THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 7, Issue of February 12, pp. 4254–4258, 1999
© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

Identification and Functional Expression of the Plasma Membrane Calcium ATPase Gene Family from *Caenorhabditis elegans**

(Received for publication, October 14, 1998, and in revised form, November 23, 1998)

Alexander Kraev, Natalia Kraev, and Ernesto Carafoli‡

From the Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), 8092 Zürich, Switzerland and the Department of Biological Chemistry, University of Padova, 35121 Padova, Ialy

Calcium-pumping ATPases are an essential component of the intracellular calcium homeostasis system and have been characterized in a large variety of species and cell types. In mammalian genomes, these proteins are encoded by gene families whose individual members feature complex tissue-specific expression and alternative splicing. In the search for a less complex system that is more amenable to genetic manipulation, we have identified a family of three genes (*mca-1*, *mca-2*, and *mca-3*) encoding putative calcium ATPases in the *Caenorhabditis elegans* Genome Project data and completed their transcript structure. In this work, we report the cloning and functional expression of the *mca-1* gene, which encodes a calcium-stimulated ATPase whose features resemble those of the plasma membrane calcium adenosine triphosphatase family of mammalian cells and appears to be regulated by a multipartite promoter.

Calcium-pumping ATPases have been characterized from a vast variety of eukaryotic organisms. Sequence analysis of more than 40 complete cDNA sequences and biochemical investigations of them have defined two major classes of these proteins: (*a*) those residing on the plasma membrane (PMCA),[1] which are stimulated by interaction with calmodulin (for reviews, see Refs. 1–3), and (*b*) organellar ATPases (SERCA), located at various intracellular membranes (for a review, see Refs. 4 and 5). The complexity of these protein families makes their study difficult, stressing the advantages of more accessible unicellular models that are amenable to genetic manipulation, such as yeast (for a review, see Ref. 6). Whereas the yeast model has contributed to the advancement of our understanding of the roles calcium pumps play in various cellular processes (7–10), the yeast cell, with its rigid wall, is closer to plants in that a major calcium storage organelle appears to be vacuole, rather than sarcoplasmic reticulum. This limits the projection of the yeast model to understanding the role of these proteins in a mammalian cell. Increasing evidence that the plasma membrane-based calcium transporters reside at specific subcellular structures, such as the caveolae (reviewed in Ref. 11), and other differences between animal and plant cell architecture demand a more adequate model of an animal cell than the yeast cell. One may add that despite substantial conservation of the protein structure, the modes of gene regulation of mammalian and yeast ATPase genes are likely to have very little in common. The study of the transcriptional regulation of PMCA genes is a technically challenging task (12) that is unlikely to be approached systematically before the sequence of at least one mammalian genome becomes available. Moreover, in mammalian systems, promoter studies are limited to tissues that are amenable to temporal cultivation and transient transfection. Whereas solitary examples of PMCA gene overexpression (13) and knockout (14) do exist, comprehensive genetic manipulation of mammalian families remains extremely time and labor intensive.

The nematode *Caenorhabditis elegans* is an established model for developmental and neurogenetic studies and is the first multicellular organism whose genome sequence is soon to be completed. More than half of the *C. elegans* genes have a significant match to a mammalian gene with a similar or identical function (reviewed in Ref. 15). Thus, it represents a unique model for studies of calcium homeostasis in a genetically manipulatable multicellular organism. Voltage-gated calcium channels (16, 17), ryanodine receptors (18, 19), and inositol 1,4,5-triphosphate receptors (20) have already been found in *C. elegans*. A putative SERCA-type pump was cloned from the parasitic nematode *Schistosoma mansoni* (21). In contrast to mammalian systems, an essentially complete catalog of candidate calcium transporters will be derived from genomic sequencing data in the near future. As a step toward this goal, we have characterized a family of *C. elegans* calcium ATPases, which appear to be similar to the mammalian PMCA family and are apparently regulated by multipartite promoters.

EXPERIMENTAL PROCEDURES

Isolation of cDNA Clones, Sequencing, and Computer Analysis—BLAST (22) searches of the Wormpep database with the amino acid sequence CSDKTGTLT were run on the *C. elegans* World Wide Web server at the Sanger Center (Cambridge, United Kingdom). Clones identified in the EST database were clustered using the Sequencher program (Gene Codes, Ann Arbor, MI) and further analyzed with this program and with the GCG package v.8.0 (23). Clones with the largest cDNA inserts for each cluster were obtained from Y. Kohara (Institute of Genetics, Mishima, Japan) and sequenced using IR800-labeled dye primers on a Li-Cor 4000L automated sequencer. Sequence analyses were run through the World Wide Web interface at the Swiss Bioinformatics Institute (http://www.isb-sib.ch/).

Isolation of Total RNA, cDNA Synthesis, and Reverse Transcription-PCR—Strain N2 was obtained from the *Caenorhabditis* Genetics Stock Center and propagated according to established methods. Total RNA was isolated from about 500 mg of worms using Trizol reagent (Life Technologies AG, Basel, Switzerland). 250 μg of total RNA were used to prepare polyadenylated RNA using Oligotex columns according to the manufacturer's procedures (Qiagen AG, Basel, Switzerland). RNA was eluted in 20 μl of water, split into two portions, and immediately used to synthesize cDNA using Expand reverse transcriptase (Boehringer

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ223616, AJ010708, and AJ010646.

‡ To whom all correspondence should be addressed: Laboratory of Biochemistry III, Swiss Federal Institute of Technology (ETH) Universitätsstrasse 16, CH-8092 Zürich, Switzerland. Tel.: 41-1-632-30-11/12; Fax: 41-1-632-12-13; E-mail: carafoli@bc.biol.ethz.ch.

[1] The abbreviations used are: PMCA, plasma membrane calcium adenosine triphosphatase; SERCA, sarcoendoplasmic reticulum calcium adenosine triphosphatase; EST, expressed sequence tag; PCR, polymerase chain reaction.

This paper is available on line at http://www.jbc.org

Downloaded from http://www.jbc.org/ by guest on July 25, 2018

Mannheim AG, Rotkreuz, Switzerland) and either oligo dT20-Not primer or hexamer primer (Pharmacia Biotech AG, Dubendorf, Switzerland). The completed cDNA reaction was kept at 4 °C and was typically used up within a month. Reverse transcription-PCR was carried out using the Expand Long Template PCR kit (Boehringer Mannheim) with one of several gene-specific primers and SL1-specific primer 5′-CGCGGGTTTAATTACCCAAGTTTGAG.

TABLE I
Candidate calcium ATPases found in the C. elegans Genome Project data

Gene	Chromosome	Genomic clone	EST clone	Transcript length (kb)
mca-1	IV	W09C2	yk49f8	4.4
mca-2	IV	Y76B12	yk44a6	4.0
mca-3	IV	Y67D8	yk186e4	4.6

Construction of the Full-Length Transcript and Its Expression in Insect Cells—A PCR product extending from the penultimate base preceding the AUG codon to the unique *Ava*I site was prepared from cDNA and joined to the *Ava*I-cut plasmid clone yk49f8 (obtained from Y. Kohara). To introduce a FLAG-tagging sequence, the fragment between the unique *Psh*AI site in the cDNA and the *Xba*I site after the stop codon was replaced by a double-stranded synthetic oligonucleotide, coding for the respective C-terminal sequence (VDMEDIELN) followed by the FLAG sequence, a new stop codon, and a *Xba*I site. The correct clone was selected by DNA sequencing. A pFastBac clone with the FLAG-tagged cDNA was transferred into the bacmid, following the instructions of the baculovirus expression kit manufacturer (Bac-to-Bac; Life Technologies). The positive clone DNA was quantitated by fluorometry and introduced into Sf9 cells essentially following the in-

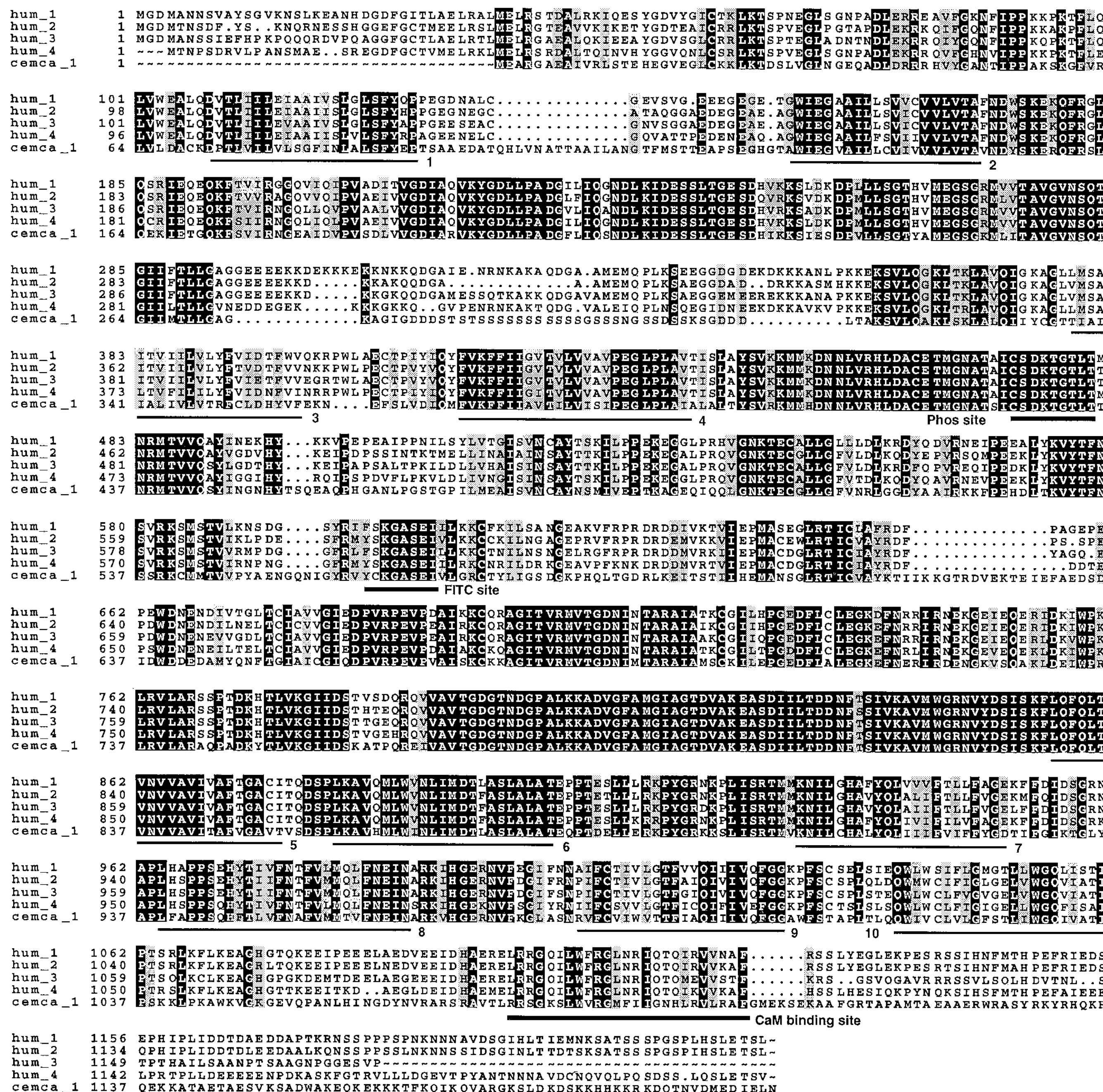

FIG. 1. **Alignment of the four human PMCA gene products (hum_1 through hum_4) with the product of *C. elegans* open reading frame W09C2.3 (cemca_1).** *Black shading* shows the sequences that are identical in all five proteins. Putative transmembrane domains are *underlined* and numbered 1 through 10. Functional sites are *underlined* with a *thick line*. The figure was compiled from the following SwissProt/TrEMBL entries: P20020 (hum_1), P23634 (hum_4), Q01814 (hum_2), Q16720 (hum_3), and O45215 (cemca_1). Alignment with only one nematode sequence is shown for clarity.

Downloaded from http://www.jbc.org/ by guest on July 25, 2018

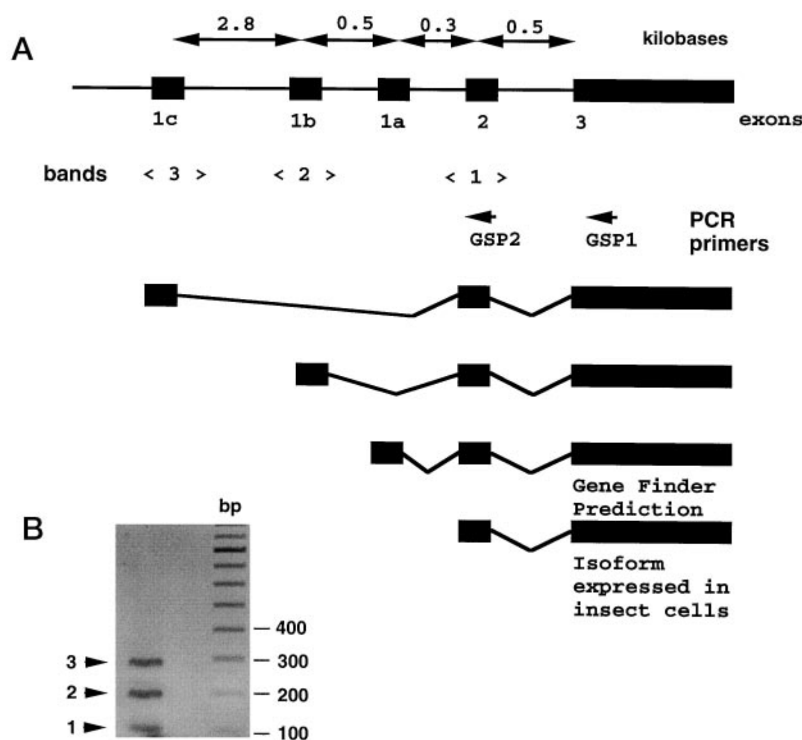

FIG. 2. **Structure of the 5′ terminus of the *mca-1* gene.** *A*, reconstruction of the gene structure and the splicing options at the 5′ terminus by comparison of the sequence data from the PCR products with the genomic sequence in cosmid clone W09C2 (accession number Z68221). Gene-specific primers (*GSP*) used for PCR are indicated by *arrows*. *B*, nested reverse transcription-PCR amplification of the alternative first exons of the *mca-1* gene.

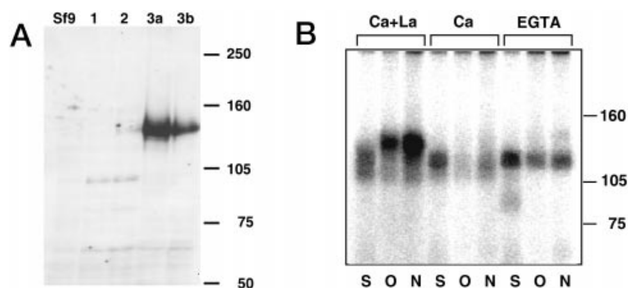

FIG. 3. **Analysis of crude membrane proteins of Sf9 cells expressing epitope-tagged *mca-1* cDNA.** *A*, Western blot analysis with the anti-FLAG antibody. *Lanes 1* and *2* contain the membranes from the primary transfected cells (a weakly reactive band of 130 kDa is seen at a longer exposure). *Lanes 3a* and *3b* contain membranes from two independent midscale recombinant virus propagations. The lane with membranes from uninfected cells is labeled *Sf9*. *B*, phosphointermediate assay for P-type ATPases. *S*, membranes from uninfected cells; *O* and *N*, two independent membrane preparations from cells infected with the recombinant virus. The cation compositions of the reaction media are indicated above the lanes.

structions supplied with the transfection reagent (Lipofectin; Life Technologies). The cells were allowed to recover in TNM FH medium containing 10% fetal calf serum and 50 μg/ml hygromycin (Sigma) for 72 h, and the virus-containing medium was collected. After a second infection with the virus, the cells were grown in the same medium and harvested after 48 h. Crude membranes were prepared essentially as described previously (24). Aliquots of the membranes (2 μl) were analyzed on 5% Protogel gels (National Diagnostics, Atlanta, GA) containing the buffer system of Laemmli (25) and electroblotted (26) onto a reinforced nitrocelulose membrane (OptiTran BA-S 85; Schleicher and Schull, Dassel, Germany). The blots were probed with the monoclonal antibody Anti-FLAG M2 (Kodak Scientific Imaging Systems, Rochester, NY) at a 1:5000 dilution using the ECL Western kit (Amersham Switzerland).

Phosphointermediate Formation of the ATPase—Aliquots of the crude membrane preparation (30 μg of protein) were incubated with assay solutions containing either various cations (100 μM $CaCl_2$, 100 μM $LaCl_3$, or both) or 5 mM EGTA and 0.3 μM [γ-^{32}P]ATP (Amersham) in a volume of 50 μl, essentially as described previously (13), and analyzed on 5% Protogel containing the buffer system of Sarkadi *et al.* (27). The gels also contained the Rainbow protein marker (Amersham). The gels were dried on Whatman 3MM paper in a gel dryer at 65 °C for 1 h and exposed to a storage phosphor screen (Kodak) for 2 h or overnight. The screen was scanned in a STORM imager and analyzed with ImageQuant 1.2 software (Molecular Dynamics, Sunnyvale, CA).

Calmodulin Binding Assay—Membrane proteins of Sf9 cells were resolved by electrophoresis in 5% Protogel gels and electroblotted onto Immobilon-P membrane (Millipore, Bedford, MA). After electroblotting, the membrane was cut into strips containing three tracks each (membrane proteins from untransfected cells, cells transfected with the *mca1* construct, and cells transfected with the human *pmca4* construct (31), respectively) and washed twice with Ca-Tris-buffered saline (25 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$, and 200 μM $CaCl_2$). The strips were incubated for 1 h in the blocking solution (Ca-Tris-buffered saline supplemented with 3% gelatine and 0.05% Tween 20), washed twice with TBST buffer (Ca-Tris-buffered saline, supplemented with 0.05% gelatine and 0.05% Tween 20), and incubated with biotinylated calmodulin dilutions in TBST buffer at a concentration ranging from 10 nM to 1 μM for 2 h at room temperature. One of the strips was incubated with 1 μM calmodulin as described above, but 200 μM $CaCl_2$ in all buffers was replaced with 6 mM EGTA. Strips were subsequently washed three times with TBST buffer, and biotin binding was visualized using a streptavidin-alkaline phosphatase conjugate (Promega, Madison, WI) at a 1:1000 dilution, essentially as suggested by the manufacturer of the conjugate.

RESULTS

Identification of a Calcium ATPase Gene Family in C. elegans—Systematic searches of *C. elegans* databases available on-line from the Sanger Center have identified three sets (Table I) of relatively abundant EST clones with a high degree of similarity to mammalian calcium ATPases of the PMCA family (Fig. 1). These sequences are located on *C. elegans* chromosome IV and show about 70% similarity to each other. Clones were obtained from the Institute of Genetics (Mishima, Japan) and completed by reverse transcription-PCR, taking into account the fact that two-thirds of the *C. elegans* mRNAs are *trans*-spliced to the same 22-nucleotide leader SL1 (28, 29). In this experiment, however, it was found that the transcripts always contained alternative 5′ ends, only one of which was predicted by GeneFinder scanning of the genomic sequence. In one case, examination of the available genomic sequence (cosmid W09C2) upstream of the predicted exon 2 (Fig. 2*A*) resulted in the unambiguous localization of all four alternative 5′ ends, which are followed by a consensus splicing signal. Thus, the

Downloaded from http://www.jbc.org/ by guest on July 25, 2018

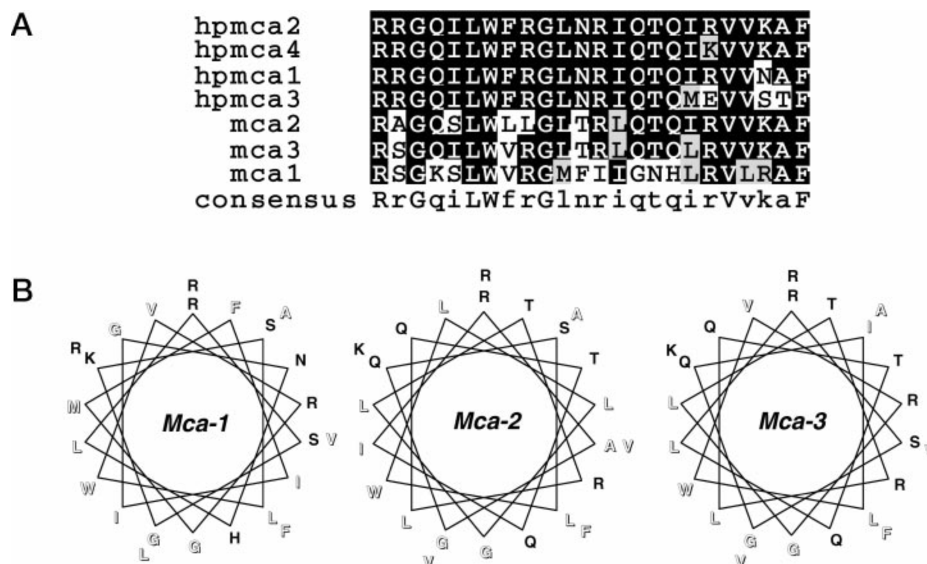

FIG. 4. **Putative calmodulin binding sites from the Mca proteins.** *A*, alignment with the sites of human PMCA proteins. *Shading* indicates positions at which 50% of the sequences match. *B*, a helical wheel representation of the putative calmodulin-binding domains of the Mca proteins. Hydrophobic amino acids are shown in *shadow lettering*.

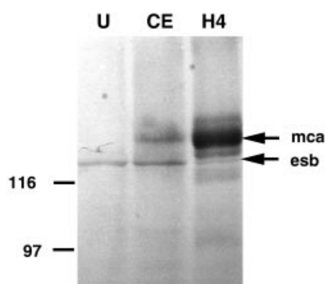

FIG. 5. **Binding of human calmodulin to the Mca1 protein expressed in Sf9 cells.** Gel-fractionated and electroblotted membrane proteins from untransfected cells (*lane U*), cells transfected with the *mca-1* construct (*lane CE*), and cells transfected with the human *pmca4* construct (*lane H4*). Sf9 cells were incubated with 10 nM biotinylated human calmodulin and 200 μM Ca^{2+}. The band that is visible only in the presence of Ca^{2+} is designated *mca*; the endogenous streptavidin-binding protein is designated *esb*. The *numbers* at the *left* show the positions of the protein marker bands. The amount of protein loaded in *lane H4* was 3-fold lower than that in *lanes U* and *CE*.

transcription of this gene is apparently driven by a multipartite promoter, which is spread over 4 kilobases. This analysis also showed that every alternative exon appended an amino acid sequence to the basic form of the protein, which is encoded in exon 2 and in the exons downstream. The shortest form of *mca-1* cDNA was used in the expression experiments.

Expression of the Complete Transcript W09C2.3 in Insect Cells Confirms Its Identity to a P-type Calcium ATPase—The shortest form, which was encoded in exons 2–10 (Fig. 2*A*), was modified by PCR to include a FLAG-tagging sequence at the C terminus, inserted in baculovirus vector pFast Bac, and introduced into the Sf9 insect cells by lipofection. A crude membrane preparation from infected cells showed a new protein band of ~130 kDa that bound monoclonal antibody M2 (Fig. 3*A*), confirming that the full-length open reading frame W09C2.3 was expressed in Sf9 cells. A crude membrane preparation from baculovirus-infected cells was then subjected to the functional assay (30) for P-type ATPases; in the case of the PMCA pump, the rapid degradation of the intermediate can typically be slowed down by lanthanum, allowing its detection in acidic gel systems (27). The results of the assay showed that a clear signal in the 130-kDa area could be detected in infected cells, even if the expression level of the new protein was low. The signal was not present in the control counterparts (Fig. 3*B*). The phosphointermediate pattern, *i.e.* an extremely weak (if any) reaction in the presence of calcium alone, the absence of reaction in the presence of EGTA, and, especially, the increase in the steady-state level of the phosphointermediate in the presence of lanthanum and calcium corresponded to that observed with pumps of the PMCA family (24, 31). An endogenous P-type ATPase of 110–120 kDa was also detected by the assay, but it was clearly Ca^{2+} independent (curiously, its phosphoenzyme formation was inhibited in cells expressing the *mca-1* construct). The nature of this putative P-type ATPase, which is routinely observed in the assay on Sf9 cells, is unknown.

Mca Gene Products Contain Calmodulin-binding Domains at their C Termini—Important features of the mammalian PMCA pumps, such as the location and spacing between the transmembrane domains, the identity of the sequence of the phosphorylation site (32) and of the FITC site (33) as well the presence of the C-terminal extension that accounts for the molecular mass of 130 kDa, as compared with those of a typical SERCA-type pump (34), all point to the conclusion that the Mca family proteins belong to the PMCA family ATPases (Fig. 1). Whereas the overall sequence identity between mammalian and nematode proteins at the 200 C-terminal amino acids appears to be low, the most pronounced region of similarity (Fig. 4*A*) corresponds to the location of the calmodulin-binding/autoinhibitory domain of the mammalian pumps (35, 36). In all three nematode sequences, this region of similarity features an obvious propensity to form an amphiphilic helix, with clustering of hydrophobic residues on one side, and a variable number of basic residues on the opposite side (Fig. 4*B*). In a membrane-based assay, the expressed *mca-1* protein actually binds human calmodulin at a concentration of 10 nM or higher (Fig. 5). This experiment clearly demonstrates the specific binding of human calmodulin to a protein, which is only present in the cells, expressing the *mca-1* construct. Moreover, this protein is indeed expected to have a very similar mobility to the expressed human PMCA4. Two kinds of control samples used in this experiment sort out the interference of an endogenous streptavidin-binding protein (Fig. 5, *esb*), because this band is the only one remaining in the presence of EGTA (not shown).

Finally, whereas a calmodulin-binding domain is not necessarily identified by its sequence similarity to another one, in this case, a significant consensus sequence can be derived (Fig. 4*A*), which suggests that the Mca family may actually contain orthologs of the PMCA family, particularly because at a >90%

Downloaded from http://www.jbc.org/ by guest on July 25, 2018

completion of the nematode genome, no other suitable candidates could be found.

DISCUSSION

The genome projects have resulted in the identification of numerous proteins with a candidate function inferred from sequence similarity to known proteins and/or a genetically identified phenotype. In this work, database searches have revealed three groups of EST clones showing about a 65% similarity to mammalian calcium ATPases of the PMCA family. The location and spacing between the major sequence motifs of the *mca-1* protein unequivocally place it in the PMCA family and rule out the possibility that it belongs to a SERCA-type family or to any novel Ca^{2+}-ATPase family not found thus far in mammalian cells. In fact, a SERCA-type sequence is encoded on chromosome III (cosmid K9D11), whereas all three *mca* genes reside on chromosome IV. However, the *mca-1* gene has a number of features that are not found in *pmca* genes, namely, the presence of alternative splicing at the extreme N terminus, the absence of internal splicing site A found in the *pmca* genes, and the absence of alternative splicing around the calmodulin-binding domain,[2] as compared with the mammalian alternative splicing site C (37, 38). A peculiar feature of *mca-1* protein (which is not found, however, in *mca-2* and *mca-3*) is the presence of the serine-rich region, which includes 11 consecutive residues, located between transmembrane segments 2 and 3. This region replaces the stretch of alternating lysines and glutamic acid residues responsible for phospholipid sensitivity (39), which is pronounced in PMCA pumps but absent in SERCA pumps.

The calmodulin-stimulated mammalian PMCA family has been the subject of extensive studies during the last decade, after the cloning of the first cDNAs in the late 1980s. However, whereas single cloned PMCA isoforms could be expressed in a model system based on a cell line and some of their biochemical properties could be studied (for a review, see Ref. 3), the implication of their specific features at the cellular/organismal level was beyond the possibilities of the experimental system. The identification of nematode analogs of the mammalian PMCA pumps now paves the way to reverse genetic experiments on the organismal level.

At the time of writing, the *C. elegans* genome seems to contain only three genes, showing substantial similarity to the mammalian PMCA family as well as five candidate plasma membrane Na/Ca(K) exchangers.[2] Although it is not excluded that other candidate genes still reside in the gaps of the genomic data, or that they are missed in the EST data due to the toxicity of this protein or portions thereof to the *Escherichia coli* cell, the number and variety of candidate plasma membrane calcium transporters in the nematode approach the level of complexity known for mammalian systems. Experiments can now be designed to study the cell specificity of expression using the transgenic reporter gene expression approach (40, 41) and the results of systematic gene inactivation using the resources of a random knockout library produced in *C. elegans* with the use of the Tc1 transposable element (42, 43).

[2] Unpublished data.

Note Added in Proof—While this paper was in press, the genome sequence of *C. elegans* was completed (*The C. elegans* Sequencing Consortium (1998) *Science* **282,** 2012–2018).

REFERENCES

1. Carafoli, E. (1992) *J. Biol. Chem.* **267,** 2115–2118
2. Carafoli, E. (1994) *FASEB J.* **8,** 993–1002
3. Carafoli, E., Garcia Martin, E., and Guerini, D. (1996) *Experientia* (*Basel*) **52,** 1091–1100
4. MacLennan, D. H., Toyofuku, T., and Lytton, J. (1992) *Ann. N. Y. Acad. Sci.* **671,** 1–10
5. MacLennan, D. H., Rice, W. J., and Green, N. M. (1997) *J. Biol. Chem.* **272,** 28815–28818
6. Catty, P., and Goffeau, A. (1996) *Biosci. Rep.* **16,** 75–85
7. Cunningham, K. W., and Fink, G. R. (1994) *J. Exp. Biol.* **196,** 157–166
8. Antebi, A., and Fink, G. R. (1992) *Mol. Biol. Cell* **3,** 633–654
9. Cunningham, K. W., and Fink, G. R. (1994) *J. Cell Biol.* **124,** 351–363
10. Cunningham, K. W., and Fink, G. R. (1996) *Mol. Cell. Biol.* **16,** 2226–2237
11. Monteith, G. R., and Roufogalis, B. D. (1995) *Cell Calcium* **18,** 459–470
12. Hilfiker, H., Strehler-Page, M.-A., Stauffer, T. P., Carafoli, E., and Strehler, E. E. (1993) *J. Biol. Chem.* **268,** 19717–19725
13. Guerini, D., Schröder, S., Foletti, D., and Carafoli, E. (1995) *J. Biol. Chem.* **270,** 14643–14650
14. Kozel, P. J., Friedman, R. A., Erway, L. C., Yamoah, E. N., Liu, L. H., Riddle, T., Duffy, J. J., Doetschman, T., Miller, M. L., Cardell, E. L., and Shull, G. E. (1998) *J. Biol. Chem.* **273,** 18693–18696
15. Ahringer, J. (1997) *Curr. Opin. Genet. Dev.* **7,** 410–415
16. Schafer, W. R., and Kenyon, C. J. (1995) *Nature* **375,** 73–78
17. Lee, R. Y., Lobel, L., Hengartner, M., Horvitz, H. R., and Avery, L. (1997) *EMBO J.* **16,** 6066–6076
18. Maryon, E. B., Coronado, R., and Anderson, P. (1996) *J. Cell Biol.* **134,** 885–893
19. Sakube, Y., Ando, H., and Kagawa, H. (1997) *J. Mol. Biol.* **267,** 849–864
20. Baylis, H., Furuichi, T., Yoshikawa, F., Mikoshiba, K., and Satelle, D. (1998) *European Worm Meeting*, T-7, The Sanger Center, Cambridge UK
21. de Mendonca, R. L., Beck, E., Rumjanek, F. D., and Goffeau, A. (1995) *Mol. Biochem. Parasitol.* **72,** 129–139
22. Altschul, S. F., Gish, W., Miller, W., and Myers, E. W. (1990) *J. Mol. Biol.* **215,** 403–410
23. Devereux, J., Haeberli, P., and Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395
24. Heim, R., Iwata, T., Zvaritch, E., Adamo, H. P., Rutishauser, B., Strehler, E. E., Guerini, D., and Carafoli, E. (1992) *J. Biol. Chem.* **267,** 24476–24484
25. Laemmli, U. K. (1970) *Nature* **227,** 680–685
26. Towbin, H., Staehlin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
27. Sarkadi, B., Enyedi, A., Földes-Papp, Z., and Gardos, G. (1986) *J. Biol. Chem.* **261,** 9552–9557
28. Davis, R. E., Singh, H., Botka, C., Hardwick, C., el Meanawy, M. A., and Villanueva, J. (1994) *J. Biol. Chem.* **269,** 20026–20030
29. Davis, R. E. (1996) *Parasitol. Today* **12,** 33–40
30. Pedersen, P. L., and Carafoli, E. (1987) *Trends Biochem. Sci.* **12,** 146–150
31. Hilfiker, H., Guerini, D., and Carafoli, E. (1994) *J. Biol. Chem.* **269,** 26178–26183
32. James, P., Zvaritch, E., Shakhparonow, M. I., Penniston, J. T., and Carafoli, E. (1987) *Biochem. Biophys. Res. Commun.* **149,** 7–12
33. Filoteo, A. G., Gorski, J. P., and Penniston, J. T. (1987) *J. Biol. Chem.* **262,** 6526–6530
34. Verma, A. K., Filoteo, A. G., Stanford, D. R., Weiben, E. D., Penniston, J. T., Strehler, E. E., Fisher, R., Heim, R., Vogel, G., Mathews, S., Strehler-Page, M.-A., James, P., Vorherr, T., Krebs, J., and Carafoli, E. (1988) *J. Biol. Chem.* **263,** 14152–14159
35. Vorherr, T., James, P., Krebs, J., Enyedi, A., McCormick, D. J., Penniston, J. T., and Carafoli, E. (1990) *Biochemistry* **29,** 355–365
36. Vorherr, T., Kessler, T., Hofmann, F., and Carafoli, E. (1991) *J. Biol. Chem.* **266,** 22–27
37. Strehler, E. E., Strehler-Page, M.-A., Vogel, G., and Carafoli, E. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 6908–6912
38. Keeton, T. P., Burk, S. E., and Shull, G. E. (1993) *J. Biol. Chem.* **268,** 2740–2748
39. Niggli, V., Adunyah, E. S., and Carafoli, E. (1981) *J. Biol. Chem.* **256,** 8588–8592
40. Fire, A. (1986) *EMBO J.* **5,** 2673–2680
41. Fire, A., Harrison, S., and Dixon, D. (1990) *Gene* (*Amst.*) **93,** 189–198
42. Korswagen, H. C., Durbin, R. M., Smits, M. T., and Plasterk, R. H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14680–14685
43. Plasterk, R. H., Thijssen, K., Hazendonk, E., van der Horst, M., Korswagen, R., and Jansen, G. (1998) *European Worm Meeting*, T-17, The Sanger Center, Cambridge UK

Downloaded from http://www.jbc.org/ by guest on July 25, 2018

Identification and Functional Expression of the Plasma Membrane Calcium ATPase Gene Family from *Caenorhabditis elegans*

Alexander Kraev, Natalia Kraev and Ernesto Carafoli

J. Biol. Chem. 1999, 274:4254-4258.
doi: 10.1074/jbc.274.7.4254

Access the most updated version of this article at http://www.jbc.org/content/274/7/4254

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 24 of which can be accessed free at
http://www.jbc.org/content/274/7/4254.full.html#ref-list-1

Downloaded from http://www.jbc.org/ by guest on July 25, 2018