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Expression of Unconventional Myosin Genes During Neuronal Development in Zebrafish

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Abstract

Neuronal migration and growth cone motility are essential aspects of the development and maturation of the nervous system. These cellular events result from dynamic changes in the organization and function of the cytoskeleton, in part due to the activity of cytoskeletal motor proteins such as myosins. Although specific myosins such as Myo2 (conventional or muscle myosin), Myo1, and Myo5 have been well characterized for roles in cell motility, the roles of the majority of unconventional (other than Myo2) myosins in cell motility events have not been investigated. To address this issue, we have undertaken an analysis of unconventional myosins in zebrafish, a premier model for studying cellular and growth cone motility in the vertebrate nervous system. We describe the characterization and expression patterns of several members of the unconventional myosin gene family. Based on available genomic sequence data, we identified 18 unconventional myosin- and 4 Myo2-related genes in the zebrafish genome in addition to previously characterized myosin (-1, -2, -3, -5, -6, -7) genes. Phylogenetic analyses indicate that these genes can be grouped into existing classifications for unconventional myosins from mouse and man. In situ hybridization analyses using EST probes for 18 of the 22 identified genes indicate that 11/18 genes are expressed in a restricted fashion in the zebrafish embryo. Specific myosins are expressed in particular neuronal or neuroepithelial cell types in the developing zebrafish nervous system, spanning the periods of neuronal differentiation and migration, and of growth cone guidance and motility.

Keywords

cytoskeleton; unconventional myosin; neuronal migration; axon guidance; growth cone motility; zebrafish; commissure; spinal cord; motor neuron; neural crest; somite; ear; eye; morphogenesis; in situ hybridization; phylogenetic tree; hindbrain; forebrain; midbrain; cranial muscles

1. INTRODUCTION

During embryogenesis, neuronal cell bodies and growth cones migrate extensively to establish precisely connected networks with target tissues. The functional properties of

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neural networks underlying physiology and behavior are critically dependent upon accurate positioning of neuronal cell bodies, and their axonal and dendritic processes. Not surprisingly, a number of brain disorders result from the loss of or aberrant migration of neurons and defective axon guidance (Copp and Harding, 1999; Gleeson and Walsh, 2000; Oster and Sretavan, 2003; ten Donkelaar et al., 2004). A large number of extracellular cues and their receptors have been intensively studied, and demonstrated to play essential roles in regulating the migration of neuronal growth cones and cell bodies (Chilton, 2006). Furthermore, the biochemical and molecular interactions between the signal transduction cascades initiated by these guidance cues and the cytoskeletal machinery that controls dynamic cellular and growth cone behaviors are being elucidated (Guan and Rao, 2003; Kalil and Dent, 2005). Nevertheless, the roles of specific components of the actin and microtubule cytoskeletons in generating particular responses to extracellular cues remain obscure. We are interested in characterizing the functions of the unconventional, non-muscle myosins in neuronal migration and axon guidance, and in elucidating whether these activities are regulated by guidance cues in the developing nervous system.

Unconventional non-muscle myosins are actin-binding motor proteins that lack the tail domain of conventional muscle myosin (myosin 2), and function in numerous processes including cell migration in lower eukaryotes, intracellular motility and trafficking, and sensory transduction (Libby and Steel, 2000; Tuxworth and Titus, 2000; Wu et al., 2000; Soldati, 2003; Hirokawa and Takemura, 2003). Furthermore, unconventional myosins have been localized to neuronal growth cones and regulate growth cone motility in vitro (Wang et al., 1996; Evans et al., 1997; Suter et al., 2000; Deifenbach et al., 2002; Sousa et al., 2006). Phylogenetic analysis of the catalytic head domain (containing the actin- and ATP-binding sites) of all available myosin heavy chain sequences shows that there are at least 18 families of myosins, including conventional (muscle) Myo2, several myosins found only in fungi or other lower eukaryotes (Myosins 4, 11, 12, 13, 14, and 17), and the plant-specific Myo8 (Hodge and Cope, 2000; Sellers, 2000; Berg et al., 2001; Reddy and Day, 2001; Volkmann et al., 2003). In addition to differences in the catalytic head domain, unconventional myosins belonging to various families differ in the presence and arrangement of specific domains and motifs that likely confer unique functions on each class of proteins (Fig. 1; Wu et al., 2000; De La Cruz and Ostap, 2004). Whereas only 4–6 unconventional myosin families have been identified in unicellular organisms and invertebrates like *Caenorhabditis*, comprehensive phylogenetic analyses have identified over 10 unconventional myosin families in *Drosophila* and mammals (Berg et al., 2001).

There has been no systematic characterization of unconventional myosins in zebrafish. Mutations generating defects in sensory neuron development and function have led to the cloning of members of the Myo6 and Myo7 families (Ernest et al., 2000; Kappler et al., 2004; Seiler et al., 2004; Coffin et al., 2007). Since the zebrafish embryo is an excellent model for studying cell migration and axon guidance (Kuwada, 1995; Hutson and Chien, 2002), the potential roles of unconventional myosins in these dynamic cellular processes can be readily investigated. Therefore, we have carried out an extensive characterization of the expression of unconventional myosin genes in the developing zebrafish nervous system. Our results demonstrate that specific myosins are expressed in particular neuronal or neuroepithelial cell types, spanning the periods of neuronal differentiation and migration, and of growth cone guidance and motility.

2. RESULTS AND DISCUSSION

2.1 Phylogenetic analysis of zebrafish unconventional myosins

Exhaustive search of the zebrafish genome assembly (Zv6, March 2006 and Zv7, July 2007) identified a total of 22 myosin genes representing classes 1, 2, 5, 9, 10, 15, 16 and 18.

Zebrafish myosins 3, 6 and 7 were excluded from analyses because they have been described previously, whereas other myosin classes (4, 8, 11, 12, 13, 14, and 17) have been described only in fungi, other lower eukaryotes, or plants (Berg et al., 2001). In order to group the newly identified zebrafish (*Danio rerio*, Dr) myosins into appropriate classes, we constructed a phylogenetic tree with human (*Homo sapiens*, Hs) and mouse (*Mus musculus*, Mm) orthologs using amino acid sequences of the highly conserved core motor domains of various myosin classes, equivalent to residues 88–780 of chicken skeletal myosin II (Hodge and Cope, 2000; Berg et al., 2001). Alignment of core domains was performed in Megalign using Clustal-W method (Reddy and Day, 2001; see Methods for additional details). A rectangular tree for the aligned sequences rooted to Myo1 (Fig. 2) was generated by maximum parsimony method (Jiang and Ramachandran, 2004; see Methods). The tree identifies the presence of at least two genes each in the Myo1b, Myo1e, Myo5, Myo9a, Myo15 and Myo18 classes and three genes in the Myo10 class in the available zebrafish genome. Our study also identifies four conventional *myo2* genes corresponding to different heavy chain genes as shown in the tree (Fig. 2). While most zebrafish myosins identified in this study group with one of the classes of human and mouse myosins with >95% bootstrap value, the core motor domains of zebrafish Myo9a1, Myo9a2 and Myo10b group with Myo16 and Myo18 with weak support (51% bootstrap value; Fig. 2). However, when the entire polypeptide sequences are used for the phylogenetic analysis, these genes group with their Myo9a and Myo10 orthologs (100% bootstrap value; cladogram not shown), respectively, due to the presence of class specific domains (Fig. 1).

2.2 Expression analysis of zebrafish myosins

We carried out a detailed analysis of the expression patterns of various myosins for which ESTs were available in 2004 (Table 1). This includes all zebrafish genes described in Figs. 1 and 2, except *myo1b1*, *myo1e2*, *myo5b*, and *myo15b*, for which no ESTs were available, and *myo3a* (Thisse and Thisse, 2005), *myo6* and *myo7* whose expression patterns were previously described (Ernest et al., 2000; Seiler et al., 2004; Kappler et al., 2004). We mostly examined expression patterns in embryos fixed at 18, 24, 30, and 36 hpf. Expression at earlier stages was not systematically examined since we are primarily interested in potential roles for these molecules in neuronal migration and axon guidance, and older ages were examined as needed. The myosin genes exhibited restricted patterns of expression in various tissues. In the tissues examined, *myo5a*, *myo10a*, *myo1b1* were expressed at all ages tested; *myo1e1* was expressed only between 30–36 hpf; *myo10b* and *myo18a* were strongly expressed between 18–24 hpf, and weaker by 30 hpf; *myo15a* was expressed only between 12–18 hpf; *myo2hc1* was expressed strongly between 15–18 hpf, and weaker by 24–30 hpf; *smyhc1*, *myo2hc2* and *myo2hc9* were expressed in similar patterns between 18–36 hpf, with *myo2hc2* and *smyhc1* expressed at least until 72 and 120 hpf, respectively.

2.2.1 Expression in Hindbrain segments—Two myosins are transiently expressed in a segmented manner in specific rhombomeres. At 18 hpf, *myo10b* is strongly expressed in r5, and more weakly in r2, r3 and r4, and at the midbrain-hindbrain boundary (Fig. 3A). At 12 hpf, *myo15a* is expressed in r3, r5, and at the midbrain-hindbrain boundary (Fig. 3B), with weaker expression at 18 hpf (data not shown). Thus, these genes are expressed in cells exhibiting sorting behaviors to set up well-defined rhombomere boundaries (Cooke et al., 2005). From 30–36 hpf, *myo1e1* is expressed in a small patch of mesendodermal cells located at the somite 2–3 boundary (Fig. 3C), either the developing pancreas (Argenton et al., 1999; Stafford and Prince, 2002) or the pronephros (Drummond et al., 1998).

2.2.2 Expression in Hindbrain neurons—Three myosins are expressed in specific neuronal populations in the hindbrain. At 18 hpf, *myo1b2* is expressed in a subset of the cranial sensory ganglia, as well as in a small group of unidentified neurons in r2 (Fig. 3D).

Analysis of *myo1b2* expression in the hedgehog pathway mutant *detour* (*gli1*^{-/-}; Vanderlaan et al., 2005) indicates that these cells are not cranial motor neurons (data not shown). At 36 hpf, *myo1b2* is expressed by a small number of cells within each rhombomere, with strong expression in cells flanking the boundaries of rhombomeres 5 and 6 (Fig. 3G). At 18 hpf, *myo5a* is expressed in a subset of the cranial ganglia, and in differentiating neurons in every rhombomere (Fig. 3E), similar to expression of the neurogenesis marker huC (Bingham et al., 2003). By 24 hpf, *myo5a* expression expands into a continuous column, as additional neurons differentiate in these regions (Fig. 3H). At 18 hpf, *myo10a* is expressed in the trigeminal ganglion, and is also expressed at high levels in the lateral line ganglia by 30 hpf (Figs. 3F, I). In addition, *myo10a* is expressed weakly by trigeminal and facial branchiomotor neurons from 18–30 hpf, and by other differentiating neurons by 30 hpf (Fig. 3I). The expression of these myosins in cranial ganglia and in differentiating neurons coincides with axonogenesis or soma migration in these neuronal populations (Metcalf et al., 1990; Higashijima et al., 2000).

2.2.3 Expression in the Forebrain and midbrain—Two myosins, *myo5a* and *myo10a*, are expressed in specific populations of forebrain and midbrain neurons (Fig. 4). In the forebrain, *myo5a*- and *myo10a*-expressing cells from 18–30 hpf (Fig. 4A–C; data not shown) appear to correspond to the telencephalic and diencephalic neurons that generate the anterior and post-optic commissures, respectively (Chitnis et al., 1990; Wilson et al., 1991). The *myo5a*- and *myo10a*-expressing cells in the midbrain are likely the nucleus of the medial longitudinal fascicle (nucMLF; Chitnis et al., 1990; Fig. 4A–C), and this was confirmed by anti-tubulin antibody labeling of the MLF in embryos processed for *myo10a* in situ (Fig. 4D). Myosin gene expression in telencephalic and nucMLF neurons from 18–30 hpf coincides with the period of growth cone motility and axon guidance in these neurons. *Myo2hc1* is strongly expressed at the midbrain-hindbrain boundary at 18 hpf, and becomes restricted to a narrow domain in the caudal optic tectum by 32 hpf (Fig. 4E, F).

2.2.4 Expression in the Otic vesicle—In addition to *myo6b* and *myo7a*, whose expression patterns have been previously described (Ernest et al., 2000; Seiler et al., 2004; Kappler et al., 2004), *myo10b* and *myo2hc1* are also expressed in the otic vesicle. However, their expression is not restricted to the sensory neurons of the epithelium as for *myo6b* and *myo7a*. At 18 hpf, *myo2hc1* transcripts are localized to the apical regions of cells throughout the otic epithelium (Fig. 5B, D), whereas *myo10b* transcripts are localized to apical regions of cells only in the rostroventral epithelium (Fig. 5A, C; see also Fig. 3A). These genes are expressed in the otic vesicle from 15–24 hpf, during morphogenesis of the vesicle (Kimmel et al., 1995).

2.2.5 Expression in the Eye—Three myosins are expressed in the eye, two in the developing lens (*myo1b2*, *myo10a*), and one in the retina (*myo2hc1*). Interestingly, *myo1b2* is expressed by cells only in the medial half of the developing lens (Fig. 6A, B), whereas *myo10a* is expressed only in the dorsal half of the developing lens (Fig. 6C; see also Fig. 4C). These localized patterns may reflect the progression of differentiation of lens fibers, or the inability of transcripts to distribute uniformly within differentiating fibers. At 18 hpf, *myo2hc1* is strongly expressed in the medial layer of the optic cup (Fig. 4E) during the period of eye morphogenesis (Li et al., 2000), and becomes restricted to the ganglion cell layer by 32 hpf (Figs. 4F and 6D), when retinal axons are extending into the brain (Hutson and Chien, 2002).

2.2.5 Expression in the Spinal cord and Trunk—As expected, *myo2hc2* is expressed strongly in all muscles (Figs. 7A and 8), and our probe labels expressing tissue more effectively than previously described (Peng et al., 2002). Interestingly, only one other

myosin among 18 tested was expressed in the presumptive musculature. At 18 hpf, *myo18a* is expressed throughout the caudal (newborn) somites, and continues to be expressed strongly in cells at the boundaries between the rostral (older) somites (Fig. 7B). At 18 hpf, *myo1b2* is expressed in the superficial cells of the somites (Fig. 7C, D), and weakly expressed in a few cells in the spinal cord, likely neurons. At 18 hpf, *myo5a* is expressed by several presumptive neurons throughout the spinal cord, except in the most dorsal and ventral regions (Fig. 7E, F). By 30 hpf, *myo5a*-expressing cells are located mostly in the dorsal half of the neural tube, with several putative migrating neural crest cells in characteristic locations beside the somites (Fig. 7G, H). At 18 hpf, *myo10a* is expressed by cells in the dorsal spinal cord in putative neural crest cells (Fig. 7I, J). These results indicate that specific myosins are expressed in neurons and neural crest cells during the period of cell migration and axon guidance.

2.2.6 Expression of *myo2hc2* in cranial muscles—Previous work (Peng et al., 2002) noted weak *myo2hc2* (*myhz2*) expression in two cranial muscles. We found that *myo2hc2* is expressed robustly in all cranial muscles (Fig. 8). By comparing the expression pattern to that of anti-myosin antibody labeling of cranial muscles (Schilling et al., 1997), we identified most of the muscles in the zebrafish head at 72 hpf. Our *myo2hc2* probe represents an excellent marker for studies of cranial muscle biology.

3. EXPERIMENTAL PROCEDURES

3.1 Animals

Maintenance of zebrafish stocks, and collection and development of embryos in E3 embryo medium were carried out as described previously (Westerfield, 1995; Chandrasekhar et al., 1997; Bingham et al., 2002). Throughout the text, the developmental age of the embryos corresponds to the hours elapsed since fertilization (hours post fertilization, hpf, at 28.5°C).

3.2 Cloning and phylogenetic analysis of zebrafish unconventional myosins

We used the nucleotide and amino acid sequences for the various unconventional myosins from human and mouse databases (Hodge and Cope, 2000, and <http://www.mrc-lmb.cam.ac.uk/myosin/>; Berg et al., 2001) to search the zebrafish EST databases at Washington University and RZPD for orthologs. Using this information, we obtained EST clones for the various myosins from RZPD (Berlin, Germany), Open Biosystems (Huntsville, AL) and Dr. Jinrong Peng (IMCB, Singapore) (Table 1). The EST clones were verified by sequencing prior to making in situ probes. The EST sequences were used to BLAST the latest assembly of the zebrafish genomic sequence (Zv7, July 2007) using BLASTN to retrieve the most complete annotated cDNA sequences for the various myosin genes. The newly identified zebrafish and previously described human and mouse myosins were BLASTed against chicken skeletal myosin 2 to delineate the core domain (composed of the actin- and ATP-binding sites) corresponding to amino acids 88–780 of the chicken protein. The core domains of zebrafish, human and mouse myosins were aligned using Clustal-W method in Megalign program of LASERGENE (DNASTAR Inc, Madison, WI; Reddy and Day, 2001). The aligned core domains were further analyzed using Mac PAUP 4.0 b10 (<http://paup.csit.fsu.edu/>; Jiang and Ramachandran, 2004) to generate a phylogenetic tree (Fig. 2). The heuristic search option was used in maximum parsimony method to create a tree with strict consensus. Bootstrap analyses were replicated 1000 times to obtain a robust support value at each node which indicates the percent times that the grouping is supported out of 1000 trials (Baker and Titus, 1997; Hodge and Cope, 2000; Sellers, 2000). Bootstrap values of greater than 70 indicate a strongly supported node with 95% probability (Hills and Bull, 1993).

3.3 Histological Procedures

The EST clones were verified by sequencing prior to probe synthesis. Synthesis of the digoxigenin-labeled probes, and whole-mount in situ hybridization was carried out as described previously (Bingham et al., 2003). Given the large number of clones and time points, embryos of different ages were pooled for the hybridization step following protease treatment. Whole-mount immunohistochemistry (tubulin and GFP antibodies) was performed as described previously (Vanderlaan et al., 2005). For combined in situ and antibody labeling, embryos were first processed for in situ before antibody labeling. Embryos were devalued, mounted in 70% glycerol, and examined with an Olympus BX60 microscope. At least ten embryos were examined for each gene and time point. Images were acquired using an Optronics camera and Scion Imaging Software on a MacOS computer equipped with an LG3 frame grabber. Images were processed in Adobe Photoshop to adjust brightness and contrast only.

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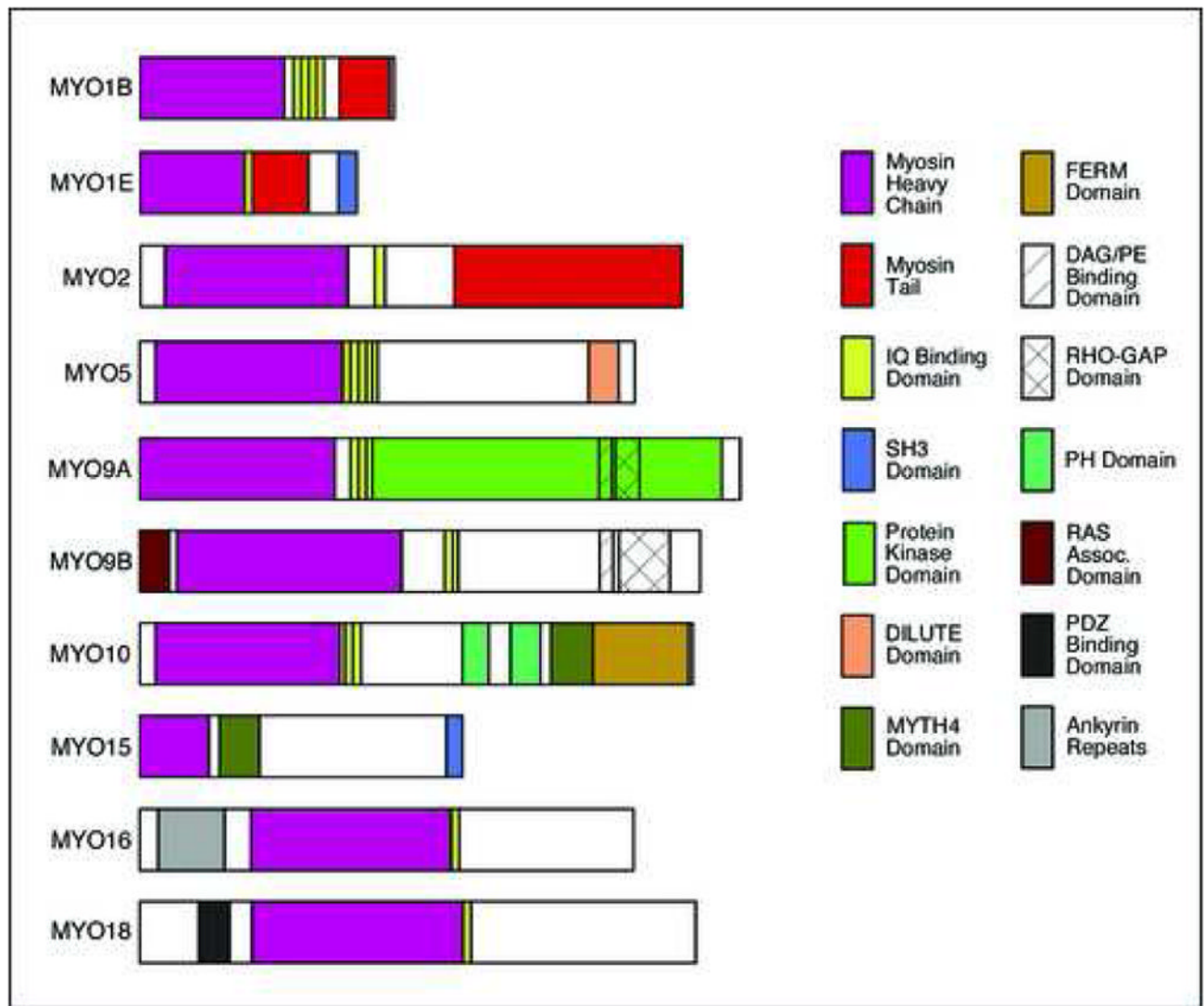


Figure 1. Domain organization of myosins examined in this report. Myosins are classified into several families based upon the occurrence and distribution of identified domains and sequence motifs in the heavy chain polypeptide. The nomenclature follows that for human and mouse proteins (Berg et al., 2001). This list contains only those myosins for which ESTs were obtained and expression analyzed in zebrafish embryos. The myosin head domain containing the actin- and ATP-binding sites (purple) is largely conserved, with differences identifying various classes. Other domains and motifs are found in only a subset of the proteins.

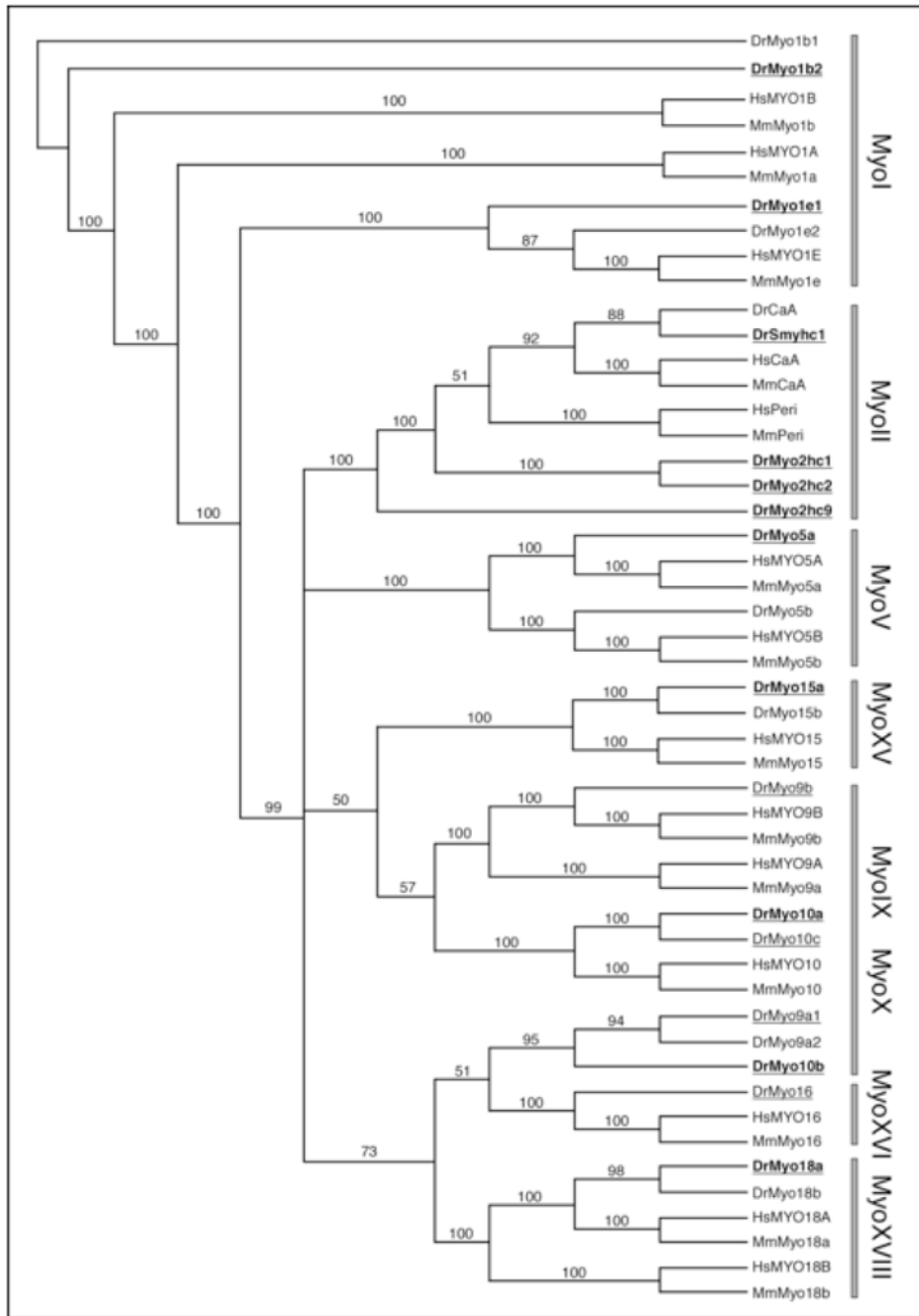


Figure 2. Phylogenetic relationship of unconventional myosin family members between zebrafish (*Danio rerio*), mouse (*Mus musculus*), and man (*Homo sapiens*). Rectangular tree rooted to Myo1, with bootstrap values for family members that were examined in this report (see Methods for details). Myosins for whom ESTs were characterized are identified by underlines. Those myosins among these that were expressed in zebrafish embryos (15–36 hpf) are identified by bold type (Table 1). Zebrafish myosins outside these two categories but included in this phylogenetic tree were identified solely from database searches. Abbreviations: CaA, cardiac myosin A; Peri, Perinatal skeletal myosin (Myhc8); Smyhc, Slow myosin heavy chain.

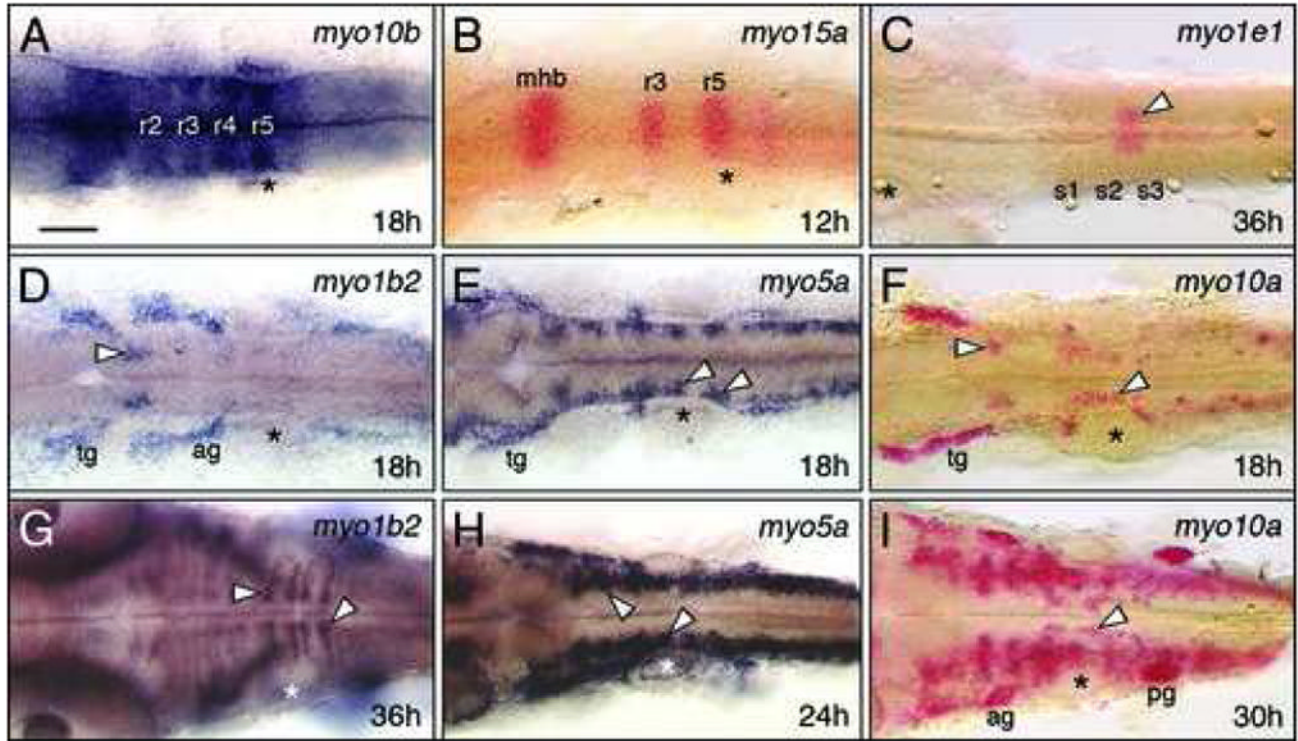


Figure 3.

Myosins exhibiting restricted expression patterns in the hindbrain. All panels show dorsal views of the hindbrain with anterior to the left. Asterisks mark the otic vesicle in each panel. (A, B) *myo10b* is expressed at varying levels in rhombomeres 2–5 (A), and *myo15a* is expressed in r3 and r5 (B), with both expressed at the midbrain-hindbrain boundary (mhb). (C) *myo1e1* is weakly but specifically expressed in a mesendodermal domain spanning the 2nd and 3rd somites (arrowhead). (D, G) At 18 hpf, *myo1b2* is expressed in sensory ganglia (tg, ag) and a cluster of cells in r2 (arrowhead, D). By 36 hpf (G), there is prominent expression in cells (arrowheads) at the boundaries of r5 and r6. (E, H) At 18 hpf, *myo5a* is expressed in sensory ganglia, and in differentiating neurons (arrowheads, E) within every rhombomere. By 24 hpf (H), expressing cells form a continuous column along the neural tube (arrowheads). (F, I) At 18 hpf, *myo10a* is expressed in the sensory ganglia (tg), and in the branchiomotor neurons in r2 and r5 (arrowheads, F). By 30 hpf (I), expression persists in the sensory ganglia and migrating motor neurons (arrowhead), and has expanded to large clusters of cells in the anterior hindbrain. tg, trigeminal ganglion, ag/pg, anterior and posterior lateral line ganglion. Scale bar, 75 μm .

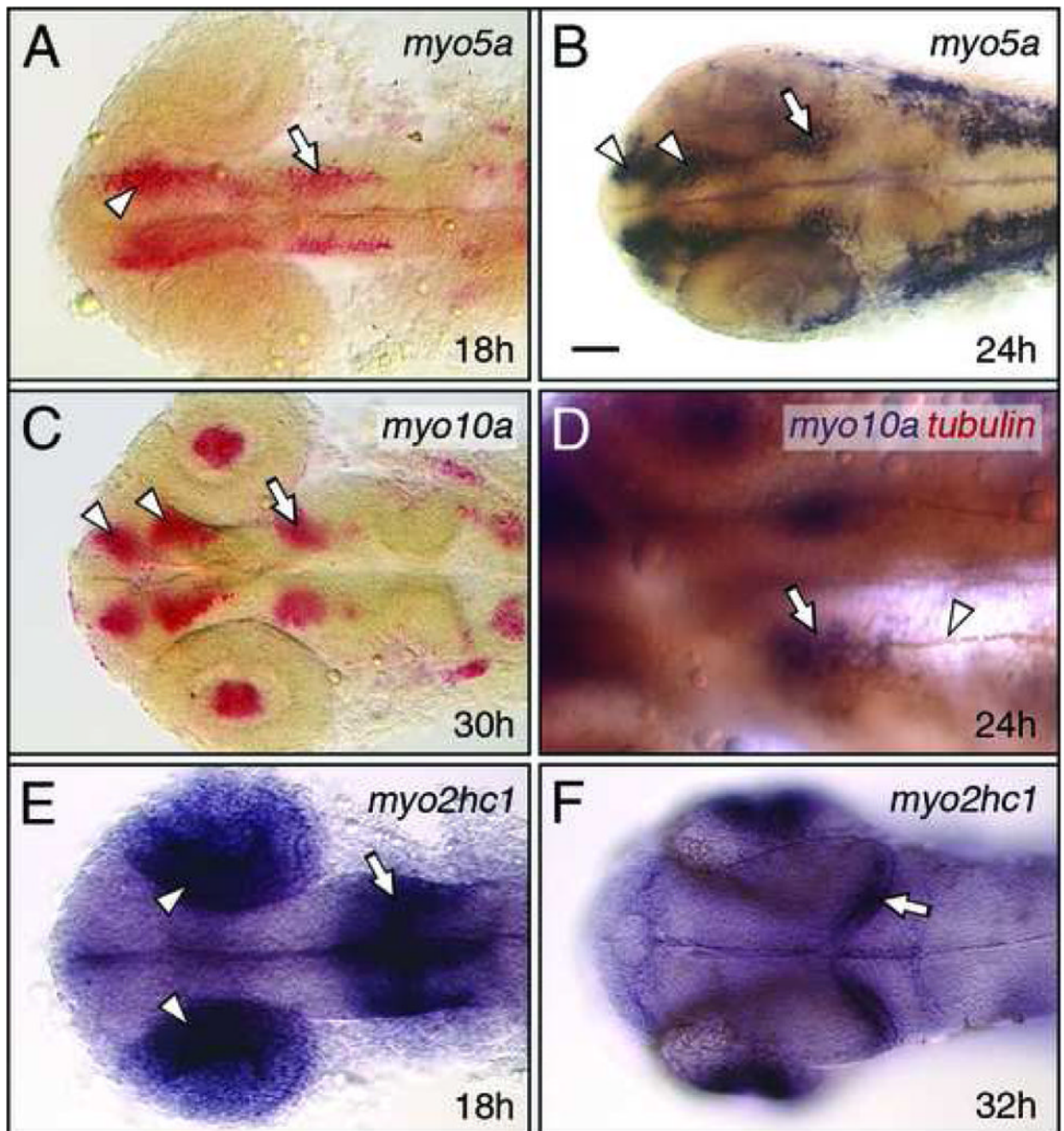


Figure 4.

Myosin expression in the forebrain and midbrain. All panels show dorsal views of the head with anterior to the left. (A, B) At 18 hpf, *myo5a* is expressed in nascent neurons (arrowhead, A) in the forebrain, and in the nucMLF neurons (arrow) in the midbrain. By 24 hpf (B), forebrain expression has refined into distinguishable clusters representing the neurons of the anterior and post-optic commissures (arrowheads, B), and is maintained in the nucMLF neurons (arrow). (C) At 30 hpf, *myo10a* is expressed in the neurons of the nucMLF (arrow), and of the anterior and post-optic commissures (arrowheads), and in the lens (see Fig. 6). (D) Double-labeling shows that tubulin antibody-labeled axons (arrowhead) of the MLF arise from the cluster of *myo10a*-expressing neurons (arrow),

corresponding to the nucMLF. (E, F) At 18 hpf, *myo2hc1* is expressed in the medial layer of the optic cup (arrowheads), and at the mid-hindbrain boundary (arrow). By 32 hpf (F), expression is restricted to the retinal ganglion layer (see Fig. 6), and to the caudal edge of the developing optic tectum (arrow). Scale bar, 25 μm (D), 50 μm (A–F).

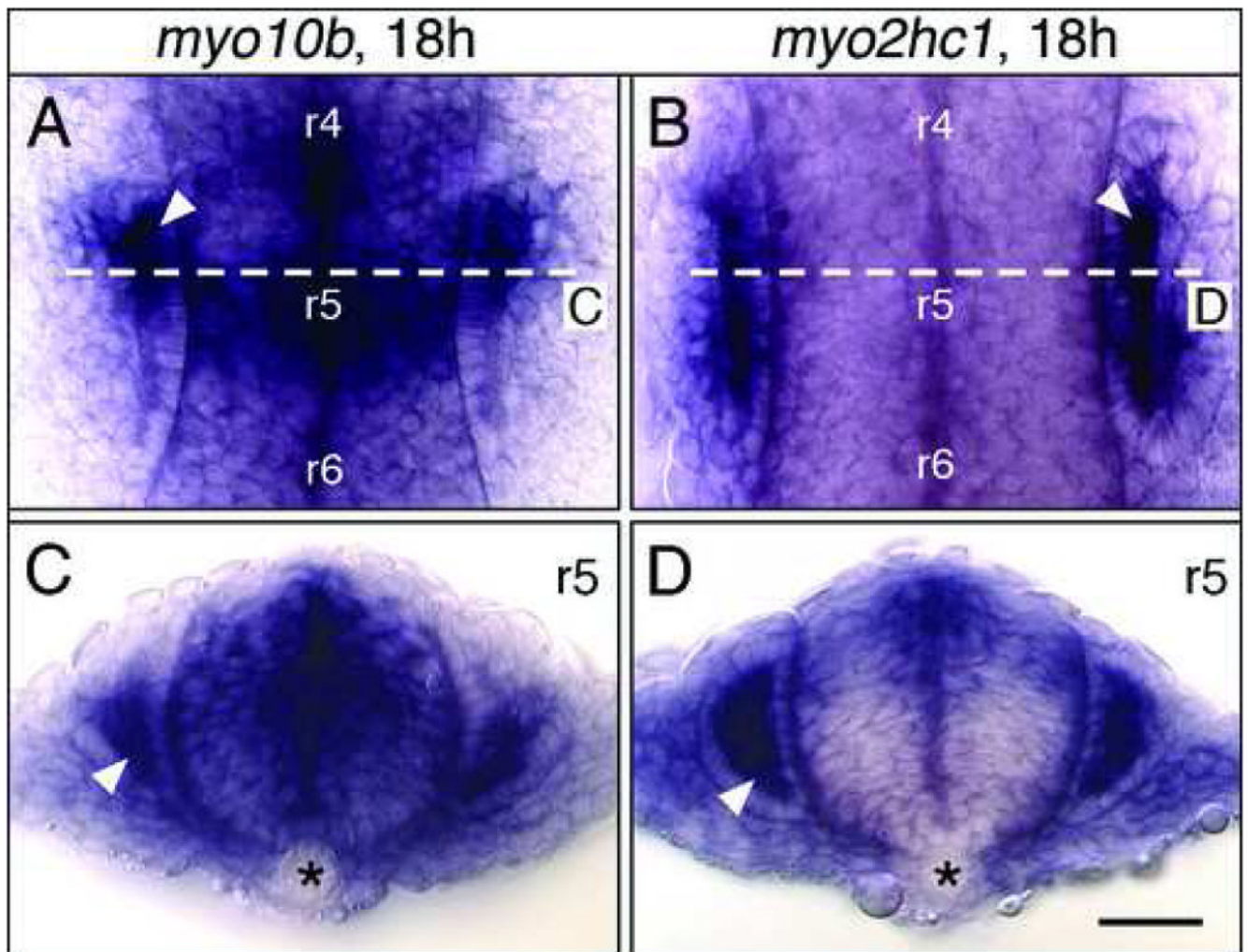


Figure 5. Myosin expression in the developing ear. Upper panels show dorsal views, with anterior at top. Lower panels show cross-sections (dorsal up) at the levels indicated in the upper panels. (A, C) *myo10b* is expressed in epithelial cells (arrowhead) lining the anterior third (A), and the ventral half (C) of the otic vesicle. (B, D) *myo2hc1* is expressed in the epithelial cells of the otic vesicle (arrowheads), adjacent to the hindbrain. Asterisks (C, D) indicate the notochord. Scale bar, 30 μ m.

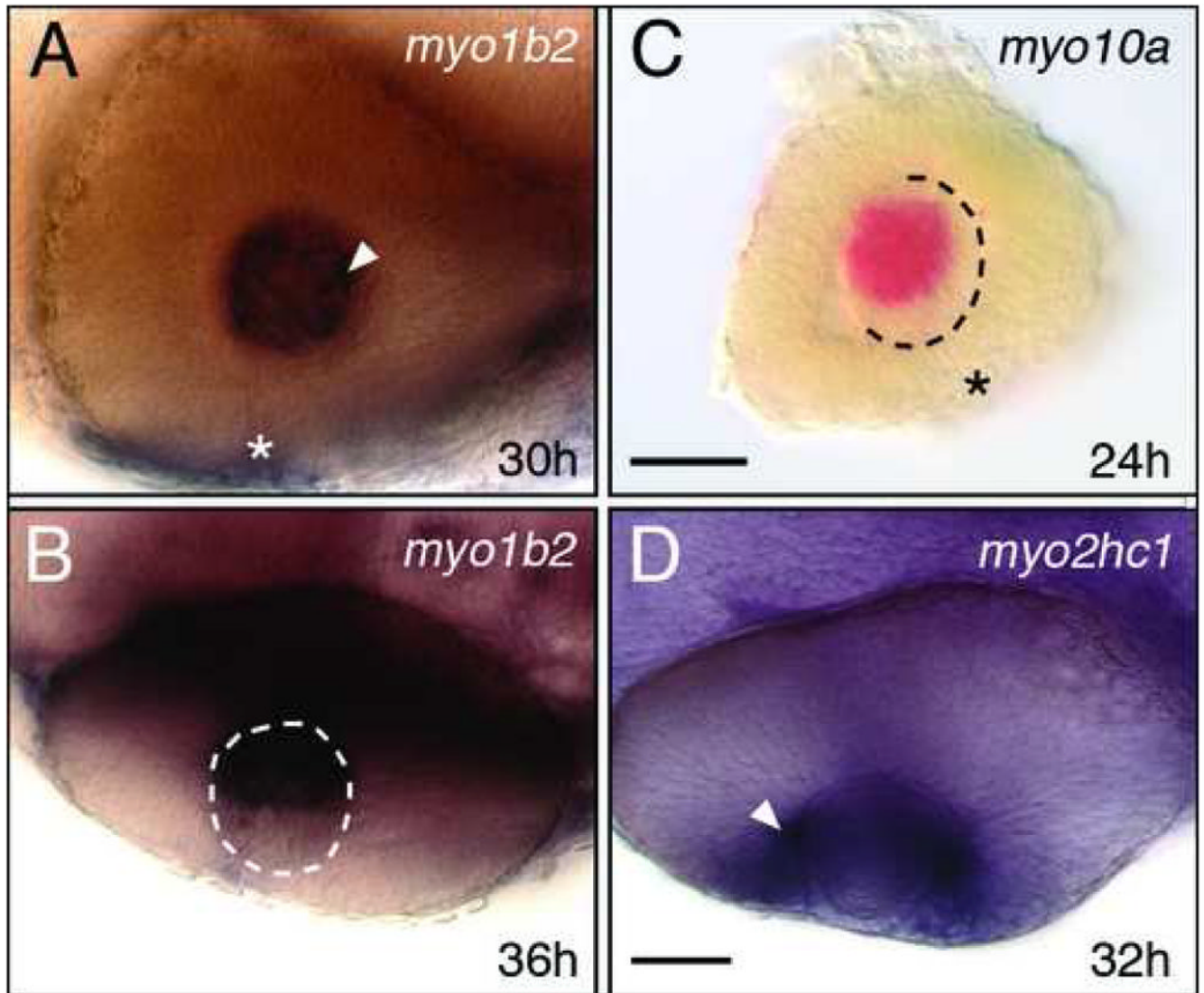


Figure 6. Myosin expression in the eye. (A, B) *myo1b2* is expressed in the differentiating lens fibers (arrowhead, A) located in the medial half of the lens (B, dorsal view). (C) *myo10a* is expressed in a subset of lens cells at 24 hpf, and at 30 hpf (see Fig. 4C). (D) *myo2hc1* is expressed in retinal ganglion cells (arrowhead) adjacent to the lens. Asterisks (A, C) indicate the choroid fissure. Scale bar (A, B, D), 40 μm . Scale bar (C), 30 μm .

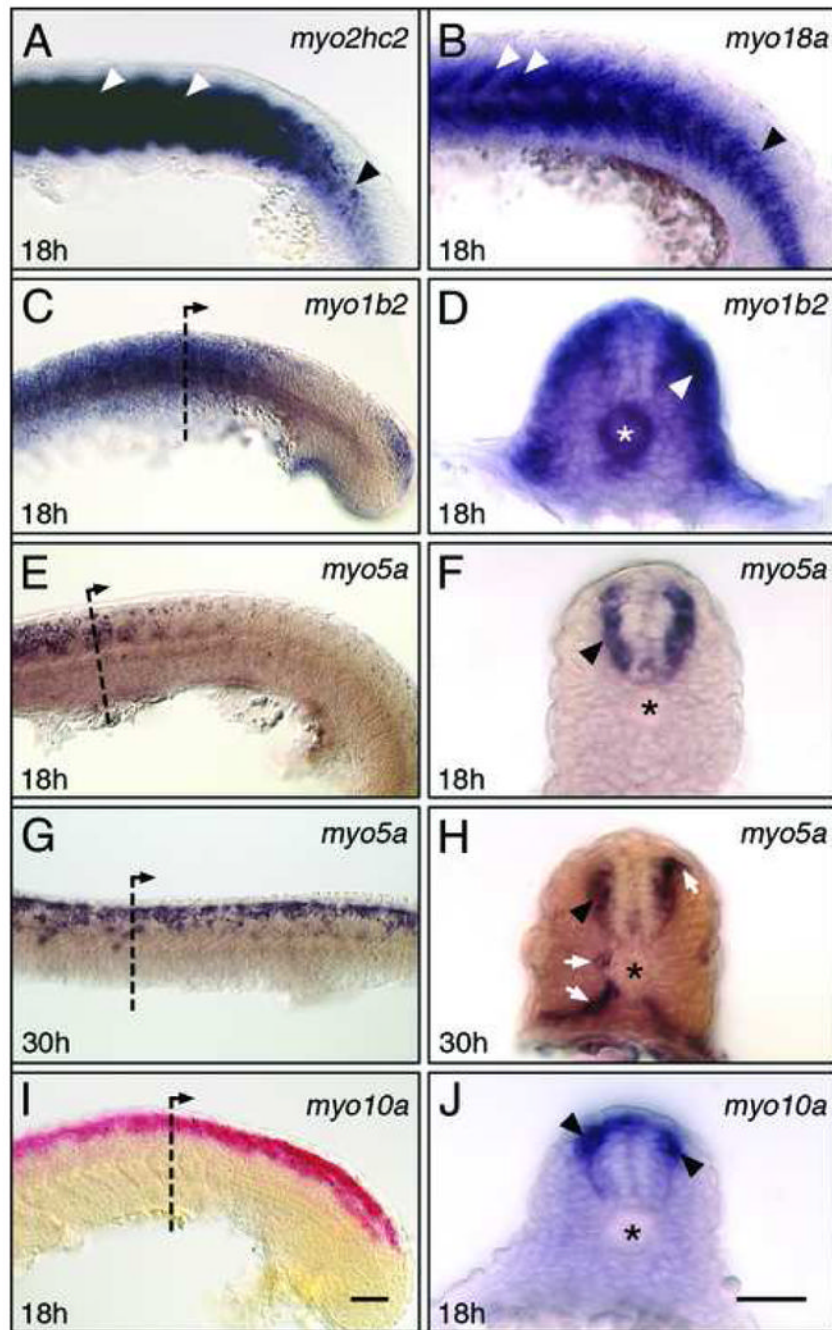


Figure 7.

Myosin expression in the trunk and spinal cord. Panels A–C, E, G, I show side views with anterior to left. Panels D, F, H, J show cross-sections at levels indicated in C, E, G, I, respectively. (A) *myo2hc2* is strongly expressed in the differentiating muscles of the somites (white arrowheads), but only weakly in the newly formed somites (black arrowhead). (B) *myo18a* is expressed in the mesenchyme of newly-formed and differentiating somites (black arrowhead), and strongly in the cells at the boundaries of older somites (white arrowheads). (C, D) *myo1b2* is expressed broadly in the trunk, excluding the presomitic mesoderm (C). Expressing cells are located superficially within the somitic mesoderm (arrowhead, D), and in the notochord (asterisk). (E, F) At 18 hpf, *myo5a* expression is restricted to the spinal

cord at anterior trunk levels (E), and represents differentiating neurons (arrowhead, F). (G, H) At 30 hpf, *myo5a* is expressed by scattered cells located in the dorsal half of the spinal cord (G). A cross-section in the anterior trunk (H) shows that expressing cells include neurons (arrowhead) and migrating neural crest cells (arrows). (I, J) *myo10a* is expressed in a continuous column of cells in the dorsal half of the spinal cord (I), including neurons (arrowheads, J). Asterisks in D, F, H, J indicate the notochord. Scale bar (I), 50 μm (A–C, E, G, I). Scale bar (J), 50 μm (D, F, H, J).

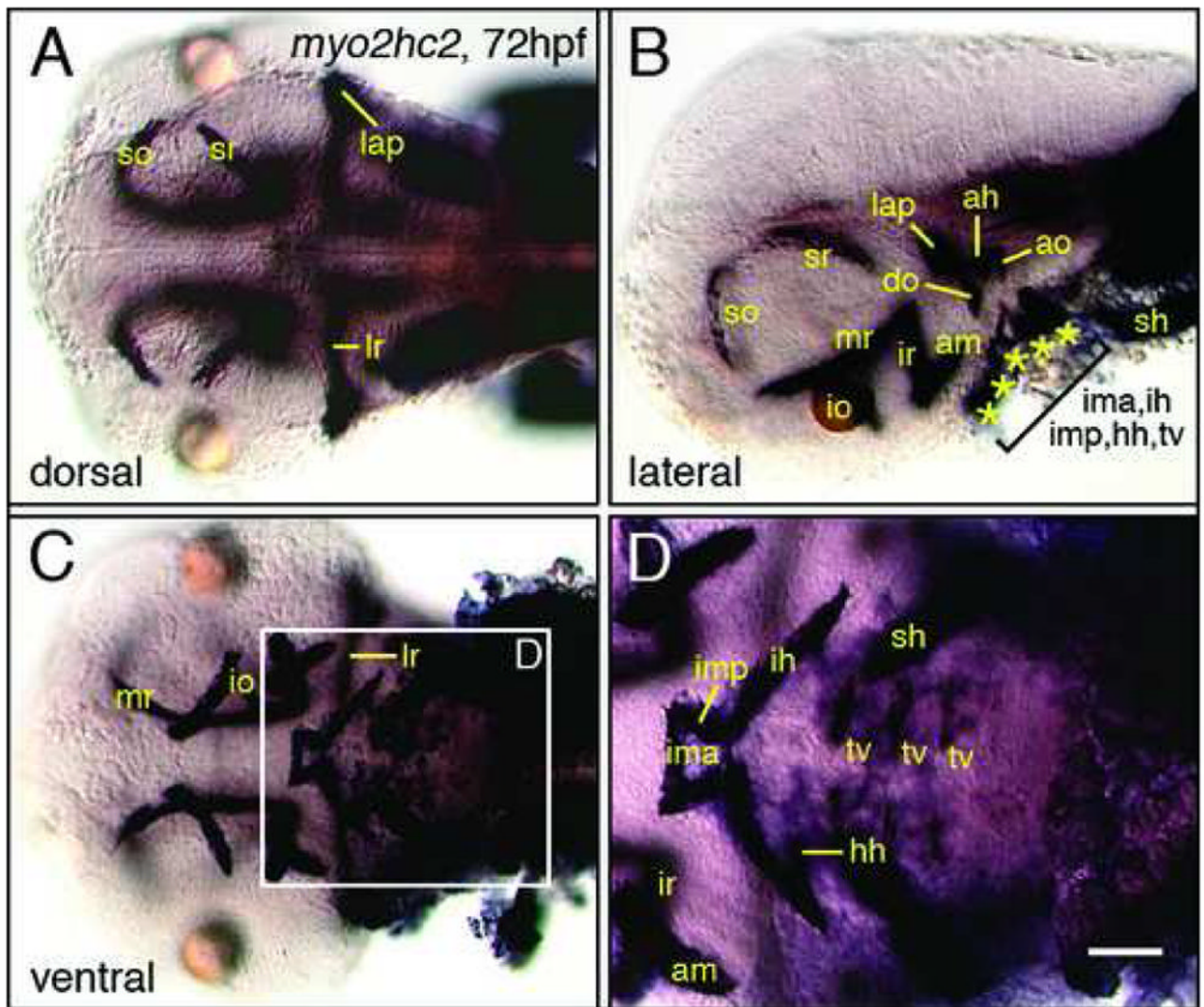


Figure 8. *Myosin2* expression in head muscles. (A) In a dorsal view, some eye (so, sr, lr) and jaw (lap) muscles can be identified. (B) In a side view, several eye (so, sr, mr, io, ir, am) and jaw (ah, ao, do, lap) muscles can be distinguished. (C, D) In a ventral view, several eye (am, io, ir, lr, mr), jaw (ima, imp, ih, hh) and gill (tv, sh) muscles can be identified. Scale bar, 100 μm (A–C), 50 μm (D).

Table 1

Zebrafish Myosin Genes Characterized in this Report

Gene Ensemble ID	Chromosome	ESTs (source) *, %	Length of ORF	% Identity to Human	Mouse	Gene Expressed
<i>myo1b2</i> ENSDARG00000024694	9	MPMGp609P206Q8 (B) MPMGp609G1826Q8 (B) AIG (A)	Partial (90%)	81.4	81.5	Yes
<i>myo1e1</i> ENSDARG00000036179	7	MPMGp609L0725Q8 (B) MPMGp609L0281Q8 (B) MPMGp637H137Q2 (B) IMAGp998P112400Q3 (B) IMAGp998G1514300Q3 (B)	Full-length	76.3	76.3	Yes
<i>myh1</i> ENSDARG00000071430	24	MPMGp609B1310Q8 (B)	Full-length	84.8	84.6	Yes
<i>myo2hc1 (myhz1)</i> ENSDARG00000067990	5	MPMGp609P1624Q8 (B)	Full-length	80.8	82.1	Yes
<i>myo2hc2 (myhz2)</i> ENSDARG00000012944	5	MPMGp609M1825Q8 (B)	Full-length	80.7	82.1	Yes
<i>myo2hc9 (myhz9)</i> ENSDARG0000001014	3	IMAGp998D0714289Q3 (B)	Full-length	84.3	81.9	Yes
<i>myo5a</i> ENSDARG00000061635	18	129-E08-02 (S)	Full-length	79.9	79.9	Yes
<i>myo6a</i> ENSDARG00000044016	20	IMAGp998H1712050Q3 (B)	Full-length	80.9	80.6	No
<i>myo9a1</i> ENSDARG00000019585	7	46-E08-02 (S)	Partial (90%)	56.7	56.3	No
<i>myo9a2</i> ENSDARG00000063639	25	IMAGp998N0912391Q3 (B)	Partial (80%)	54.8	54.3	No
<i>myo9b</i> ENSDARG00000062159	10	MPMGp609G0210Q8 (B)	Full-length	57.3	58.1	No
<i>myo10a</i> ENSDARG00000062580	6	IMAGp998M2314313Q (B)	Partial (98%)	61.6	62.1	Yes
<i>myo10b</i> ENSDARG00000017004	2	MPMGp609N1624Q8 (B) MPMGp609C1781Q8 (B) 88-A02-02 (S)	Partial (84%)	68.3	67.7	Yes
<i>myo10c</i> ENSDARG00000063138	9	112-H11-02 (S)	Full-length	62.1	62.6	No

Gene Ensembl ID	Chromosome	ESTs (source) *, %	Length of ORF	% Identity to		Gene Expressed
				Human	Mouse	
<i>myo15a</i> ENSDARG00000059709	3	62-G10-02 (S)	Full-length	62.2	61.2	Yes
<i>myo16</i> ENSDARG00000004512	9	86-H05-02 (S) 100-C07-02 (S)	Full-length	59.8	57.8	No
<i>myo18a</i> ENSDARG000000061862	15	DKFZp717N112Q2 (B)	Full-length	78	76.8	Yes
<i>myo18b</i> ENSDARG000000061225	10	54-D04-02 (S) 98-C07-02 (S)	Partial (87%)	73.7	73.3	No

*, Source, B: RZPD, Berlin; S: Dr. Jinrong Peng, IMCB, Singapore; A: Research Genetics (Invitrogen), Alabama.

% For genes with multiple ESTs, all ESTs were tested, and generated similar in situ results.