com/locate/ybbrc

Cloning of a sea urchin sarco/endoplasmic reticulum Ca^{2+}ATPase

Herath Jayantha Gunaratne *, Victor D. Vacquier

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0202, USA

Received 24 October 2005
Available online 14 November 2005

Abstract

Sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA), a vesicular integral membrane protein, is the best-characterized member of the P-type ion translocating ATPase superfamily. Here we describe the cloning and structural analysis of a sea urchin SERCA (suSERCA) cloned from testis cDNA. The ~112 kDa suSERCA is 1022 amino acids with ~70% identity and 80% similarity to all known mammalian SERCA isoforms. suSERCA shares all the structural features of mammalian SERCAs, including domains: A, actuator; N, nucleotide-binding; and P, phosphorylation, and also 10 transmembrane helices. Like human SERCA2, the suSERCA has a possible 11th transmembrane segment in its extreme C-terminus. The alignment of three sequences (suSERCA, human SERCA2, and rabbit SERCA1a) shows that the Ca^{2+} binding residues and kinks (required to form the ion-binding pocket) are 100% conserved. The annotated suSERCA gene consists of 24 exons separated by 23 introns and is ~30 kb. Western blots show that suSERCA is present in sea urchin eggs and testis, but not in mature spermatozoa. Treatment of live sperm with SERCA inhibitors has no effect on intracellular calcium, suggesting the absence of SERCA in sea urchin spermatozoa.

Keywords: Calcium homeostasis; Protein topology; Transporters; Spermatozoa

Calcium ATPases (Ca^{2+} pumps) play a major role in Ca^{2+} homeostasis. Their activity is needed for viability and also to mediate Ca^{2+} signaling [1]. Three Ca^{2+} ATPases, named plasma membrane Ca^{2+} ATPase (PMCA)[1], sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), and secretory pathway Ca^{2+} ATPase (SPCA), are known in animal cells. PMCA exports cytosolic Ca^{2+} to the outside of cells, whereas the other two sequester Ca^{2+} within intracellular organelles. Among the three Ca^{2+} ATPases, SERCA governs the major cytoplasmic Ca^{2+}-vesicle sequestering mechanism, which regulates Ca^{2+} levels in the cytosol. By maintaining high luminal Ca^{2+} levels, SERCAs support post-translational modification and transit of newly synthesized proteins across the sarco/endoplasmic reticulum (SER) [2], maintain the ability of the SER to control intracellular signaling by ryanodine and inositol triphosphate receptors [3], and regulate Ca^{2+} influx via store-operated Ca^{2+} channels [4]. Three mammalian SERCAs (SERCA1–3) encoded by three genes (*ATP2A1–3*) are known, which form alternatively spliced isoforms that show distinct patterns of tissue expression [5–10].

The crystal structures of several conformations of rabbit skeletal muscle SERCA (rSERCA1a) are known [11–15]. The structures led to the formulation of models, suggesting how different parts of the protein relate to its activity [11–15]. Here we describe cloning of a SERCA from sea urchin (suSERCA) and discuss its structural conservation. The gene structure, phylogenetic analysis, expression in gametes, and effect of SERCA inhibitors on intracellular

* Corresponding author. Fax: +1 858 534 7313.
E-mail address: hgunarat@ucsd.edu (H.J. Gunaratne).

[1] *Abbreviations:* AR, acrosome reaction; ASW, artificial sea water; CPA, cyclopiazonic acid; DMSO, dimethyl sulfoxide; NCKX, plasma membrane K^+ dependent Na^+/Ca^{2+} exchanger; PMCA, plasma membrane Ca^{2+} ATPase; SER, sarco/endoplasmic reticulum; SERCA, sarco/endoplasmic reticulum Ca^{2+}ATPase; SPCA, secretory pathway Ca^{2+} ATPase; TG, thapsigargin.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.11.033

calcium levels in sea urchin spermatozoa are also presented.

Material and methods

Cloning. The full-length suSERCA cDNA was cloned by PCR amplification using a *Strongylocentrotus purpuratus* testis cDNA library in Lambda Zap II (Stratagene) as template. Testis cDNA was also synthesized from total testis RNA by standard methods. RT-PCR was performed using exact match primers based on human SERCA sequences from a BLAST search at http://www.ncbi.nlm.nih.gov/blast/ and from the Sea Urchin Genome Project (http://www.hgsc.bcm.tmc.edu/projects/seaurchin/). PCR products were cloned into pCR-XL-TOPO (Invitrogen) and sequenced with gene specific primers and vector primers. The exon–intron map of suSERCA was constructed from the assembled sea urchin genome (http://www.hgsc.bcm.tmc.edu/projects/seaurchin/). Sites and motifs were identified using Prosite (http://ca.expasy.org). The suSERCA is deposited in GenBank under Accession No. DQ233466.

Sequence and structural analysis. MacVector and BioEdit were used to make alignments and hydropathy plots. The suSERCA sequence was threaded onto the crystal structure of rabbit skeletal muscle SERCA, chain 1A ([15], NCBI Protein Structural Data Base, Accession No. 1XP5) using the ESyPred3D server at http://www.fundp.ac.be/urbm/bioinfo/esypred/ [16].

Phylogenetic analysis. Complete sequences were used to construct a neighbor-joining phylogenetic tree of SERCA proteins (1000 replications) using Mega 3 software [17]. GenBank accession numbers for the SERCA sequences used are: *Danio rerio* (drSERCA), AAH85636; *Drosophila melanogaster* (dmSERCA), P22700; *Gallus gallus* (ggSERCA), NP990222; *Heliothis virescens* (hvSERCA), AAD09820; human (hSERCA1–3), O14983, P16615, Q93084; *Makaira nigricans* (mnSERCA), AAB08098; *Panulirus argus* (paSERCA), CAH10336; *Paramecium tetraurelia* (ptSERCA), CAA76764; *Placopecten magellanicus* (pmSERCA), AAC63909; *Plasmodium falciparum* (pfSERCA), BAD73967; *Procambarus clarkia* (pcSERCA), AAB82291; rabbit-chain A (rSERCA1), 1IWO; *Rana clamitans* (rcSERCA), CAC20853; and *Trypanosoma cruzi* (tcSERCA), AAD08694. The sea urchin plasma membrane Ca^{2+} ATPase (suPMCA, GenBank Accession No. DQ009662) was used as an out-group.

Western blotting. Sea urchins were spawned by injection of 0.5 M KCl into adults to collect sperm and eggs. Gametes were stored on ice <12 h before use. Minced whole testis was used to prepare the testis sample. All samples were dissolved in 10% SDS, boiled for 5 min, and precipitated in 80% acetone. Proteins were estimated by the BCA method (Pierce), separated by SDS/PAGE (10%) (10 μg protein per lane), and transferred to a nitrocellulose membrane. Blots were blocked with 5% milk in TBST (25 mM Tris, pH 8, 150 mM NaCl, and 0.05% v/v Tween 20) for 1 h followed by incubation with monoclonal antibody Y1F4 anti-SERCA antibody (a gift from J.M. East, Southampton University, UK) for either 1.5 h at room temperature or overnight at 4 °C. Proteins were detected with an HRP-conjugated goat anti-mouse secondary antibody and developed with SuperSignal West Dura Extended Duration Substrate (Pierce) according to manufacturer's protocol.

Measurement of intracellular Ca^{2+}. Sea urchin sperm were loaded with fura-2-AM (Molecular Probes) and washed as described [18]. Artificial seawater (ASW) was formulated as follows: 486 mM NaCl/10 mM $CaCl_2$/10 mM KCl/27 mM $MgCl_2$/29 mM $MgSO_4$/2.5 mM $NaHCO_3$/10 mM Hepes, adjusted to pH 7.9 with 1 N NaOH. For Ca^{2+} measurements, 50 μl of fura-2-loaded sperm (4×10^8 cells/ml) were diluted into 1.45 ml ASW in a FluoroMax-2 fluorometer with excitation at 340 and 380 nm and emission at 500 nm (16 °C). Thapsigargin (TG) and cyclopiazonic acid (CPA) (Sigma) were dissolved in DMSO to 5 mM and the volume of DMSO added to the cuvette never exceeded 0.6% (v/v). The ratio of emission intensities at 340 and 380 nm excitation (F340/F380) reports relative intracellular Ca^{2+} concentrations.

Results

suSERCA sequence

The open reading frame of full-length suSERCA is 3066-bp, encoding a 1022 amino acid protein with a calculated molecular mass of ~112 kDa (Fig. 1). The alignment with rabbit SERCA1a (rSERCA1a) and human SERCA2 (hSERCA2) shows extreme conservation. There is 70% identity for hSERCA2 and 69% for rSERCA1a. In the first 10 putative transmembrane segments (M1–M10 underlined), the percent identity is ~85% with both the rabbit and the human proteins. There are 11 protein kinase-C phosphorylation sites in suSERCA (12 in rSERCA1a, and 11 in hSERCA2). There are no classical protein kinase-A phosphorylation sites in these three sequences. There are four sites for potential N-linked glycosylation in suSERCA (seven in rSERCA1a and five in hSERCA2). These PKC sites and glycosylation sites are all in the putative cytoplasmic loops. The 10 transmembrane domains of all SERCAs are underlined in Fig. 1. The hydrophobic C-terminus of suSERCA and hSERCA2 (Fig. 1) could be a 11th transmembrane domain, as shown in the hydropathy plot (Fig. 2).

suSERCA domains

The major structural features of suSERCA were analyzed by comparison to the crystal structure of rabbit SERCA1a. The comparison shows that suSERCA has all the features of typical SERCAs. These include three cytoplasmic loops, 10 transmembrane α-helices, and five luminal loops. The larger luminal loop between M7 and M8 is characteristic of SERCAs (Fig. 3). The cytoplasmic motifs are designated: A, actuator; N, nucleotide binding; and P, phosphorylation. The N domain, which contains the adenosine binding site, interrupts the two segments that form the P domain. The γ-phosphate of ATP binds to Asp^{351} of the rabbit and human proteins; in suSERCA, this is Asp^{352} (Fig. 1). Ca^{2+} binding amino acids are in M4–M6 and M8, and are 100% identical among the three sequences (Fig. 1, asterisks). The rSERCA1a crystal structure shows that M4 and M6 are interrupted, forming kinks that act as ion-binding pockets [12,19]; these kinks are 100% identical among these three sequences (Fig. 1).

Gene structure

An exon–intron map was constructed by comparison of the SERCA cDNA to the sea urchin genome. The NCBI gene list shows that there is only one SERCA gene in this species. suSERCA consists of 24 exons (Fig. 4A). The gene region coding for the N-terminal half of suSERCA has longer introns than the region coding for the C-terminal half. The first two exons are found in one sea urchin genome contig. (Spur_v1.5/Scaffold59529). All other exons are found in another contig, (Spur_v1.5/Scaffold1777)

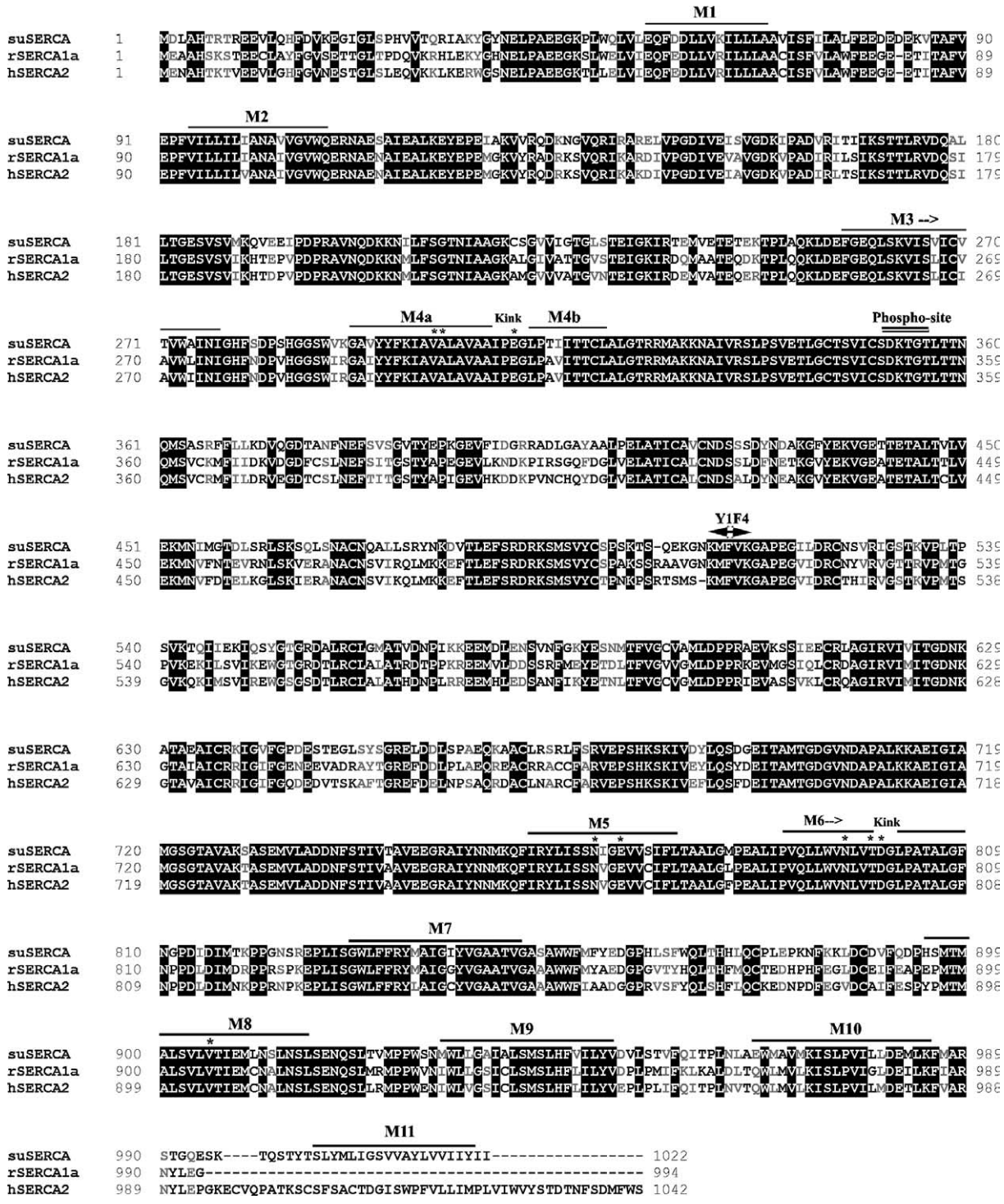

Fig. 1. Sequence alignment of suSERCA, rabbit SERCA1a and human SERCA2. Putative transmembrane segments are labeled M1–M11. Ca^{2+} binding residues (*) and kinks that form ion-binding pockets are marked. ATPase phosphorylation site and the anti-SERCA epitope are labeled as Phospho-site and Y1F4.

(*S. purpuratus* Genome Assembly of 2005-07-18). Representation of each exon in the protein structure is depicted in Fig. 4B. Exons 1–9 cover the A domain and first four transmembrane helices. Exons 10–19 represent the catalytic core (P and N domains) and exons 20–24 cover the C-terminus containing 6.5 transmembrane helices.

Phylogenetic analysis

Phylogenetic analysis based on full-length SERCAs (Fig. 5) shows that suSERCA branches at the base of the neighbor-joining tree of vertebrate SERCA orthologs. This branching topology is consistent with the evolutionary

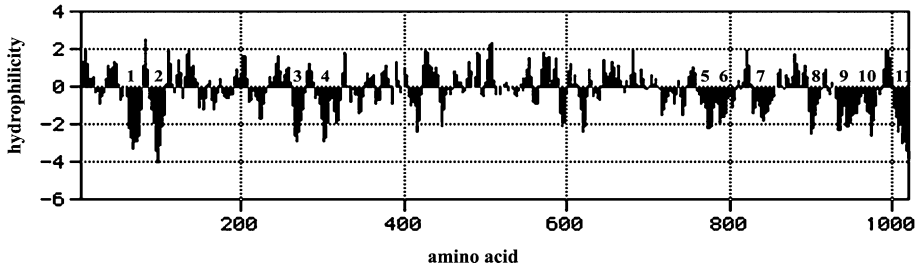

Fig. 2. Kyte–Doolittle hydropathy plot of suSERCA with a window size of 10, showing 11 putative transmembrane segments.

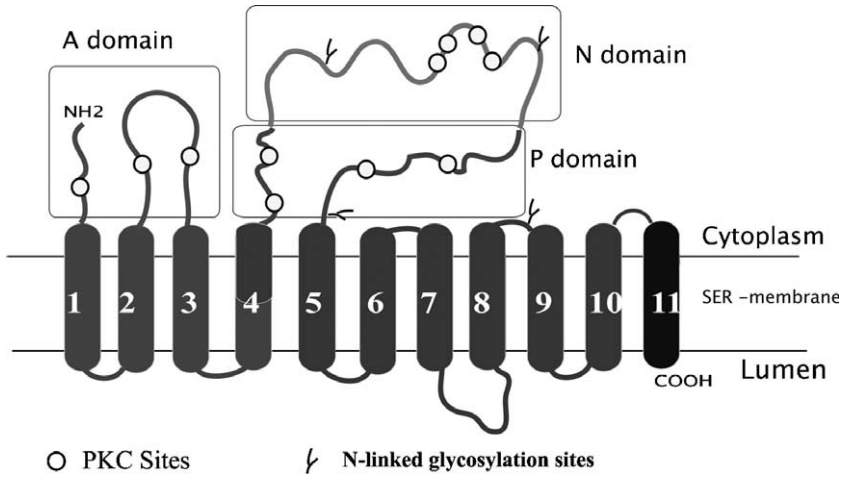

Fig. 3. Topology of suSERCA. Three major domains; actuator (A), nucleotide (N), and phosphorylation (P), which are on the cytoplasmic face, are boxed. Eleven putative PKC-sites and four potential N-linked glycosylation sites are all in cytoplasmic loops. Predicted 11 transmembrane helices are numbered.

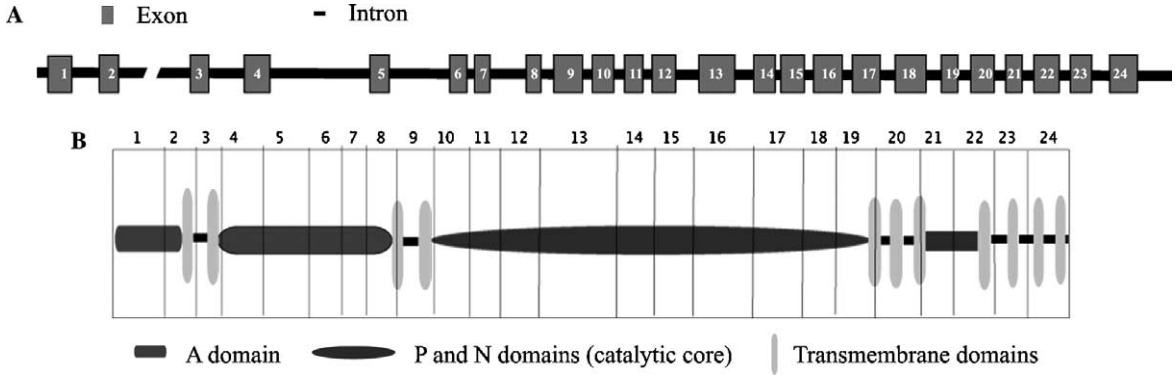

Fig. 4. suSERCA exon–intron structure. There are 24 exons in suSERCA (A). Relative lengths (not to exact scale) and position were gathered by comparing the cDNA sequence to the genome sequence (Genome Assembly database of 2005-07-18). The positioning of each exon is mapped onto the suSERCA protein (B).

position of sea urchins (Phylum Echinodermata) as basal deuterostomes.

Western blotting

Immunoblot analysis of suSERCA was performed using SERCA specific monoclonal antibody to the epitope, KMFVK, which is present in most SERCAs (Fig. 1, in suSERCA positions 511–515) [20]. At equal protein concentrations, strong signals from eggs and weaker signals from whole testis were detected at ~115 kDa. No reaction occurred with mature sea urchin spermatozoa (Fig. 6).

Ca^{2+} measurement

The effects of two SERCA inhibitors were investigated. The concentration ranges of 0.5–10 μM thapsigargin (TG) and 10–100 μM cyclopiazonic acid (CPA) were used to

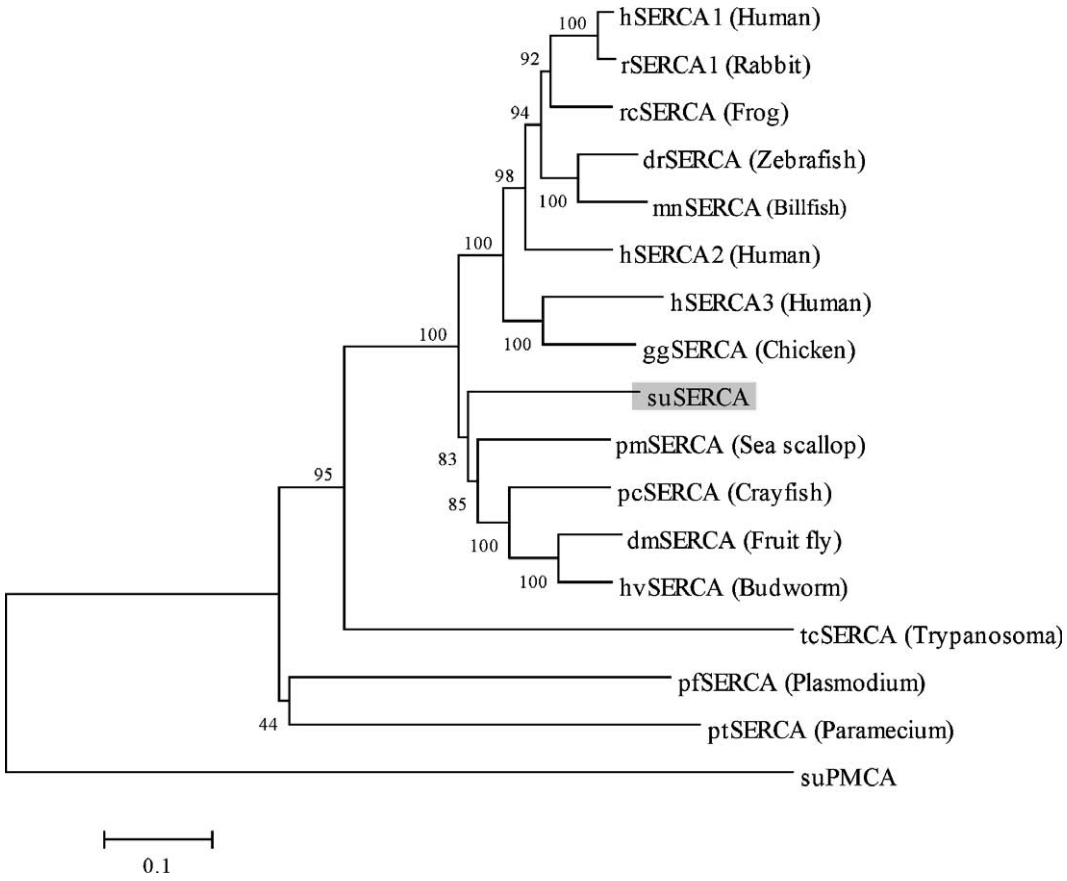

Fig. 5. A neighbor-joining tree of selected SERCAs and suSERCA. The tree was constructed with full-length sequences using suPMCA as the out-group. Values at branch points are bootstrap percentages with 1000 replications. Scale bar indicates percent amino acid difference with Poisson correction.

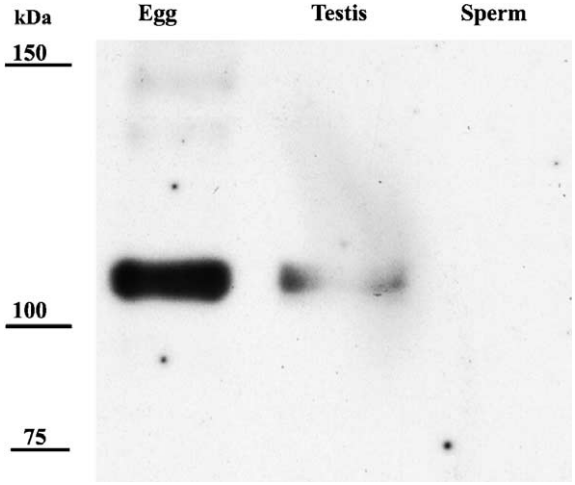

Fig. 6. Western blot of suSERCA. A band at ~115 kDa was detected in the egg and testis samples, but not in the mature sperm sample, with anti-SERCA (Y1F4A) antibody. Ten microgram of each protein was loaded in each lane.

monitor intracellular Ca^{2+} in fura-2 loaded sperm. Concentrations from 0 to 2.5 μM TG had no effect on intracellular Ca^{2+}. At 10 μM TG, increases in intracellular Ca^{2+} began to occur. However, even at 100 μM CPA, no elevation of Ca^{2+} was observed.

Discussion

Ca^{2+} activates many cellular responses when it enters the cytosol. However, it must be rapidly returned to sub-micromolar levels by sequestration in vesicles. SERCA plays a major role in many cell types by pumping Ca^{2+} from the cytosol into vesicles against the Ca^{2+} concentration gradient. Here we present the first cloning of a SERCA from a sea urchin and characterize its structural features in comparison to mammalian SERCAs. The suSERCA has extensive sequence homology to all known vertebrate SERCAs. The major domains of A, P, and N, the transmembrane helices, and the ion-binding segments are all well conserved. In mammals, two Ca^{2+} ions are moved across the membrane into the vesicle per one ATP-hydrolyzed [11–15,21]. We assume from structural conservation that suSERCA exhibits the same stoichiometry. The Ca^{2+} pumping process is associated with H^+ counter-transport and possibly water transport [22]. This suggests that SERCA might contribute to the regulation of other cellular parameters such as intracellular pH.

Although 10 transmembrane segments are the common structural theme for Ca^{2+} ATPases, some SERCA orthologs (e.g., hSERCA2) are thought to have a 11th transmembrane segment at the extreme C-terminus [23].

Hydropathy analysis of suSERCA (Fig. 2) suggests a 11th transmembrane segment.

We found that suSERCA is present in eggs and testis tissue, but not in mature spermatozoa. This is consistent with the fact that sea urchin eggs contain a large population of Ca^{2+} sequestering vesicles that are used in pharmacological studies to assay compounds that release Ca^{2+} from vesicles [24]. We assume that there are many somatic cells in the testis that would contain Ca^{2+} sequestering vesicles, which is the reason for the weaker positive signal on the Western blot (Fig. 6). With the exception of the giant fused mitochondrion and the acrosome vesicle, the sea urchin sperm contains no other membrane vesicles. Failure to detect suSERCA in mature sperm by Western blotting, and also by immunofluorescence, is consistent with this cytological deficit.

Measurements of intracellular Ca^{2+} in sea urchin sperm were studied using TG, a known inhibitor of SERCA. TG up to 2.5 μM had no effect on intracellular Ca^{2+} levels, but 10 μM TG did elevate internal Ca^{2+}. In mammalian somatic cells, the concentration of TG to completely inhibit SERCA is 0.3–1.0 μM [25]. CPA, another inhibitor of SERCA, that completely inhibits mammalian SERCA at 10–20 μM [26], had no effect on internal Ca^{2+} in sea urchin sperm even at 100 μM. These results are consistent with previously reported observations on human and mouse sperm [27,28], suggesting that a SERCA pump protein is not expressed in mature spermatozoa. Sea urchin sperm keep their intracellular Ca^{2+} levels in the 0.1 μM range while swimming in seawater, which is 10 mM Ca^{2+}. Two ion transporters used to keep Ca^{2+} low are the plasma membrane Ca^{2+} ATPase, which is concentrated in the sperm head as compared to the flagellum [29], and a K^+ dependent Na^+/Ca^{2+} exchanger (NCKX) that is concentrated in the sperm flagellum as compared to the sperm head [18]. The presence of a secretory pathway Ca^{2+} ATPase (SPCA) in sea urchin sperm has yet been documented, although we have cloned SPCA from testis cDNA (GenBank Accession No. NP_001028821). SPCA has been shown to regulate Ca^{2+} levels in human sperm [27]. Thus, PMCA and SPCA, but not SERCA, along with NCKX, might all regulate Ca^{2+} in animal spermatozoa.

Acknowledgments

This research was supported by NIH Grant HD12986 to V.D.V. Drs. C. Lambert, M. Nomura, G. Moy, and S. Publicover are thanked for their help and advice.

References

[1] V. Prasad, G.W. Okunade, M.L. Miller, G.E. Shull, Phenotypes of SERCA and PMCA knockout mice, Biochem. Biophys. Res. Commun. 322 (2004) 1192–1203.

[2] G.R. Cooper, C.O. Brostrom, M.A. Brostrom, Analysis of the endoplasmic reticular Ca^{2+} requirement for alpha1-antitrypsin processing and transport competence, Biochem. J. 325 (1997) 601–608.

[3] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signaling: dynamics, homeostasis and remodeling, Nat. Rev. Mol. Cell Biol. 4 (2003) 517–529.

[4] A.M. Hofer, C. Fasolato, T. Pozzan, Capacitative Ca^{2+} entry is closely linked to the filling state of internal Ca^{2+} stores: a study using simultaneous measurements of ICRAC and intraluminal [Ca^{2+}], J. Cell Biol. 140 (1998) 325–334.

[5] D.H. MacLennan, C.J. Brandl, B. Korczak, N.M. Green, Amino-acid sequence of a ($Ca^{2+} + Mg^{2+}$)-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its cDNA sequence, Nature 316 (1985) 696–700.

[6] C.J. Brandl, N.M. Green, B. Korczak, D.H. MacLennan, Two Ca^{2+}-ATPase genes: homologies and mechanistic implications of deduced amino acid sequences, Cell 44 (1986) 597–607.

[7] A.M. Gunteski-Hamblin, J. Greeb, G.E. Shull, A novel Ca^{2+} pump expressed in brain, kidney, and stomach is encoded by an alternative transcript of the slow-twitch muscle sarcoplasmic reticulum Ca-ATPase gene, J. Biol. Chem. 263 (1988) 15032–15040.

[8] J. Lytton, D.H. MacLennan, Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene, J. Biol. Chem. 263 (1988) 15024–15031.

[9] S.E. Burk, J. Lytton, D.H. MacLennan, G.E. Shull, cDNA cloning, functional expression and mRNA tissue distribution of a third organellar Ca^{2+} pump, J. Biol. Chem. 264 (1989) 18561–18568.

[10] L. Dode, C. De Greef, I. Mountian, M. Attard, M.M. Town, R. Casteels, F. Wuytack, Structure of the human sarco/endoplasmic reticulum Ca^{2+}-ATPase 3 gene: promoter analysis and alternative splicing of the SERCA3 pre-mRNA, J. Biol. Chem. 273 (1998) 13982–13994.

[11] C. Toyoshima, M. Nakamura, H. Ogawa, Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution, Nature 405 (2000) 647–655.

[12] C. Toyoshima, M. Nakamura, Structural changes in the calcium pump accompanying the dissociation of calcium, Nature 418 (2002) 605–611.

[13] Toyoshima, M. Nakamura, T. Tsuda, Luminal gating mechanism revealed in calcium pump crystal structures with phosphate analogues, Nature 432 (2004) 361–368.

[14] T.L. Sørensen, R.C. Nielsen, J.V. Møller, P. Nissen, Phosphoryl transfer and calcium ion occlusion in the calcium pump, Science 304 (2004) 1672–1675.

[15] C. Olesen, T.L. Sørensen, R.C. Nielsen, J.V. Møller, P. Nissen, Dephosphorylation of the calcium pump coupled to counter ion occlusion, Science 306 (2004) 2251–2255.

[16] C. Lambert, N. Leonard, X. De Bolle, E. Depiereux, ESyPred3D: prediction of proteins 3D structures, Bioinformatics 18 (2002) 1250–1256.

[17] S. Kumar, K. Tamura, M. Nei, MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and sequence alignment, Briefing in Bioinformatics 5 (2004) 150–163.

[18] Y-.H. Su, V.D. Vacquier, A flagellar K(+)-dependent Na(+)/Ca(2+) exchanger keeps Ca(2+) low in sea urchin spermatozoa, Proc. Natl. Acad. Sci. USA 99 (2002) 6743–6748.

[19] K.J Sweadner, C. Donnet, Structural similarities of Na,K-ATPase and SERCA, the Ca^{2+}-ATPase of the sarcoplasmic reticulum, Biochem. J. 356 (2001) 685–704.

[20] R.E. Tunwell, J.W. Conlan, I. Matthews, J.M. East, A.G. Lee, Definition of surface-exposed epitopes on the (Ca^{2+}-Mg^{2+})-ATPase of sarcoplasmic reticulum, Biochem. J. 279 (1991) 203–212.

[21] C. Toyoshima, H. Nomura, Y. Sugita, Structural basis of ion pumping by Ca^{2+}-ATPase of sarcoplasmic reticulum, FEBS Lett. 555 (2003) 106–110.

[22] K. Obara, N. Miyashuta, C. Xu, I. Toyoshima, Y. Sugita, G. Inesi, C. Toyoshima, Structural role of counter transport revealed in Ca^{2+} pump crystal structure in the absence of Ca^{2+}, Proc. Natl. Acad. Sci. USA 102 (2005) 14489–14496.

[23] D. Bayle, D. Weeks, G. Sachs, The membrane topology of the rat sarcoplasmic and endoplasmic reticulum calcium ATPases by

in vitro translation scanning, J. Biol. Chem. 270 (1995) 25678–25684.
[24] A. Galione, M. Ruas, NAADP receptors, Cell Calcium 38 (2005) 273–280.
[25] M. Treiman, C. Caspersen, S.B. Christensen, A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases, Trends Pharmacol. Sci. 19 (1998) 131–135.
[26] N. Demaurex, D.P. Lew, K.H. Krause, Cyclopiazonic acid depletes intracellular Ca^{2+} stores and activates an influx pathway for divalent cations in HK-60 cells, J. Biol. Chem. 267 (1992) 2318–2324.
[27] C. Harper, L. Wootton, F. Michelangeli, L. Lefievre, C. Barratt, S. Publicover, Secretory pathway Ca^{2+}-ATPase (SPCA1) Ca^{2+}-pumps, not SERCAs, regulate complex [Ca^{2+}-](i) signals in human spermatozoa, J. Cell Sci. (2005) 1673–1685.
[28] G. Wennemuth, D.F. Babcock, B. Hille, Calcium clearance mechanisms of mouse sperm, J. Gen. Physiol. 122 (2003) 115–128.
[29] H.J. Gunaratne, A.T. Neill, V.D. Vacquier, Plasma membrane calcium ATPase is concentrated in head of sea urchin spermatozoa, J. Cell Physiol. (2005), in press.