The Sarco-Endoplasmic Reticulum Ca\(^{2+}\) ATPase Is Required for Development and Muscle Function in Caenorhabditis elegans*

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The sarco-endoplasmic reticulum Ca\(^{2+}\)-transport ATPase (SERCA) loads intracellular releasable Ca\(^{2+}\) stores by transporting cytosolic Ca\(^{2+}\) into the endoplasmic (ER) or sarcoplasmic reticulum (SR). We characterized the only SERCA homologue of the nematode Caenorhabditis elegans, which is encoded by the sce-1 gene. The sce-1 transcript is alternatively spliced in a similar mode as the vertebrate SERCA2 transcript, giving rise to two protein variants: CeSERCAa and CeSERCAb. These proteins showed structural and functional conservation to the vertebrate SERCA2a/b proteins. The CeSERCA isoforms were primarily expressed in contractile tissues. Loss of CeSERCA through gene ablation or RNA interference resulted in contractile dysfunctioning and in early larval or embryonic lethality, respectively. Similar defects could be induced pharmacologically using the SERCA-specific inhibitor thapsigargin, which bound CeSERCA at a conserved site. The conservation of SERCA2 homologues in C. elegans will allow genetic and chemical suppressor analyses to identify promising drug targets and lead molecules for treatment of SERCA-related diseases such as heart disease.

Intracellular organelles play a critical role in the regulation of cytosolic Ca\(^{2+}\) levels by acting both as sinks for removal of Ca\(^{2+}\) from the cytoplasm and as sites of storage for its subsequent release. Ca\(^{2+}\) cycling between the cytoplasm and the sarcoplasmic reticulum (SR) is tightly linked to the contraction-relaxation cycle of myocytes. Contraction occurs upon release of Ca\(^{2+}\) from the SR into the cytosol, whereas relaxation is mediated by the rapid removal of cytosolic Ca\(^{2+}\), which is predominantly re-sequestered into the SR by the Ca\(^{2+}\) pumps of the SERCA gene family.

In higher vertebrates, SERCA Ca\(^{2+}\)-pump isoforms are encoded by three genes, recently designated ATP2A1-3 (reviewed in Refs. 1 and 2). Further isoform diversity at the protein level is created by alternative processing of the primary gene transcripts at a conserved 3' terminus. Alternative processing is regulated in a developmental or tissue-specific manner. SERCA1 is expressed specifically in adult (SERCA1a) or neonatal (SERCA1b) fast-twitch skeletal muscle. SERCA2a is expressed in the heart and in slow-twitch muscle, whereas SERCA2b is the main isoform expressed in smooth muscle and in all non-muscle tissues examined. SERCA3 isoforms are expressed in various non-muscle cells. Various pathologies have been linked to defects in SERCA-encoding genes. Mutations in ATP2A1, encoding SERCA1, cause an autosomal recessive form of Brody disease (3), whereas the loss of one functional allele of ATP2A2 encoding SERCA2 causes Darier-White disease (4). In addition, reduction in cardiac-specific SERCA2a function has been associated with relaxation abnormalities in heart failure (Ref. 2 and references therein). Mouse models with altered levels of SERCA2a or of its regulator phospholamban (PLB) further support a role for SERCA2a in cardiac function (5–10). Moreover, increasing contractility by alteration of SERCA2a or PLB levels in myocardium could prevent the onset of cardiac failure in different models of the disease (11, 12). These studies carry the prospect that modulation of SERCA2a or PLB activity can correct cardiac dysfunction in heart failure.

The nematode Caenorhabditis elegans is an established model organism amenable to genetic and pharmacological analysis. Here we report characterization of the unique C. elegans SERCA homologue. The two protein isoforms, CeSERCAa and CeSERCAb, show differential functional characteristics and expression patterns. Down-regulation of CeSERCA results in defects in various muscle and myoepithelial tissues, demonstrating an indispensable role for CeSERCA in behavior, growth, and reproduction.

EXPERIMENTAL PROCEDURES

Expression of CeSERCA in COS Cells—Standard molecular biological techniques were used to construct plasmids. A NotI-KpnI restriction fragment (3.2 kb) containing the entire coding sequence of CeSERCAb (Kohara clone yk7581) was blunt-ended and ligated into the blunt-ended BamHI-XhoI sites of pcDNA3 mammalian expression vector (Invitrogen). The CeSERCAa expression vector was constructed by replacing the CeSERCAb-specific tail for a PCR-amplified CeSERCAa-specific tail by ApaI-BstXI swapping. Transient DNA transfections were carried out in COS-1 cells with FuGENE6 transfection reagent (Roche Molecular Biochemicals) according to the manufacturer's instructions. Microsomes from COS-1 cells overexpressing CeSERCa/b isoforms were prepared as described earlier (13).

Immunochromeny—Antisera were raised to synthetic peptides CeSERAb (VEAPIKDIDKR) and CeSERAb (AVYTPDLHLNEI) by EUROGENTEC, Seraing, Belgium. The pan-specific SERCA antisemur 809-27 (14) was kindly provided by J. V. Møller (University of Aarhus,
Denmark). Immunocytochemical experiments on COS-1 cells transfected with CeSERCAa/b isoforms were performed as described by Wuytack et al. (15). Cells were stained 60 h after transfection. Primary anti-CeSARCA antisera were used at 1:100 and 1:1000 dilutions. Stained samples were treated with ProLong™ nuclear stain (Molecular Probes Inc.) to retard photobleaching. Western blotting was performed as reported previously (15).

\[ Ca^{2+} \] Uptake and Phosphorylation Assays—Oxalate-stimulated ATP-dependent \( Ca^{2+} \) uptake in microsomal fractions was measured at 27 °C by a Millipore filtration method as described earlier (16). Phosphorylations were done as described in Verboomen et al. (17). Microsomal fractions were incubated in phosphorylation buffer containing 1 mM EGTA or 0.1 mM EGTA + 0.9 mM Ca\(^{2+}\)–EGTA. The reaction was stopped after 20 s. Signal intensities were quantified by means of a Phospho-Imager (STORM 840, Molecular Dynamics Inc., Sunnyvale, CA).

Strains and Alleles—C. elegans strains were maintained according to Brenner (18) and grown at 20 °C. The strain C. elegans sca-1 (ok190) was back-crossed seven times against the wild-type strain N2 and unc-49(e382) using PCR to follow the lethal mutation.

Cloning and Transformation Rescue of sca-1—A genomic sca-1 subclone, pGR1, was generated by inserting a 12-kb SphiI fragment from the cosmids K11D9 into pUC18. A 3.3-kb MluI–Smal fragment was removed to yield pGK7. This clone contains 5.6 kb of sequence upstream of the translational start codon and 1.1 kb of sequence downstream of the translational stop codon of CeSARCAa.

Germ-line transformation rescue of the sca-1(ok190) null mutant was performed by injecting pGK7 at 10 and 20 ng/μl along with pFP4 (rol-6 marker) and the pGK10 (P\(_{pca-1}\)::GFP) construct described below. A total of 10 lines were established that all rescued sca-1(ok190). Isoform-specific constructs (pDW2651 and pDW2652) were made by inserting the pGK10 promoter fragment and the cDNAs amplified from the mammalian expression vectors into pPD49.26. These constructs were injected at 20 ng/μl. From each construct 5 lines were obtained, that all but one, rescued ok190.

Construction of Green Fluorescent Protein (GFP) Reporter Genes—To construct P\(_{pca-1}\)::GFP (pGK10), a 5.0-kb fragment of the sca-1 upstream sequence ending at the translational start site was amplified from pGK7 and cloned into the SalI and BamHI sites of pD95.75.5 For CeSARCAa::GFP (pGK26) and CeSARCa::GFP (pGK27), standard overlap extension PCR was performed to obtain products consisting of three subfragments: the C terminus of the proper isoform, GFP, and the 3′-untranslated region of that isoform. These were swapped into pGK7 using Apal–PacI for CeSARCAa and Apal–SalI for CeSARCa, respectively. Constructs were injected at 20 ng/μl. For each construct at least three lines were obtained.

RNA Interference—The 727-base pair EcoRI–HindIII fragment from exon 5 was cloned into pGEM3 to yield pGK6. RNA was transcribed in vitro from linearized pGK6 using the MEGAscript™ SP6 and T7 kits (Ambion), mixed, and dissolved in injection buffer (66 mM potassium phosphate (pH 7.5), 10 mM potassium citrate (pH 7.5), 6.6% polyethylene glycol 3550). Double-stranded RNA was injected into gravid adults, which were transferred to fresh plates 3 h after injection to remove embryos resulting from eggs fertilized before the injection. Injections were done at three different times, and each time at least 10 animals were injected.

Thapsigargin-resistant Strains—The mutation F259V was introduced into pGK7 using the QuikChange site-directed mutagenesis Kit (Stratagene) with Pfu DNA polymerase and primers gk33 (CAACA-GAAGTTGGACAGTCCGAGAATCTTTC) and gk34 (GAAGAGTTGTCTTCGGACTGGCATCCACTGTTG). The resulting plasmid (pGK28) was able to rescue sca-1(ok190) (data not shown). pGK28 was injected at 20 ng/μl in N2 together with 20 ng/μl pGK10 and 160 ng/μl pUC18. Three strains were obtained: UG528, UG529, and UG530. UG528 and UG530 showed comparable resistance to thapsigargin; UG529 was somewhat less resistant.

Thapsigargin Plate Assay—12-Well plates with standard NGM agar (2 ml) were seeded with a thin lawn of cold-sensitive OP50-derived bacteria that were completely covered with a 10-μl drop of thapsigargin (Alomone Labs, Jerusalem, Israel) dissolved in MeSO 1 day before the test. The thapsigargin concentrations mentioned in the text assume complete diffusion of thapsigargin in the plate. To follow the growth of wild-type and thapsigargin-resistant animals, thapsigargin plates were seeded with ~50 L1 larvae obtained after bleeding and hatching in M9, 4 wells/strain/concentration. The stage of each animal was determined after 3 days of growth at 20 °C. As a way of quantifying growth, each stage was assigned an arbitrary value (1 for adult, 0.5 for L4, 0.25 for L3, and 0.125 for L2). The “relative growth score” reflects the average stage at which animals reached. When all animals become adult, the resulting score is 1.0. When half of the animals reach the L3 and half reach the L4 stage, the resulting score becomes 0.735.

RESULTS

CeSARCA Is Highly Conserved—The C. elegans genome contains only one gene encoding a SERCA homologue. This gene was originally identified by the C. elegans sequencing consortium (19) and annotated K11D9.2. In this paper, we will refer to the gene as sca-1 and to the corresponding protein as CeSARCA. CeSARCA shows about 70% amino acid identity and 80% similarity to the three human SERCA proteins. The phosphorylation domains of CeSARCA and human SERCA2 share 81% amino acid identity, the ATP binding domains share 59% amino acid identity, and the anchor domain shares 67% amino acid identity. The predicted amino acid sequence of CeSARCA possesses all residues defined as essential for phosphorylation (Asp-351), ATP binding (such as Phe-487, Lys-515, and Lys-492) and Ca\(^{2+}\) binding/translocation in membrane-spanning segments 4, 5, 6, and 8 (for review see e.g. Ref. 20). C. elegans SERCA does not contain the putative cytosolic interaction site for phospholamban, which is found both in vertebrate SERCA1 and SERCA2 but is lacking in vertebrate SERCA3 and in other invertebrate SERCAs (21). It should be noted in this respect that the gene encoding PLB or a PLB-like protein should be found in C. elegans nor has such a gene been reported in any other invertebrate.

The sca-1 gene consists of eight exons and seven introns (Fig. 1A). The positions of four of these introns in the nematode gene are conserved in all three vertebrate SERCA genes; the worm introns 3, 4, 5, and 6 correspond to the vertebrate SERCA
introns 1, 5, 16, and 19, respectively. The transcripts of the sca-1 gene, like those of the three vertebrate SERCA genes, can be alternatively spliced, whereby the position of the alternative intron is conserved as well. Alternative splicing of the sca-1 transcript follows a similar mode as that of vertebrate SERCA2, which differs from that of SERCA1 or SERCA3. In sca-1, exon seven contains an optional internal 5′ splice donor site, which can be spliced to the 3′ splice acceptor site, marking the beginning of exon eight (Fig. 1A). Such a splicing results in the formation of the short isoform (1004 amino acids), which we name CeSERCAa following the nomenclature in the SERCA field. The absence of this splicing leads to expression of the CeSERCAb variant (1059 amino acids). As in vertebrate SERCA2b, the CeSERCAb-specific tail comprises a hydrophobic stretch (1006-SAISLLAWVSVT-1026) with the propensity to form an additional transmembrane domain. Four lines of evidence indicate that both predicted CeSERCA proteins are indeed expressed in C. elegans. First, multiple cDNA clones of both isoforms are present in the Kohara cDNA collection (www.ddbj.nig.ac.jp/htmls/c-eleganshtml/CE_INDEX.html). Second, both transcripts were detected using reverse transcription-PCR on whole worm mRNA. Third, both proteins were detected by Western blotting on fragmented membranes prepared from whole worms (Fig. 2). Fourth, GFP reporter constructs specific for each of the splice variants were expressed in vivo (see Fig. 7).

CeSERCAa and CeSERCAb Function as P-type Ca2+ Pumps—COS-1 cells were transiently transfected with cDNAs encoding each CeSERCA isoform. Immunocytochemical staining with isoform-specific polyclonal antisera or with a polyclonal pan-specific SERCA antiserum revealed that both CeSERCA proteins were expressed at high levels and were properly targeted to the ER compartment (Fig. 3A). Western blots of microsomes prepared from these cells showed immunoreactive bands with an electrophoretic mobility of around 100 kDa, with CeSERCAb showing a slightly higher apparent molecular mass than CeSERCAa (Fig. 3B). From these blots it can also be concluded that in the COS cells the CeSERCAs were expressed at levels at least 10-fold higher than the endogenous SERCA2b.

Microsomal vesicles were isolated from the transfected COS cells to measure the time course of oxalate-stimulated Ca2+ uptake. Ca2+ transport into the vesicles was strictly dependent on the presence of ATP. The apparent affinity for Ca2+ (K0.5) of CeSERCAa was found to be 2-fold lower than that of CeSERCAb (K0.5 = 0.80 versus 0.40 μM; Fig. 4A). This difference is similar to that seen for vertebrate SERCA2a and SERCA2b (17). Thus, both the mode of splicing and its structural and functional consequences at the protein level appear to be evolutionarily conserved.

As other P-type ATPases (reviewed in Ref. 20), CeSERCA formed a phosphoprotein intermediate (Fig. 4B). Phosphorylation depended on the presence of Ca2+ and was much more pronounced in microsomes from CeSERCA-expressing COS cells than in microsomes from untransfected controls, in accordance with the strong overexpression of the CeSERCA isoforms in the COS cells.

C. elegans SERCA Is Essential for Larval Development—A deletion allele of the sca-1 gene was isolated using a PCR-based sb-selection protocol (kindly provided by the C. elegans knockout consortium). Subsequent sequence analysis revealed that this allele, designated ok190, lacked 12 base pairs of intron 4 and 1690 base pairs of exon 5 (Fig. 1A). The deleted region encompasses the third and fourth transmembrane domain (the latter of which contains residues critical for Ca2+ binding/translocation), the ATP binding domain, and the phosphorylation domain including the aspartyl phosphorylation site, ascertaining that ok190 represents a sca-1 null allele.

Animals heterozygous for ok190 appeared as wild type. Animals that were homozygous for ok190, however, arrested in the first larval (L1) stage, showed slow and irregular pharyngeal pumping and sluggish body movement, and became stunted (Fig. 5A). Their body cavity became filled with fluid, indicative of defective osmo-regulation. ok190 larvae rescued by the wild-type sca-1 gene on an extra-chromosomal transgene developed into apparently wild-type adults that were, however, sterile or produced very few offspring. The gonads of these adults contained endomitotic oocytes, indicative of failing ovulation (Fig. 5B).

C. elegans SERCA Is Essential for Embryonic Development—To perform RNA interference (22), double-stranded RNA was prepared in vitro from a 727-base pair genomic fragment (Fig. 1A) and injected into the gonadal syncytium of
CeSERCAa or CeSERCAb were incubated with /H9253 mediate. Microsomes transfected with empty vector (control, Ca2+ presence of 5 mM ATP. Ca2+ higher, whereas L1 larvae developed normally into healthy, gous but then arrested at the L1 stage, closely resembling homozygous adults became sterile (Fig. 5). The progeny of injected animals was severely affected. About half of them successfully completed embryonic development but then arrested at the L1 stage, closely resembling homozygous ok190 larvae. The other half of the progeny arrested already during embryogenesis, usually at the 2–3-fold stage (Fig. 5C). Similar results were reported by Cho et al. (23), although embryonic defects generally occurred at an earlier stage.

C. elegans SERCA Is Inhibited by Thapsigargin—Thapsigargin is a highly potent and highly specific inhibitor of SERCA pumps (24, 25). L4 larvae or young adults that were transferred to plates containing 1.25 \( \mu \text{M} \) thapsigargin as young adults. Complete cessation of pharyngeal pumping was determined after 30 min, loopy movement after 4 h, reduced fertility, gut morphology and protruding vulva after 24 h. Thapsigargin also affected growth. Wild-type L1 larvae essentially arrested or died at the L3 or L4 stage when transferred to plates containing 1.25 \( \mu \text{M} \) thapsigargin or higher, whereas L1 larvae developed normally into healthy, slow, pale, and thin as a consequence of limited food intake and displayed a protruding vulva (Fig. 6; Table I). The animals became slower and irregular. In addition, thapsigargin affected animal movement and defecation cycles (Table I). The effect was reversible; after 1 h, pharyngeal pumping resumed, albeit completely suppressed pharyngeal pumping (Table I). The effect was conserved in CeSERCA. First, the epoxide derivative of thapsigargin did not cause any defect under the same experimental conditions, not even at 64-fold higher concentrations (data not shown). As the result of a very small structural change, thapsigargin epoxide has a SERCA affinity of at least 5 orders of magnitude lower than thapsigargin and, thus, serves as a negative control for thapsigargin inhibition (26).

Two lines of evidence support the view that CeSERCA is the in vivo target for thapsigargin and that the binding site for thapsigargin is conserved in CeSERCA. First, the epoxide derivative of thapsigargin did not cause any defect under the same experimental conditions, not even at 64-fold higher concentrations (data not shown). As the result of a very small structural change, thapsigargin epoxide has a SERCA affinity of at least 5 orders of magnitude lower than thapsigargin and, thus, serves as a negative control for thapsigargin inhibition (26).

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FIG. 6. Effect of thapsigargin on C. elegans growth and behavior. A, wild-type N2 adult exposed to 5 μM thapsigargin for 24 h, starting as young adult. B, N2 adult expressing transgenic thapsigargin-resistant CeSERCA259V, under the same conditions as in A. C, amount of progeny produced by wild-type animals exposed to thapsigargin from the L4 stage onwards. The number of progeny was counted for 24 individual animals (12 animals for 0 and 0.05 μM). The sigmoidal curve is best-fitted to the median values of each concentration. D, development of L1 larvae chronically exposed to thapsigargin. Stages were given an arbitrary weight as described under “Experimental Procedures.” At higher thapsigargin concentrations, wild-type N2 animals (diamonds) died as L3 or L4, whereas N2 animals carrying CeSERCA259V on an extrachromosomal transgene (triangles) nearly all became adults. Their non-transgenic sisters (squares) behave like N2. Scale bars, 100 μm.

FIG. 7. Expression pattern of sca-1. A, expression of CeSERCAa::GFP in the posterior pharynx (open arrow, isthmus; closed arrow, terminal bulb), head muscles (triangles), and intestine (arrowheads). B, expression of CeSERCAb::GFP in the excretory canal (arrowheads). Background staining originates from out of focus body-wall muscle. C and D, CeSERCAa::GFP is expressed in the gonadal sheath cells (filled arrowheads) but not in the spermatheca or the uterine sheath cells. CeSERCAb::GFP is expressed in the gonadal sheath cells (filled arrowheads), spermatheca (open arrow), uterine sheath cells (open arrowheads), and the vulva muscles (arrows). The triangles indicate body-wall muscle staining. E and F, expression of CeSERCAa::GFP and CeSERCAb::GFP in the body wall muscles. Staining is localized to dense bodies (arrow) and membranous structures in the cell (arrowheads). Scale bars, 50 μm.

Function of sca-1 SR/ER Ca2+ ATPase in C. elegans

array. Transgenic young adults were considerably less responsive to thapsigargin than wild-type young adults (Fig. 6B and Table I). Also, transgenic L1 larvae developed into healthy, gravid adults at high thapsigargin concentrations that caused developmental arrest and lethality of wild-type or non-transgenic L1 (Fig. 6D).

C. elegans SERCA Is Expressed in Contractile and Non-contractile Tissue—To determine the expression pattern of sca-1, we made a number of GFP reporter constructs. The isoform-specific reporter constructs CeSERCAa::GFP and CeSERCAb::GFP were obtained by insertion of GFP (28) at the C-terminal end of either coding region in the rescuing sca-1 genomic clone (Fig. 1B). A third construct, P_{sca-1}::GFP, contained sca-1 upstream sequence transcriptionally fused to GFP (Fig. 1B).

All three reporter constructs were expressed in all major contractile tissues, starting during embryogenesis and continuing until adulthood (Fig. 7 and data not shown). Fluorescence was particularly pronounced in striated muscle (body wall muscles used for locomotion), non-striated muscle (pharyngeal muscles used for pharyngeal pumping, vulval and uterine muscles used for egg laying, the sphincter muscle and anal depressor used for defecation), and in myoepithelial cells (gonadal sheath cells used for ovulation). Furthermore, all three constructs showed expression in the intestine.

Some cells expressed CeSERCAb::GFP but not CeSERCAa::GFP. These include the somatic cells of the spermatheca and the excretory canal and the uterine sheath cells (Fig. 7, B–D). No cells were found that expressed CeSERCAa::GFP but not CeSERCAb::GFP.

Fluorescence from P_{sca-1}::GFP was evenly distributed over the cytosol. However, both CeSERCAa::GFP and CeSERCAb::GFP showed specific subcellular localization. Both fusion proteins were found in a tubular meshwork that was most distinct in the body wall muscle cells. This staining was continuous with staining at or near the dense bodies, structures functionally analogous to vertebrate Z-lines (Fig. 7, E and F). In body wall muscle cells and other cells, staining was also often localized in internal vesicles and membrane-like structures, including the nuclear envelope.
DISCUSSION

In this paper we have described the C. elegans SERCA gene sca-1 and its two protein products. Alternative splicing occurs at a homologous position in the worm transcript as in vertebrate SERCA1–3 transcripts, according to a mode typical for SERCA2. It leads to the production of two protein variants, CeSERCAa and CeSERCAb, which structurally and functionally resemble the SERCA2a and SERCA2b isoforms, pointing to an evolutionarily conserved mechanism and functional significance of the isoform diversity. Both CeSERCA isoforms transported Ca\(^{2+}\) into the microsomal vesicles of COS-1 cells in an ATP-dependent and oxalate-stimulated manner, which was associated with the formation of a Ca\(^{2+}\)-dependent phosphoprotein intermediate. Similarly to mammalian SERCA2a and SERCA2b, the longer \(b\) isoform showed a 2-fold higher apparent affinity for Ca\(^{2+}\) than the a isoform. However, in contrast to SERCA2 expression pattern, the two CeSERCA isoforms were expressed in a highly overlapping pattern.

We employed three complementary approaches to inactivate CeSERCA. Direct gene targeting, RNA interference, and treatment with the SERCA-specific inhibitor thapsigargin all resulted in developmental arrest and lethality. The observation that RNA interference caused embryonic lethality, whereas the ok190 null allele caused larval lethality, argues for a maternal component of CeSERCA expression, which is required during early embryogenesis, and a zygotic component, which is required during larval development. ok190 embryos receive CeSERCA from their heterozygous mothers but fail to produce CeSERCA themselves and, hence, arrest as L1 larvae. Injection of double-stranded RNA into wild-type mothers already receive CeSERCA-deficient animals. Apparently, the maternal component and, hence, results in embryonic arrest.

Besides its absolute requirement for normal development, inhibition of CeSERCA resulted in a variety of behavioral defects, reflecting the widespread expression of CeSERCA. The pumping and movement defects in ok190 larvae and after thapsigargin treatment show that Ca\(^{2+}\) from internal stores plays an important role in pharyngeal and body wall muscle activity. Both pharyngeal pumping and locomotion are impaired but not eliminated in animals deficient for CeSERCA. Apparently, Ca\(^{2+}\) release from intracellular stores is required for full muscle function but is not essential for excitation-contraction coupling, as is also suggested by the relatively mild phenotypes observed in mutants of the main Ca\(^{2+}\) release channel of the SR, the ryanodine receptor (29, 30). Perhaps Ca\(^{2+}\) entry through the plasma membrane is sufficient to induce pharyngeal and body wall muscle contraction. The smaller size and higher surface-to-volume ratio compared with vertebrate muscle cells could make nematode muscle cells relatively less dependent on internal Ca\(^{2+}\) stores. Furthermore, the diminished Ca\(^{2+}\)-loading state of internal stores may increase Ca\(^{2+}\) entry at the plasma membrane via store-activated Ca\(^{2+}\) entry.

CeSERCA-deficient animals show defective defecation, a process that is coordinated by inositol-trisphosphate receptor-mediated Ca\(^{2+}\) oscillations generated in the posterior intestinal cells (31). The defective defecation found in CeSERCA-deficient animals could therefore be caused by the absence of these Ca\(^{2+}\) oscillation cycles by defective body wall or enteric muscle contraction or both. The process of ovulation requires the concerted action of oocyte signaling, spermathecal dilation, and sheath cell contraction (32, 33). Mutants of two other Ca\(^{2+}\)-handling proteins, the inositol triphosphate receptor, expressed in the spermatheca (34), and troponin T, expressed in the sheath cells (35), also display ovulation defects. Any of these events could be perturbed in CeSERCA-deficient animals. Osmo-regulation is mediated by the excretory cell. Dysfunction of this cell, which expresses CeSERCAb, is therefore the likely cause of the osmoregulatory defects found in CeSERCA-deficient animals.

Thus, loss of CeSERCA activity leads to a variety of phenotypes that could be used as a starting point for further analysis. C. elegans is a model organism amenable to high-throughput genetic and pharmacological screening. Because SERCA is likely to be a difficult target for classical in vitro drug screening, C. elegans-based models could prove to be particularly useful in the identification of compounds against SERCA-related diseases, such as heart disease.

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