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Neuronal expression of class 6 semaphorins in zebrafish

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ABSTRACT

Semaphorins are a large family of guidance molecules identified by an extracellular SEMA domain. Classes 1 and 2 are derived from invertebrates, classes 3–7 are vertebrate and class 8 (v) are viral semaphorins. Class 6 semaphorins are reported to have a wide variety of roles including in axon guidance, transcriptional regulation and cancer. Here we report the identification and expression of four class 6 semaphorins (6A, 6Ba, 6Bb and 6Dl) in three stages of larval development in zebrafish (24, 48 and 72 hours postfertilization). Our data indicate that each of the class 6 semaphorins shows a distinct pattern of expression in the developing nervous system that is dynamic over the first 3 days of embryonic development. These data suggest that the individual class 6 semaphorins have diverse roles in nervous system development.

Semaphorins (semas) are a large class of transmembrane and secreted molecules. They are easily identified by a highly conserved, extracellular 500 amino acid region referred to as the SEMA domain. Semaphorins were first identified in invertebrates with the cloning of the gene encoding the grasshopper *sema1* transmembrane protein (previously named fasciculin) that guides pioneering axons into the developing limb bud (Kolodkin et al., 1992). Currently, there are eight known classes of semaphorins (1–8), with classes 1 and 2 derived from invertebrates, 3–7 derived from vertebrates, and class 8 (v) being viral (Yazdani and Terman, 2006). Semaphorins bind to and signal through a plexin receptor. In the case of class 3 semaphorins, signaling generally requires the co-receptor neuropilin (Nakamura et al., 2000). There are four classes of plexin receptors (A–D) and many subfamily members in each class (Negishi et al., 2005).

Class 6 semaphorins have a transmembrane domain (Yazdani and Terman, 2006), and are reported to bind to PlexinA2 (Suto et al., 2007; Chetodal and Renaud, 2008; Tawarayama et al., 2010) and PlexinA4 receptors (Suto et al., 2007; Haklai-Topper et al., 2010). In mammals, Sema6A has a variety of functions that range from localizing the nucleus within the cell (Chetodal and Renaud, 2008; Renaud et al., 2008), cytoskeleton interactions (Prislei et al., 2008) and axon guidance (Xu et al., 2000; Suto et al., 2007; Runker et al., 2008). Sema6B is known to participate in transcriptional regulation (Collet et al., 2004; Murad et al., 2006, 2011), cancer (Correa et al., 2001), and axon guidance (Tawarayama et al., 2010). Similarly, mammalian Sema6D has roles in cardiac patterning (Toyofuku et al., 2004), cancer (Zhao et al., 2006), and immune responses (O'Connor et al., 2008).

Given the evident importance of class 6 semaphorins in a variety of systems, detailed expression analysis in the versatile model organism, the zebrafish, will aid in the discovery of novel functions of these transmembrane molecules. Six class 6 semaphorins have been identified in zebrafish; Sema6A, Sema6Ba, Sema6Bb, Sema6Dl, Sema6D (Kimura et al., 2007), and Sema6E (Taniguchi et al., 2011). Because of the expected roles for Sema6 in neuronal migration, axon guidance and synaptic connectivity we report complete, annotated, comparative neuronal expression of zebrafish *sema6A*, *sema6Ba*, *sema6Bb*, and *sema6Dl*, focusing on stages of embryonic development when neurons are migrating and neuronal connections are forming (24–72 hours postfertilization (hpf)). Each *sema6* has a distinct spatio-temporal expression pattern, arguing that the class 6 semaphorins have diverse roles in nervous system development.

1. Results and discussion

1.1. Phylogenetic tree of class 6 semaphorins

We performed a phylogenetic analysis of the nucleotide sequences of class 6 semaphorin genes using ClustalW (Fig. 1). All of the zebrafish (*Danio rerio*, Dr) sequences clustered with ortholo-

Abbreviations: hpf, hours postfertilization; sema, semaphorin; DIG, digoxygenin.

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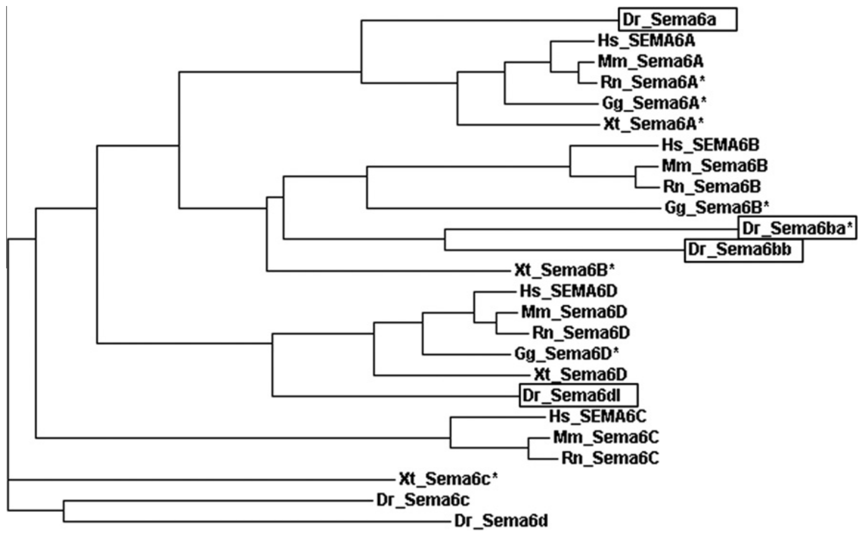

Fig. 1. Phylogenetic tree showing alignment of class 6 semaphorins using predicted (indicated by ∗) and annotated sequences from chicken (*Gallus gallus*, Gg), frog (*Xenopus tropicalis*, Xt), human (*Homo sapiens*, Hs), mouse (*Mus musculus*, Mm), rat (*Rattus norvegicus*, Rn), and zebrafish (*Danio rerio*, Dr). Accession numbers are available in Supplementary Fig. 1.

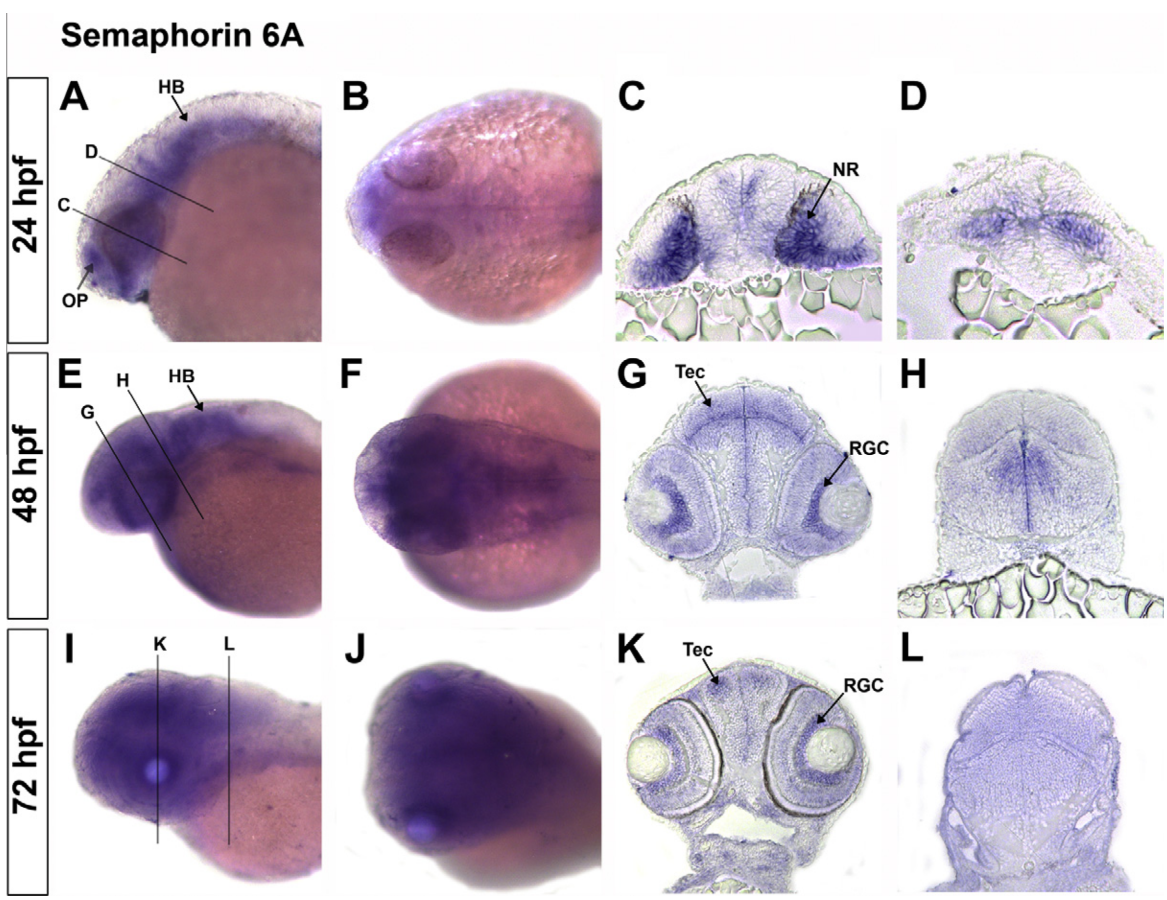

Fig. 2. In situ hybridization of *sema6A* gene expression at 24 hpf (A–D), 48 hpf (E–H) and 72 hpf (I–L). Lateral views (A, E, and I) with anterior to the left, dorsal at the top, dorsal views (B, F, and J) with anterior to the left, rostral transverse brain sections (C, G, and K) and caudal transverse brain sections (D, H, and L) with dorsal at the top. Parallel lines indicate where sections were imaged. Arrows indicate expression in the hindbrain (HB), olfactory pit (OP), neural retina (NR), retinal ganglion cells (RGC), and optic tectum (Tec).

gous semaphorins from other species. Interestingly, the sema6Dl sequence we examined here clusters with sema6D genes from other species, while a previously identified zebrafish sema6D gene is more closely related to sema6C genes from other species than sema6D genes. Thus there is some uncertainty about the true ortholog of the zebrafish semaphorin 6D gene. Obtaining full