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Sequence, annotation and developmental expression of the sea urchin Ca^{2+}-ATPase family

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Abstract

Whole genome sequence data permit the study of protein families regulating cellular homeostasis during development. Here we present a study of the sea urchin Ca^{2+}-ATPases made possible by the Sea Urchin Genome Sequencing Project. This is of potential interest because adult sea urchins, their gametes and embryos live in the relatively high Ca^{2+} concentration of 10 mM. Three Ca^{2+}-ATPases regulate Ca^{2+} levels in animal cells: plasma membrane Ca^{2+}-ATPase (PMCA), sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and secretory pathway Ca^{2+}-ATPase (SPCA). The primary structures of Sp-PMCA and Sp-SERCA in the sea urchin, *Strongylocentrotus purpuratus* (Sp), have been published. Here, we present the primary structure of Sp-SPCA, which is 912 amino acids and has 66% identity and 80% similarity to human SPCA1. Southern blots and genome analysis show that Sp-SPCA is a single copy gene. Each Sp Ca^{2+}-ATPase is highly conserved when compared to its human ortholog, indicating that human and sea urchin share structurally similar energy driven Ca^{2+} homeostasis mechanisms that have been maintained throughout the course of deuterostome evolution. Annotation using the assembled sea urchin genome reveals that Sp-SPCA, Sp-PMCA and Sp-SERCA have 23, 17 and 24 exons. RT-Q-PCR shows that transcripts of Sp-SPCA are at low levels compared to Sp-PMCA and Sp-SERCA. Gradual increases in Sp-PMCA and Sp-SERCA mRNA begin at the 18 hour hatched blastula stage and peak 4-5-fold higher by 25 h at the mid to late blastulae stage.

Keywords: Ca^{2+}-ATPase genes; Calcium homeostasis; Protein domains; Developmental regulation; Embryos

1. Introduction

Three Ca^{2+}-ATPases regulate intracellular Ca^{2+} concentrations in animal cells: plasma membrane Ca^{2+}-ATPase (PMCA), sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and secretory pathway Ca^{2+}-ATPase (SPCA) (Prasad et al., 2004). PMCAs are found in the plasma membrane and transport Ca^{2+} extracellularly. SERCAs sequester Ca^{2+} in intracellular vesicles and are the major sequestering mechanism regulating Ca^{2+} in the cytosol. Among Ca^{2+}-ATPases, the SPCA family is the least known. The *PMR1* (plasma membrane Ca^{2+}-ATPase-related) gene of the yeast, *Saccharomyces cerevisiae*, was the first SPCA family member to be described (Rudolph et al., 1989). PMR1 protein is localized mainly in membranes of the yeast Golgi apparatus (Antebi and Fink 1992).

In humans, there are four distinct PMCA genes (*ATP2B1-4*), which encode roughly 40 alternatively spliced variant PMCA proteins (Brandt et al., 1992; Strehler and Zacharias, 2001; Żylińska et al., 2002). Three human SERCA proteins (SERCA1-3) are encoded by three genes (*ATP2A1-3*), which form many alternatively spliced isoforms that show distinct patterns of tissue expression (MacLennan et al., 1985; Brandl et al., 1986; Gunteski-Hamblin et al., 1988; Lytton and MacLennan., 1988; Burk et al., 1989; Dode et al., 1998). In humans, the two *PMR1* genes are known as *ATP2C1* and *ATP2C2,* whose proteins SPCA1 and

Abbreviations: NCKX, K^+-dependent Na^+/Ca^{2+} exchanger; PMR; plasma membrane Ca^{2+}-ATPase-related protein; PMCA; plasma membrane Ca^{2+}-ATPase; SERCA; sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; Sp; *Strongylocentrotus purpuratus*; SPCA; secretory pathway Ca^{2+}-ATPase.

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SPCA2, contain all the conserved domains of a typical P-type ATPase (Baelen et al., 2004). In the sea urchin, the primary structures of PMCA and SERCA, which have significant conservation to their human orthologs, have been described (Gunaratne et al., 2006; Gunaratne and Vacquier, 2006a). The Sea Urchin Genome Project made possible the discovery and analysis of these three energy driven Ca^{2+} pumps in the most basal of all yet known deuterostome genomes. Here we describe the primary structure of the sea urchin Sp-SPCA, and present comparisons of protein and gene structural features, together with expression data on all three Ca^{2+}-ATPases during embryogenesis.

2. Materials and methods

2.1. Cloning and sequence analysis

The full-length Sp-SPCA cDNA was cloned by PCR amplification using a *Strongylocentrotus purpuratus* testis cDNA library in Lambda Zap II (Stratagene) as template. Testis cDNA was synthesized from total testis RNA by standard methods. RT-PCR was done using primers designed from a BLAST search of human SPCA sequences from NCBI (http://www.ncbi.nlm.nih.gov/blast) and 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. Transmembrane helices were predicted with HMMTOP Version 2.0 located at http://www.enzim.hu/hmmtop/index.html with the help of hydropathy analysis (MacVector). BioEdit was used for alignments. Sites and motifs were identified using Prosite (http://ca.expasy.org). The Sp-SPCA sequence is deposited in GenBank under accession number DQ150587.

2.2. Southern blots

Ten micrograms *S. purpuratus* genomic DNA was digested and electrophoresed in 0.7% agarose and transferred to a nylon membrane, which was probed with a fragment (500 bp) of the 5'-region within the open reading frame of Sp-SPCA cDNA, which was random–prime labeled with [^{32}P]–dCTP (DECAprime II Kit, Ambion). Hybridization was at 65 °C and washings were done by standard methods.

2.3. Annotation

The copy number, length and number of exons/introns of each gene was determined using the *S purpuratus* multiple-contig linear assembly as of 2006.09.20.

(http://www.hgsc.bcm.tmc.edu/projects/seaurchin/). CLUSTALW (MacVector) was used to make nucleotide alignments.

2.4. Phylogenetic analysis

Full-length sequences were used to construct a neighbor-joining phylogenetic tree of selected Ca^{2+}-ATPase proteins (1000 replications) using Mega 3 software (Kumar et al., 2004). GenBank accession numbers for the protein sequences used are:

2.4.1. SPCAs

C. elegans (ceSPCA), NP001021862; *Drosophila melanogaster* (dmSPCA), NP730745; *Gallus gallus* (ggSPCA, XP426010 (predicted); *Homo sapiens* (hSPCA1), AAP30008; *S. cerevisiae* (scPMR1), NP011348; *Schistosoma mansoni* (smPMR), AAK50768; *S. purpuratus* (Sp-SPCA), AAZ77788; *Xenopus laevis* (xlSPCA), ABD98688.

2.4.2. PMCAs

C. elegans (ce-mca1), NP001023426; *Danio rerio* (drPMCA), XP687129 (predicted); *D. melanogaster* (dmCG21265-PC), NP_001014689; *G. gallus* (ggPMCA4, XP418055 (predicted); *H. sapiens* (hPMCA1a), NP001001323; *Paramecium tetraurelia* (ptPMCA), AAB81284; *Procambarus clarkia* (pcPMCA), AAR28532; *Rana clamitans* (rcPMCA), AAK11272; *S. cerevisiae* (scPMC1), CAA96706; *S. purpuratus* (Sp-PMCA), NP001028822.

2.4.3. SERCAs

C. elegans (ceSERCA), 499386; *Danio rerio* (drSERCA), AAH85636; *D. melanogaster* (dmSERCA), P22700; *G. gallus* (ggSERCA), B40812; *H. sapiens* (hSERCA2), P16615; *P. tetraurelia* (ptSERCA), CAA76764; *P. clarkia* (pcSERCA), AAB82291; *R. clamitans* (rcSERCA), CAC20853; *S. mansoni* (smCA), AAC72756; *S. purpuratus* (Sp-SERCA), ABB51168; *Ustilago maydis* (umSERCA), CAE11789. *S. purpuratus* potassium dependent Na^{+}/Ca^{2+} exchanger (Sp-NCKX), AAL75810, was used as an out-group.

2.5. Culture of embryo and preparation of cDNAs

Sea urchin embryos were cultured with constant 60 rpm stirring at 16 °C in Millipore filtered seawater at 0.5% egg volume to seawater (Foltz et al., 2004). Total RNA was extracted from 100 µl of embryo pellet using the RNAeasy kit (QIAGEN, Cat # 74104). Synthesis of cDNA was performed with 2 µg of total RNA using the Super-Script II polymerase and oligo (dT)12–18 primers by standard procedures (Invitrogen) in a final volume of 20 µl.

2.6. Real-time quantitative PCR (RT-Q-PCR)

Primers were designed to amplify the three Sp Ca^{2+}-ATPase gene fragments as follows (5' to 3'): PMCAf (forward), TGACCCTGGATGAGTGGATGTG and PMCAr (reverse), TTGGAGACCTGACTGGAAAGC GTG (272 bp product); SPCAf, GATTATCTTGGAGCGGACCCTG and SPCAr, CTGAACAAATGCCACTGTGACAAC (255 bp product); SERCAf, CCTCATCTCTCATTTTGGCAGC and SERCAr, CTGGAACACGGTTGATAGGA CG (288 bp product); UBIQf, CGAGTATTTGCCAGATGTGAACCC and UBIQr, ATTGGATTTTTTGCCCCTGC (233 bp product) for Sp-ubiquitin (GenBank accession number M61772). These primer pairs were used to PCR amplify cDNA prepared from eggs and embryos. To construct standard curves, gel purified (QIAGEN) template cDNA quantities were prepared by serial dilution. RTQ-PCR was performed on a LightCycler II (Roche) instrument with LightCycler FastStart DNA Master^{PLUS}