

Characterization and phylogenetic analysis of a cDNA encoding an EF-hand domain-containing calumenin-like protein in *Strongyloides ratti*

Abbas Jolodar^{1*}

¹ Associate Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received: 24.07.2025

Accepted: 16.10.2025

Abstract

Strongyloides ratti serves as an experimental model for diagnosing strongyloidiasis in humans. The secretory EF-hand-containing calumenin-like protein functions as calcium signaling molecule from rat was characterized. An 849-nucleotide cDNA fragment was amplified using RT-PCR, and a GenBank search revealed that the amplified product shared 98.54% identity with an incomplete mRNA sequence for *S. ratti*. Using this fragment as a probe, a search was conducted in the Expressed Sequence Tags (EST) database, identifying a 998 bp cDNA fragment through overlapping regions designated as SrCAL. The encoded protein consists of 314 amino acids and has a molecular weight of 36.74 kDa, and an isoelectric point (pI) of 4.61. The sequence includes a signal peptide, which has a cleavage site located between amino acids 21 and 22. A protein domain database search identified five highly conserved EF-hand calcium-binding motifs (cd16226), classifying SrCAL within the Efh_CREC_Calumenin-like protein superfamily, spanning amino acids 29 to 296. Structural prediction revealed that SrCAL is composed of 39.18% alpha-helices, 18.21% beta-strands, and 42.61% loops. Three dimensional (3D) structural modeling focused on 232 amino acids, covering 74% of the total coding sequence, with 99.8% confidence, showing strong structural resemblance to the crystal structure of an EF-hand protein from *Danio rerio*. Genetic distance and phylogenetic analysis revealed that SrCAL shares the lowest genetic distance (0.19%) with the only known protein sequence isolated from *S. ratti* (XP_024499492.1). SrCLA plays a crucial role in calcium signaling and may serve as a housekeeping gene with strong potential as a nematode-specific drug target.

Key words: Nematodes, EF-hand domain, *Strongyloides ratti*, RT-PCR, Calumenin-like proteins

* **Corresponding Author:** Abbas Jolodar, Associate Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: jolodara@scu.ac.ir



© 2020 by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (<http://creativecommons.org/licenses/by-nc/4.0/>).

References

- Anandarajah, E. M., Ditgen, D., Hansmann, J., Erttmann, K. D., Liebau, E. (2016). SPARC (secreted protein acidic and rich in cysteine) of the intestinal nematode *Strongyloides ratti* is involved in mucosa-associated parasite-host interaction. *Molecular and Biochemical Parasitology*, 207(2), 75-83.
- Buonfrate, D., Bisanzio, D., Giorli, G., Odermatt, P., Fürst T (2020). Greenaway C, French M, Reithinger R, Gobbi F, Montresor A, Bisoffi Z. The Global Prevalence of *Strongyloides stercoralis* Infection. *Pathogens*, 9(6), 468.
- Chazin, W. J. (2011). Relating form and function of EF-hand calcium binding proteins. *Accounts of chemical research*, 44(3), 171-9.
- Cho, J. H., Song, H. O., Singaravelu, G., Sung, H., Oh, W. C. (2009). Pleiotropic roles of calumenin (*calu-1*), a calcium-binding ER luminal protein. *Caenorhabditis elegans*. *FEBS Letters*, 583(18), 3050-3056.
- Choi, T. W., Cho, J. H., Ahnn, J., Song, H. O. (2018) Novel Findings of Anti-Filarial Drug Target and Structure-Based Virtual Screening for Drug Discovery. *International Journal of Molecular Sciences*, 19(11), 3579.
- Day, I. S., Reddy, V. S., Shad Ali, G. (2002). Analysis of EF-hand-containing proteins in *Arabidopsis*. *Genome Biology*, 3(10), RESEARCH0056.
- Finkelman, F. D., Shea-Donohue, T., Morris, S. C., Gildea, L., Strait, R., Madden K. B., Schopf, L., Urban, J. F. (2004). Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunological Review*, 201, 139-55.
- Grabarek, Z. (2006). Structural basis for diversity of the EF-hand calcium-binding proteins. *Journal of molecular biology*, 359(3), 509-25.
- Heiman, R. G., Atkinson, R.C., Andruss, B. F., Bolduc, C., Kovalick, G. E. (1996). Spontaneous avoidance behavior in *Drosophila* null for calmodulin expression. *Proceedings of the National Academy of Sciences of the United States of America*, 93(6), 2420-5.
- Honore, B., Vorum, H. (2000). The CREC family, a novel family of multiple EF-hand, low-affinity Ca(2+)-binding proteins localized to the secretory pathway of mammalian cells. *FEBS Letters*, 466(1), 11-8.
- Hotez, P. J., Brindley, P. J., Bethony, J. M., King, C. H., Pearce, E. J. (2008). Helminth infections: the great neglected tropical diseases. *The Journal of Clinical Investigation*, 118(4), 1311-21.
- Jantsch-Plunger, V., Glotzer, M. (1999). Depletion of syntaxins in the early *Caenorhabditis elegans* embryo reveals a role for membrane fusion events in cytokinesis. *Current Biology*, 9(14), 738-45.
- Karabinos, A. (2015). RNA interference and protein expression of the four calmodulin-like genes in *Caenorhabditis elegans*. *Biologia*, 70(6), 826-830.
- Kelley, L. A., Sternberg, M. J. (2009). Protein structure prediction on the Web: a case study using the Phyre server. *Nature Protocols*, 4(3), 363-71.
- Lamande, S. R., Bateman, J. F. (1999). Procollagen folding and assembly: the role of endoplasmic reticulum enzymes and molecular chaperones. *Seminars in Cell and Developmental Biology*, 10(5), 455-64.
- Maizels, R. M., Pearce E. J., Artis, D., Yazdanbakhsh, M., Wynn, T. A. (2009). Regulation of pathogenesis and immunity in helminth infections. *Journal of Experimental Medicine*, 206(10), 2059-66.
- Mason, L., Tribolet, L., Simon, A., von Gnielinski, N., Nienaber, L. (2014). Probing the equatorial groove of the hookworm protein and vaccine candidate antigen, Na-ASP-2. *International Journal of Biochemistry & Cell Biology*, 50, 146-55.
- Nakayama, S., Kawasaki, H., Kretsinger, R. (2000). Evolution of EF-Hand Proteins. In: Carafoli, E., Krebs, J. (eds). *Calcium Homeostasis. Topics in Biological Inorganic Chemistry*, Springer, Berlin, Heidelberg. 3, 29-58.
- Nagamune, K., Moreno, S. N., Chini, E. N., Sibley, L. D. (2008). Calcium Regulation and Signaling in Apicomplexan Parasites. In: Burleigh, B.A., Soldati-Favre, D. (eds) *Molecular Mechanisms of Parasite Invasion. Subcellular Biochemistry*, Springer, New York, NY. 47, 70-81.
- Orans, J., Johnson, M. D., Coggan, K. A., Sperlazza, J. R., Heiniger, R. W., Wolfgang, M. C., Redinbo, M. R. (2010). Crystal structure analysis reveals *Pseudomonas* PilY1 as an essential calcium-dependent regulator of bacterial surface motility. *Proc Natl Acad Sci U S A*, 107(3), 1065-70.

- Primm, T. P., Walker, K. W., Gilbert, H. F. (1996) Facilitated protein aggregation. Effects of calcium on the chaperone and anti-chaperone activity of protein disulfide-isomerase. *Journal of Biological Chemistry*, 271(52), 33664-9.
- Ruiz de Eguino, A. D., Machín, A., Casais, R., Castro, A. M., Boga, J. A. (1999). Cloning and expression in *Escherichia coli* of a *Fasciola hepatica* gene encoding a calcium-binding protein. *Molecular and Biochemical Parasitology* 101(1-2), 13-21.
- Saitou, N., Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.
- Tamura, K., Stecher, G., Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology Evolution*, 38(7), 3022-3027.
- Thompson, J. D., Higgins, D. G., Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673-4680.
- Viney, M., Kikuchi, T. (2017). *Strongyloides ratti* and *S. venezuelensis* - rodent models of *Strongyloides* infection. *Parasitology*, 144(3), 285-294.
- Yabe, D., Nakamura, T., Kanazawa, N., Tashiro, K., Honjo, T. (1997). Calumenin, a Ca²⁺- binding protein retained in the endoplasmic reticulum with a novel carboxyl-terminal sequence HDEF. *Journal of Biological Chemical*, 272(29), 18232-18239.
- Zhou, J., Sun, J., Huang, Y., Zhou, C., Liang, P. (2013). Molecular identification, immunolocalization, and characterization of *Clonorchis sinensis* calmodulin. *Parasitology Research*, 112(4), 1709-17.