EXPRESSION OF CYTOSKELETAL, MEMBRANE TRANSPORT, AND TRANSCRIPTION FACTOR mRNAS IN EMBRYONIC AND ADULT *ILYANASSA OBSOLETA*

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The fertilized egg of the common marine gastropod mollusc, *Ilyanassa obsoleta*, forms two microfilament contractile rings at first cleavage. The cleavage furrow ring, which bisects the animal pole of the egg in a vertical plane, constricts around the spindle midbody, and results in the division of the egg into two daughter cells. The polar lobe constriction (PLC) ring, which forms just below the equator of the fertilized egg, constricts in a horizontal plane around few microtubules to sequester the vegetal cytoplasm into a polar lobe, and then relaxes, thereby delivering the polar lobe cytoplasm to just one of the two daughter cells. In this manner first cleavage produces daughter cells of unequal size and composition. Subsequent equal and unequal cell divisions divide the embryo into micromeres and macromeres with determined developmental fates (Render, J., Dev.Biol. 189:301-310, 1997), eventually giving rise to a bilaterally symmetrical veliger larva. If the polar lobe is removed from the embryo at first cleavage, the resulting veliger larva is radially symmetrical and fails to develop a beating heart, eyes, statocysts, operculum, intestine, and external shell, whereas the velum, digestive glands, stomach, style sac, shell-secreting cells, and muscle cells do differentiate (Atkinson, J.W., J. Morphol. 133:339-352, 1971). Thus, the overall morphogenesis of the lobeless embryo is severely disturbed. Incubation of fertilized eggs in nanomolar concentrations of Ag^+ causes the PLC to constrict more quickly once it forms, to lengthen greatly, and to remain constricted, such that the polar lobe cytoplasm is severed from the embryo, creating a lobeless embryo. The mechanism by which Ag^+ alters the constriction dynamics of the PLC is not known. In order to understand how Ag^+ alters the PLC and how the consequent removal of the polar lobe cytoplasm results in subsequent veliger developmental defects, we are defining molecular markers for cytoskeletal, membrane, heat shock, and transcription factor proteins that may be involved in these alterations.

Using RT-PCR with degenerate primers designed from highly conserved regions of myosin II, we previously identified, cloned, and sequenced a 945 bp fragment from the head region of *Ilyanassa* myosin II mRNA, and showed that this sequence is expressed abundantly in adult foot muscle, heart, gill, and liver, and at a very low level in fertilized eggs prior to first cleavage. In the present study we isolated RNA from *Ilyanassa* foot muscle (RNAgents, Promega), performed 5' RACE (Gibco-BRL) with *Ilyanassa*-specific myosin II primers, and determined the sequence for the 5' 1678 bp of *Ilyanassa* myosin II cDNA. Interestingly, this region of *Ilyanassa* myosin II is most closely related to scallop smooth and striated myosin IIs, followed by mouse, rat, and human heart-specific alpha-cardiac myosin. In addition, we used *Ilyanassa* foot muscle RNA, degenerate primers, and RT-PCR to obtain, clone, and sequence a 721 bp fragment of *Ilyanassa* mRNA for tropomyosin, an actin-binding protein.

We previously employed RT-PCR with degenerate primers (sequences generously provided by Dr. David Towle, Lake Forest College and MDIBL) to obtain, clone, and sequence a fragment of the *Ilyanassa* Na^+/H^+ antiporter mRNA. The present study used a similar stratagy, with degenerate primer sequences provided by Dr. Towle, to obtain, clone, and sequence a fragment of an *Ilyanassa* vacuolar H^+/ATPase membrane transporter mRNA. Using RT-PCR with mRNA isolated from various adult *Ilyanassa* tissues (Invitrogen MicroFastTrack Kit), we demonstrated that the vacuolar H^+/ATPase transporter mRNA is strongly expressed in adult foot muscle, heart, gill, and liver of *Ilyanassa*, whereas the Na^+/H^+ antiporter mRNA is expressed strongly in adult gill and liver, but is not detected in adult foot muscle or heart muscle. Moveover, RT-PCR with RNA isolated from the fertilized egg by the LiCl method (Sambrook, J., Fritch, E.F., and Maniatis, T. Molecular Cloning. A Laboratory Manual. Second Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 11724; 17.16-17.17, 1989) revealed abundant

mRNAs for both membrane transporters, in contrast to a very low level of myosin II mRNA.

While Ag^+ has not been shown to elicit metallothionein expression in other organisms, it may cause a change in the expression of heat shock proteins. To examine this possibility, we obtained, cloned, and sequenced a 3' fragment of an *Ilyanassa* mRNA that is highly homologous in its deduced open reading frame amino acid sequence to vertebrate and invertebrate hsp 70 proteins.

The *Antennapedia* homeobox (*hox*) genes, the *engrailed* homeobox (*en*) gene, the *ets* proto-oncogene, and paired-domain (*pax*) genes encode transcription factor proteins involved in early embryogenesis and larval metamorphosis in organisms from the gastropod abalone to humans. Degenerate primers were designed based on highly conserved regions of these transcription factor proteins. RNA isolated from D6 or D9 *Ilyanassa* embryos (see below) was used with random hexamers to synthesize cDNA. PCR performed with these degenerate primers and cDNAs gave bands of the expected sizes for *hox* (129 bp), *en* (232 bp), *ets* (159 bp) and *pax* (276) cDNA fragments. These bands were then cloned and sequenced. We identified 8 transcription factor genes: 4 *hox* genes, 1 *en* sequence (previously obtained in *Ilyanassa* by others, also), 1 *ets* 1/2 sequence, and 2 *pax* genes (*pax* 6 and *pax* 2/5/8).

To examine the expression of the mRNAs for these cytoskeletal, transmembrane, heat shock, and transcription factor proteins during *Ilyanassa* veliger morphogenesis, batches of 30-80 egg capsules were collected and incubated in 50 mls MFSW (millepore-filtered sea water) containing 50 ug/ml gentamycin with slow rotation. The MFSW/gentamycin was changed every 2 days. Capsules were opened, embryos were recovered, and RNA was harvested using the LiCl method after 0 days (D0=fertilized eggs), 2 days (D2=blastula), 4 days (D4=shell field evagination), 5 days (D5=early torsion), 6 days (D6=early velum, but no directed swimming), 8 days (D8=swimming veliger, but not hatched), and 9 days (D9=hatched swimming veliger) of development. In RT-PCR with cDNA synthesized using oligo dT, primers for *Ilyanassa* myosin II gave no band from D0, D2, or D4 embryos, but gave a strong band from D5, D6, D8, and D9 embryos. In contrast, primers for the *Ilyanassa* Na^+/H^+ antiporter gave a strong band with fertilized eggs and all later stage embryos.

In the future we intend to perform 5' RACE and 3' RACE to obtain the N-terminal and C-terminal nucleotide sequences for tropomyosin mRNA in order to deduce the terminal peptide sequences for this cytoskeletal protein. We will synthesize *Ilyanassa* myosin II and tropomyosin terminal peptides, raise chicken antibodies to these peptides, and confirm their respective specificities for intact *Ilyanassa* myosin II and tropomyosin by western blotting. We will then stain control and Ag^+-treated eggs with these antibodies (Conrad, A.H., et al., Cell. Motil. Cytoskel. 27:117-132, 1994) to determine whether localizations of tropomyosin or myosin II in the PLC are altered by Ag^+-treatment. We will examine the expression of transmembrane and hsp70 mRNAs in control and Ag^+-treated D0 through D9 embryos. Fertilized eggs, preveliger and veliger embryo RNAs will be examined by RT-PCR for the presence of the transcription factor mRNAs. We will then use RNAse protection assays, with labeled probes synthesized from the cloned transcription factor PCR bands, to assess the expression of these morphogenesis-related transcription factor mRNAs in normal and Ag^+-treated embryos, to see if any of these genes are altered in their expression in the Ag^+-treated embryos.

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