Gene Expression Patterns 8 (2008) 161–170

www.elsevier.com/locate/gep

Expression of unconventional myosin genes during neuronal development in zebrafish

Vinoth Sittaramane [a,b], Anand Chandrasekhar [a,b,c,*]

[a] *Division of Biological Sciences, Room 340D Bond Life Sciences Center, 1201 E. Rollins Street, University of Missouri, Columbia, MO 65211-7310, USA*
[b] *Interdisciplinary Neuroscience Program, University of Missouri, Columbia, MO 65211, USA*
[c] *Genetics Area Program, University of Missouri, Columbia, MO 65211, USA*

Received 25 September 2007; received in revised form 27 October 2007; accepted 30 October 2007
Available online 6 November 2007

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

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

* Corresponding author. Address: Division of Biological Sciences, Room 340D Bond Life Sciences Center, 1201 E. Rollins Street, University of Missouri, Columbia, MO 65211-7310, USA. Tel.: +1 573 882 5166; fax: +1 573 884 9676.
E-mail address: AnandC@missouri.edu (A. Chandrasekhar).

1567-133X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2007.10.010

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; Diefenbach 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.

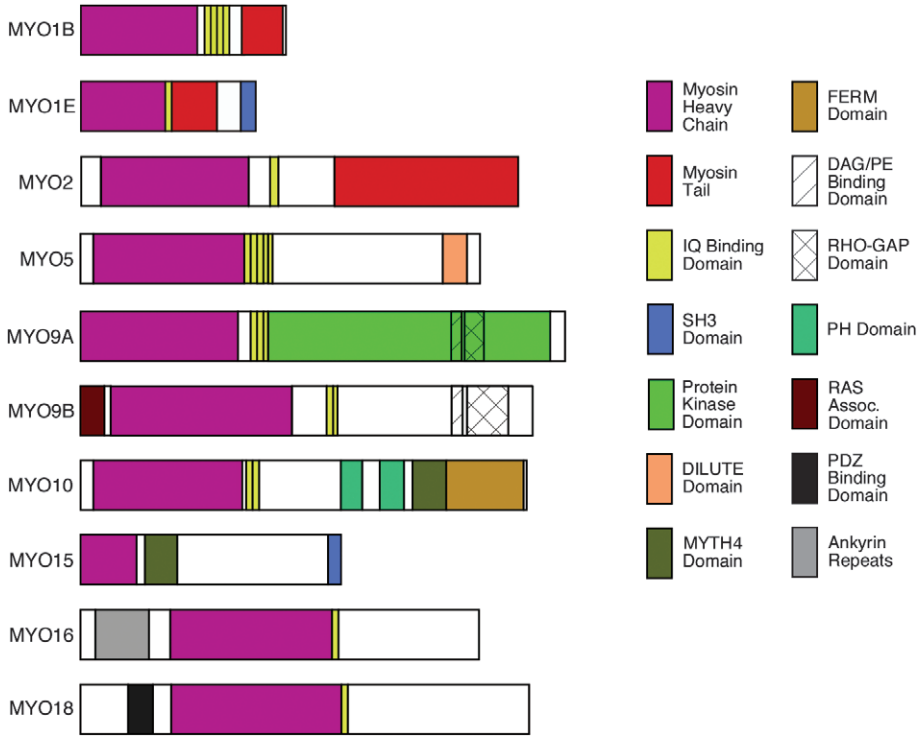

Fig. 1. Domain organization of myosins examined in this report. Myosins are classified into several families based upon the occurrence and distribution of identified domains and sequence motifs in the heavy chain polypeptide. The nomenclature follows that for human and mouse proteins (Berg et al., 2001). 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.