ATRIAL NATRIURETIC PEPTIDE (ANP) GENE EXPRESSION AS A MARKER FOR CARDIAC MUSCLE DIFFERENTIATION IN *ILYANASSA OBSOLETA*

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In the common marine mudsnail, *Ilyanassa obsoleta* (*Nassarius obsoletus*), the morphogenetic determinants regulating the differentiation of several larval tissues are localized in the polar lobe cytoplasm of the fertilized egg. Removal of the polar lobe that is formed at the time of first cleavage results in no loss of nuclei from the daughter cells, but the 2-cell embryo that remains undergoes cleavage and generates a larva that consistently forms certain tissues (lobe-independent tissues), but fails to form several others (lobe-dependent tissues). One of the tissues in the latter group appears to be the heart, based on morphological evidence, but the detection of some contractile cells indicates that functional cardiomyocytes may have differentiated normally but become scattered within the embryo (Atkinson, J.W. 1971. J. Morphol. 133:339-325). We are interested in the processes by which such differentiation factors localize in the polar lobe and regulate differentiation of specific tissues such as the heart. However, to assay the activity of such factors in embryos whose morphology is abnormal requires an assay for heart muscle development that is independent of morphological criteria and more sensitive than those available previously. To this end, we have noted the synthesis of ANP-like peptides by cardiac muscle cells in an evolutionarily broad range of species from humans to annelids (Vesely, D.L., and A.T. Giordano. 1992. Comp. Biochem. Physiol. 101C:325-329), and, therefore, have chosen to utilize available techniques of molecular biology to detect the presence of mRNAs for ANP-like peptides as a marker for cardiac muscle differentiation. We developed an assay utilizing cardiac tissue from adult snails and intend to use it to assay for heart muscle differentiation in normal and polar lobeless embryos. The active 28 [ANP] or 22 [CNP] amino acids of ANF-like peptides are very highly conserved in all species so far examined (Zivin et al. 1984. Proc. Natl. Acad. Sci. 81:6325-6329). PCR sense 17 and 22 base primers, one set unique for ANP and another unique for CNP, were designed and synthesized, based on published amino acid and nucleotide sequences for frog and mouse natriuretic peptides, respectively. Poly A^+ mRNA was isolated separately from atria and ventricles of adult *Ilyanassa* hearts using the Invitrogen Micro-FastTrack mRNA isolation procedure (Invitrogen Corp., San Diego, CA) or the Hybond mAP paper chip method (Sheardon, S.A. 1992. Trends in Genetics 8:121). After reverse transcriptase conversion to cDNAs, one ANP-specific sense primer and one CNP-specific sense primer were used with 3' RACE PCR (GIBCO BRL, Life Technologies, INC., Gaithersburg, MD) to examine the atrial and ventricle mRNAs for ANP and/or CNP messages, respectively, and the PCR products were resolved on agarose gels. Atrial mRNA revealed a strong band of about 190 base pairs and two minor bands of about 264 and 88 base pairs with the ANP primer, and a strong band of about 350 base pairs and several minor bands of 397, 228, and 138 base pairs with the CNP primer. In contrast, ventricle mRNA yielded no bands with either ANP or CNP primers. The atrial ANP and CNP bands will be reamplified with the second natriuretic-specific primer, and those bands which respond to both primers for a specific natriuretic peptide will be sequenced to confirm that they are the proper natriuretic mRNAs and to reveal the exact nucleotide sequences for *Ilyanassa* atrial natriuretic peptide(s). *Ilyanassa*-specific primers will be synthesized and used to screen other *Ilyanassa* tissues to determine whether the natriuretic mRNAs are cardiac specific. If

this is the case, then PCR analysis of the developing snail embryo will be conducted to determine when the natriuretic mRNA (and thus cardiac myocytes) first appears during embryogenesis, and how its appearance may be altered by removal of the polar lobe cytoplasm at first cleavage. Work supported by NASA-BioServe (NAGW-1197), NASA-NSCORT (NAGW-2328), and summer fellowship to A.P.S. from American Heart Assoc. (ME Affil.).