Myosin heavy chain expression in the specialized embryonic tail appendage of the skate, *Leucoraja erinacea*

Fred Schachat[1], Linhua Song[1], Thomas Koob[2] and John H. Long[3]

[1]Department of Cell Biology, Duke University Medical School, Durham, NC 27710
[2]Shriner's Hospital for Children, Tampa, FL 33607
[3]Biology Department, Vassar College, Poughkeepsie, NY 12604

The North Atlantic skate, *Leucoraja erinacea*, lays its fertilized eggs in a horny capsule. Commonly known as a mermaid's purse, the sclerotized opaque capsule conceals the embryo and protects it from predators. This protection has a cost: the capsule severely limits the diffusion of oxygen needed for growth. To obtain sufficient oxygen for development, the skate embryo propels sea water through small slits that form in the outer edge of the horns on the capsule's periphery. Effective ventilation is accomplished by steady undulatory flexures of a caudal, post-dorsal fin extension of the embryonic tail, [2] which we have designated the tail pump appendage (TPA). We predict that the TPA, which is an embryonic structure that appears to be resorbed after hatching, will express contractile proteins that differ from those of the myomeric and tail muscles, reflecting the optimization of the TPA for virtually continuous contractile activity.

Because myosin is the major determinant of the contractile properties of striated muscle, we focus here on the expression of myosin heavy chains (MYH) in the TPA. SDS-PAGE analysis was used to resolve MYHs from different skate muscles. As shown in Figure 1, when homogenates of several embryonic and adult skate muscles are electrophoresed on gels designed to separate MYHs, (Talmadge et al. [4]), a single MYH species predominates in the TPA. This MYH comigrates with the atrial MYH, but is readily resolved from those present in the embryonic pre-dorsal fin tail and adult myomeric and tail muscle. The comigration of the atrial and TPA MYHs suggested that the TPA MYH might be a member of the slow/cardiac family of MYHs that are expressed in vertebrate heart and slow striated muscle, both of which are specialized for persistent contractile activity.

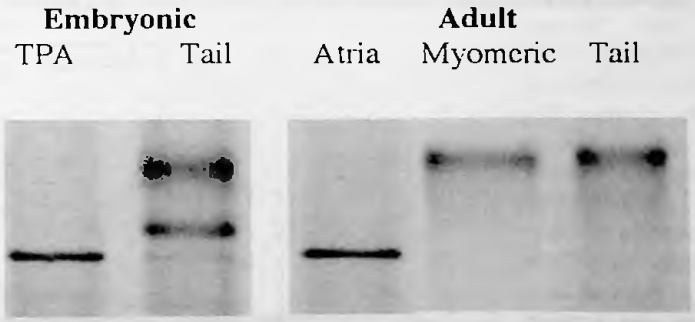

Fig. 1. SDS-PAGE electrophoresis of skate embryonic and adult myosin heavy chains. Analysis of MYHs revealed a single MYH variant in the embryonic TPA that differs in mobility from the MYHs in adult tail muscle and myomeric muscle, but is similar in mobility to that of adult atria.

The MYHs of the TPA, atrial, and other skate MYHs were further assessed by sequence analysis of RT-PCR products. Total RNA was isolated from the TPA and several embryonic and adult muscles, and cDNAs were generated using degenerate oligonucleotide primers (Ruiz-Trillo et al.[3]) that bind to highly conserved sequences flanking a functionally important and diagnostically variable region of the myosin head. cDNAs sequences were determined directly, when a single MYH predominated, or by subcloning and sequencing of several clones, when multiple MYH mRNAs were present (as described in Briggs et al. [1]). The inferred amino acid sequences are presented in Figure 2.

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Embryonic TPA   KLQQFFNHHMFVLEQEEYKKEGIEWEFIDFGMDLQACIDLIEKPMGIM
Adult atria     ........................................................
Adult epaxial   ...........................T.......A...E.......F
Adult jaw       ...........................V.T...........E.L.....F

Embryonic TPA   SILEEECMFPKASDQTFKAKLYDNHLGKSANFQKPRVVKGKPEAHFGL
Adult atria     ........................................................
Adult epaxial   ..............IS..N....Q.....N..L..KP.........S.
Adult jaw       .....Q.V....T.A....A.........N..L..KGG...A.PT.E.

Embryonic TPA   AHYAGTVDYNIMGWLQKNKDPLNETVVGLYQKSSVKLLAVLFANYAGA
Adult atria     ........................................................
Adult epaxial   .....N.....T...D..........I..F........GT.YV--.AP
Adult jaw       V......G.STQ...E.............F....QA..PI..K-----

Embryonic TPA   DGTTEKKGTK-KKGSSFQTVSALHRENLNKLMANLRSTHPHFVRCIIP
Adult atria     ..........-.............................................
Adult epaxial   EDS.K.A.-.-...G.........F....G.......T.........L..
Adult jaw       EEE.PAG.K.Q............FY..Q.....TT....A.......V.
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Fig. 2. The inferred amino acid sequences of MYH PCR products generated from different skate embryonic and adult striated muscles. The amino acid sequences of the TPA and adult atrial muscles are identical, as are their nucleic acid sequences. "." indicates sequence identity; "-" indicates the insertion of gaps for alignment by Clustal W.

Aligning these sequences with Clustal W (DNAStar, Madison, WI) reveals that those from the TPA and adult atrial MYHs are identical, as were their nucleic acid sequences (data not presented). In contrast, the TPA and adult atria sequences differ from those in the adult epaxial and jaw muscles.

Clustal W analysis was also used to determine the molecular phylogeny of the skate MYH sequences by comparing them with the corresponding human striated MYH sequences (Figure 3). This shows that the virtually continuous activity of the TPA is reflected in the expression of a member of the "slow/cardiac" family of MYHs.

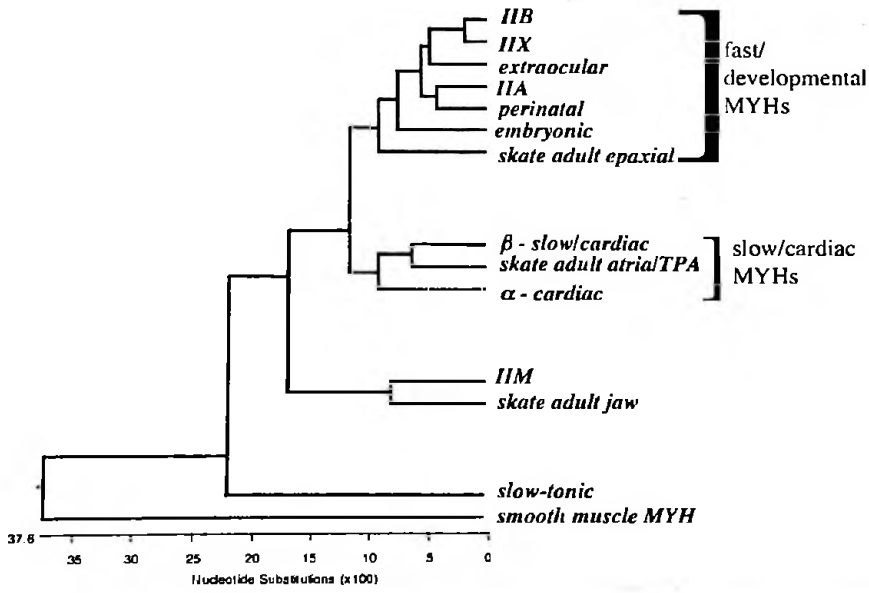

Fig. 3. Sequence relationship between the skates and human MYHs. Human was chosen for comparison because its' genome has been sequenced, and all its striated MYH genes identified. Clustal W analysis reveals that the skate adult atria/TPA sequence is most closely related to the human cardiac/slow MYHs. The families of fast/developmental and slow/ cardiac MYHs are indicated

In conclusion, these studies show that a single MYH is expressed in the specialized embryonic post-dorsal fin TPA of *L. erinacea*. This MYH differs from those that predominate elsewhere in the embryonic and adult skate tail and myomeric muscle; however, it is homologous with the vertebrate

slow/cardiac family of MYHs. These studies also reveal that the skate myomeric muscle expresses MYHs that are homologous with the fast/developmental MYHs and the IIM myosin in its jaw muscle.

The expression of a member of the slow/cardiac family of vertebrate MYHs in the TPA confirms our expectation that it is specialized for its virtually continuous contractile activity in propelling sea water though the skate egg case to ventilate the embryo.

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