MOLECULAR CHARACTERIZATION OF MYOSIN AND THE SODIUM-PROTON ANTIPORTER IN ILYANASSA OBSOLETA

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Ilyanassa obsoleta Stimpson, the common marine mud snail, offers an excellent experimental system for examining possible effects of zero gravity on embryogenesis in general and for determining cytoplasmic factors essential for heart development in particular. Large yolk platelets accumulate gravocentrically in the vegetal pole cytoplasm of the Ilyanassa egg, displacing the nucleus to the animal pole. As a result, Ilyanassa obsoleta embryos sequester their vegetal hemisphere cytoplasm in an anuclear polar lobe at the time of their acentric first cleavage, then merge that polar lobe cytoplasm with just one of the daughter cells, and eventually form their hearts (as well as other tissues) from the cellular descendants of the polar lobe cytoplasm-containing daughter cell. If the polar lobe cytoplasm is divided equally between the two first cleavage daughter cells, morphogenesis, especially of lobe-dependent structures, is disturbed in 40% of the embryos (Render, J., J. Exp. Zool. 253:30-37, 1990). If the polar lobe is removed from the embryo at the time of first cleavage, developing embryos fail to form a beating heart, eyes, statocysts, intestine, operculum, or external shell, although they do form extensive muscle tissue (Atkinson, J.W., J. Morphol. 133:339-352, 1971; Int. J. Invertr. Reprod. Dev. 9:169-178, 1986). To examine the role of cytoplasmic factors in heart muscle development, a rapid, sensitive, and specific assay for Ilyanassa cardiac myocyte differentiation must be developed. Vertebrate heart muscle cells express heart-specific isoforms of the contractile protein myosin-II that can be distinguished from other myosin isoforms by the nucleic acid/amino acid sequence of its amino-terminal globular head region (Bement, W.M., Hasson, T., Wirth, J.A., Cheney, R.E., and Mooseker, M.S. Proc. Natl. Acad. Sci. USA 91:6549-6553, 1994). As a first step in determining whether Ilyanassa obsoleta adult hearts express a heart-specific myosin isoform that may serve as a heart-specific marker for developmental studies, reverse transcriptase-polymerase chain reaction (RT-PCR) studies using degenerate primers for myosin-II were carried out on whole adult Ilyanassa obsoleta. For comparison, a non-heart-specific Ilyanassa probe was developed using degenerate primers for a sodium-proton antiporter.

Total cell RNA was isolated from a whole adult snail using the Promega RNAgents Total RNA Isolation System. Analysis of the RNA on an agarose/ formaldehyde gel revealed intact ribosomal RNA. Degenerate probes for myosin-II were prepared against the 5′ ATP binding site (5′-GARTCNGGNGCNGGNAARAC-3′) and a 3′ site of unknown function (5′-RTGRTTRAANARYTGYTG-3′), based on amino acid sequences conserved from C. elegans to rat, and synthesized by Genemed Biotechnologies, Inc., South San Francisco, CA 94080. Degenerate 5′ and 3′ oligonucleotide probes for the crab sodium-proton antiporter were generously supplied by Dr. David Towle. Ilyanassa

RNA was converted to cDNA using the GibcoBRL Superscript Preamplification System for First Strand cDNA Synthesis, and PCR was subsequently conducted using the GibcoBRL PCR Reagent System with 40 amplification cycles of 94° for 45 sec, 42° for 30 sec, and 72° for 90 sec in a Perkin Elmer Thermocycler. The myosin-II probes yielded an expected band of approximately 925 bp, whereas the sodium-proton antiporter probes yielded an expected band of 710 bp, when separated on agarose gels. These bands were then excised from the gel, cleaned using the Ambion GeniePrep Kit, ligated into gGEMt, transfected into DH5α cloned using blue-white selection, purified by 5 Prime-3 Prime Perfectprep, and sequenced by the KSU Biotechnology Sequencing Center.

Comparison of the Ilyanassa obsoleta myosin cDNA sequence with other known sequences revealed that it is most closely related to Scallop myosin-II, and that it contains sequence identities to both the striated and the smooth muscle myosin isoforms in the critical exon 5-exon 6 region that defines the difference between these two isoforms in the Scallop (Nyitray, L., Jancsó, A., Ochiai, Y., Gráf, L., and Szent-Györgyi, A.G. Proc. Natl. Acad. Sci. USA 91:12686-12690, 1994). The second most closely related myosins are the chicken and rabbit embryonic sarcomeric myosin-IIs, and the third most closely related myosins are the mouse, rat, and human alpha cardiac myosin-IIs. Current studies are underway using degenerate primers to the more 3' myosin actin-binding site to extend knowledge of the Ilyanassa myosin-II sequence to include that region. In addition, other myosin degenerate probes, shown by Blement et al., 1995, to reveal multiple additional myosin class isoforms in human and porcine cells, will be used to investigate the existence of other myosin isoforms in Ilyanassa. Comparison of the Ilyanassa obsoleta sodium-proton antiporter cDNA sequence with other known sequences revealed that it is most closely related to the rat antiporter, followed by the chinese hamster and the crab antiporters. Upon further confirmation, these Ilyanassa sequences will be submitted to Genbank, and will represent the second and third Ilyanassa sequences present in Genbank (the first is for RNA polymerase II: U10338). With this myosin-II sequence known, we are positioned to isolate Ilyanassa adult hearts and to determine, using these and other degenerate myosin probes, whether Ilyanassa hearts make tissue-specific myosin isoforms that may be used for following heart development in lobed and lobeless embryos. Research supported by NASA-NSCORT NAGW-2328.