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Temporal and tissue specific gene expression patterns of the zebrafish *kinesin-1* heavy chain family, *kif5s*, during development

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ABSTRACT

Homo- and heterodimers of Kif5 proteins form the motor domain of Kinesin-1, a major plus-end directed microtubule motor. Kif5s have been implicated in the intracellular transport of organelles, vesicles, proteins, and RNAs in many cell types. There are three mammalian KIF5s. KIF5A and KIF5C proteins are strictly neural in mouse whereas, KIF5B is ubiquitously expressed. Mouse knockouts indicate crucial roles for KIF5 in development and human mutations in KIF5A lead to the neurodegenerative disease Hereditary Spastic Paraplegia. However, the developmental functions and the extent to which individual *kif5* functions overlap have not been elucidated. Zebrafish possess five *kif5* genes: *kif5Aa*, *kif5Ab*, *kif5Ba*, *kif5Bb*, and *kif5C*. Here we report their tissue specific expression patterns in embryonic and larval stages. Specifically, we find that *kif5As* are strictly zygotic and exhibit neural-specific expression. In contrast, *kif5Bs* exhibit strong maternal contribution and are ubiquitously expressed. Lastly, *kif5C* exhibits weak maternal expression followed by enrichment in neural populations. In addition, *kif5s* show distinct expression domains in the larval retina.

1. Results and discussion

Polarized cells like oocytes and neurons rely heavily on the proper organization of their intracellular components in order to achieve their function. Subcellular localization of organelles, proteins, and RNAs is critical for functional compartmentalization in cells. Such organization can be achieved via two main mechanisms: broadly distributed cellular components experience a selective force in specific subcellular regions (*i.e.* tethering to the plasma membrane, selective degradation or stabilization etc.) or select cellular components are actively transported to specific subcellular locations. Selective transport is a common mechanism to affect cellular asymmetry that relies on a polarized array of microtubule tracks and their associated families of molecular motor proteins that travel along the microtubules (Hirokawa, 1998; Houliston and Elinson, 1991; Lafont et al., 1994; Messitt et al., 2008). The two main classes of microtubule-based molecular motors are Kinesins, composed mainly of microtubule plus-end directed motors, and Dyneins, composed mainly of microtubule minus-end directed motors (Allan, 2011; Hirokawa et al., 2009). While Dyneins rely on a complex composed of combinations of large proteins and small peptides known as the Dynactin complex to enact cargo specificity (Allan, 2011), the Kinesin superfamily of proteins (known as Kifs) is composed of around 45 different Kinesin proteins in mammals (Miki et al., 2001), each thought to have their own cargo specificity.

The Kinesin superfamily of proteins is subdivided into three branches based on the location of the motor domain: N-terminal motor domain Kifs (N-Kifs), C-terminal motor domain KIFs (C-Kifs), and middle motor domain Kifs (M-Kifs) (Miki et al., 2001). Among the N-Kifs, the best studied motors are Kif5s. Originally named Kinesin Heavy Chains (KHCs), Kif5s were the first Kinesins discovered (Vale et al., 1985). There are three mammalian KIF5s: KIF5A, KIF5B, and KIF5C. Together, these proteins form homo- and heterodimers (Kanai et al., 2000), and are also often found in a heterotetrameric protein complex together with two Kinesin Light Chains (KLCs), originally known as conventional Kinesin, but now referred to as Kinesin-1 (Bloom et al., 1988; Kuznetsov et al., 1988). While these three isoforms display striking sequence similarity, their expression patterns differ, with adult murine KIF5A and KIF5C proteins expressed only in neurons and KIF5B protein expressed ubiquitously in all tissues (Kanai et al., 2000).

Kif5s have been implicated in a wide-range of transport processes including retrograde transport of vesicles from Golgi to ER (Lippincott-Schwartz et al., 1995), anterograde transport of lysosomes to the plasma membrane (Nakata and Hirokawa, 1995), pigment dispersion in melanocytes (Hara et al., 2000), and anterograde axonal transport of organelles, proteins, vesicles, and

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RNAs in neurons (Hirokawa et al., 2010). Mouse knockouts have been generated for all three murine *Kif5s*. *Kif5A* knockout mice die shortly after birth due to failure to inflate their lungs (Xia et al., 2003). Among *Kif5s*, *Kif5B* knockout phenotypes are the most severe and these mice do not survive past 11.5 days postcoitum (Tanaka et al., 1998), making the developmental function of KIF5B difficult to study. *Kif5C* knockout mice are grossly normal and display only a modest decrease in brain size and motor neuron numbers (Kanai et al., 2000). With the exception of *Kif5B,* the relatively moderate mutant phenotypes of the other *Kif5s* suggest they may have some redundant roles.

Zebrafish possess five *kif5* genes, *kif5Aa*, *kif5Ab*, *kif5Ba*, *kif5Bb*, and *kif5C*, none of which have been carefully studied to date. To investigate the temporal and tissue specific expression patterns of the five zebrafish *kif5s* during development and in adult tissues, we have performed RT-PCR and *in situ* hybridization experiments.

1.1. Kif5 structure, phylogeny, and sequence alignment

Kif5s have a conserved stereotypical structure comprised of an N-terminal globular head/motor domain connected via a neck domain to a coiled-coil rich stalk region and a C-terminal globular tail domain (Fig. 1A). The motor domain is responsible for ATP-hydrolysis and microtubule binding allowing the complex to move along microtubules. The neck is composed of two regions, a flexible neck linker (NL) thought to be involved in velocity (Kalchishkova and Bohm, 2008) and regulation of directionality, and a coiled coil (NCC) domain thought to be important for Kif5 dimerization (Grummt et al., 1998; Marx et al., 1998). The rigid stalk is composed of two coiled coil regions (CC1/2) which allow for dimerization and two flexible hinge regions (H1/2). H2 is especially important as it allows formation of the inactive folded conformation when cargo is not bound (Friedman and Vale, 1999; Hackney et al., 1992). The C-terminal tail region is known to mediate interaction with Kinesin Light Chains (KLCs) and various cargo (Jeppesen and Hoerber, 2012).

Sequence alignment of zebrafish, human and mouse Kif5s reveals significant conservation between these proteins, particularly in the motor domain, the neck, CC1/2, H2, and the N-terminal portion of the tail (Fig. 1B). Sequence divergence among zebrafish paralogs and among mouse and human orthologs is most evident in the flexible H1 region, in the middle portion of CC2, and at the C-terminal portion of the tail. H1 is thought to allow flexibility of Kif5 dimers as they move cargo (Gutierrez-Medina et al., 2009), and so the secondary structure of these regions would be expected to be more conserved rather than the primary structure. Predictions of coiled-coil probability for the zebrafish Kif5s (zKif5s) were made using the freely available Paircoil2 program (McDonnell et al., 2006). This program predicts coiled coil domains based on amino acid sequences by using pairwise residue correlations acquired from a coiled coil database and is thought to outperform other common prediction methods. These predictions reveal that H1 has a very low probability of coiled coils (Fig. 2), allowing for flexibility in this region. The divergent sequence noticed in the middle of CC2 correlates with a small region which has zero probability of coiled coil formation in zKif5As (Fig. 2). This region may represent an additional hinge domain that is specifically found in Kif5As. Finally, the sequence differences noted in the C-terminal portion of the tail domain may lead to variability in cargo specificity.

Phylogenetic analysis of human, mouse, and zebrafish Kif5s reveals clustering of zKif5s with their respective mouse and human orthologs (Fig. 1C). zKif5Ba and zKif5Bb are most similar to their human and mouse Kif5B orthologs with 86% sequence identity. zKif5C is 78% identical to its orthologs, while zKif5Aa is 72% identical and zKif5Ab has 66% identity with mouse and human Kif5As. Furthermore, comparing the coiled coil probability profile of zKif5Ab with all other zebrafish paralogs reveals two interesting points of divergence. The first occurs in CC1 (Fig. 2, bar) where zKif5Ab appears to have a relatively low coiled coil probability compared to the its paralogs. The second occurs in CC2 where zKif5Ab once again seems to have a low coiled coil probability and also lacks the third peak present in the other profiles (Fig. 2). Among the *kif5s* duplicated in zebrafish, zKif5Ba and zKif5Bb are closely related and cluster together as expected. Interestingly, zKif5Aa and zKif5Ab appear to be more distantly related, with zKif5Aa being more closely related to human and mouse Kif5As. zKif5Ba and zKif5Bb are 90% identical to one another whereas zKif5Aa and zKif5Ab share only 67% identity. This striking difference between zKif5Aa and zKif5Ab suggests that these two genes have diverged significantly over time and may now possess non-redundant functions.

1.2. kif5s show distinct expression profiles during zebrafish development and in adult tissues

To determine the temporal expression profiles of zebrafish *kif5s* during development, RT-PCR analysis was performed on zebrafish

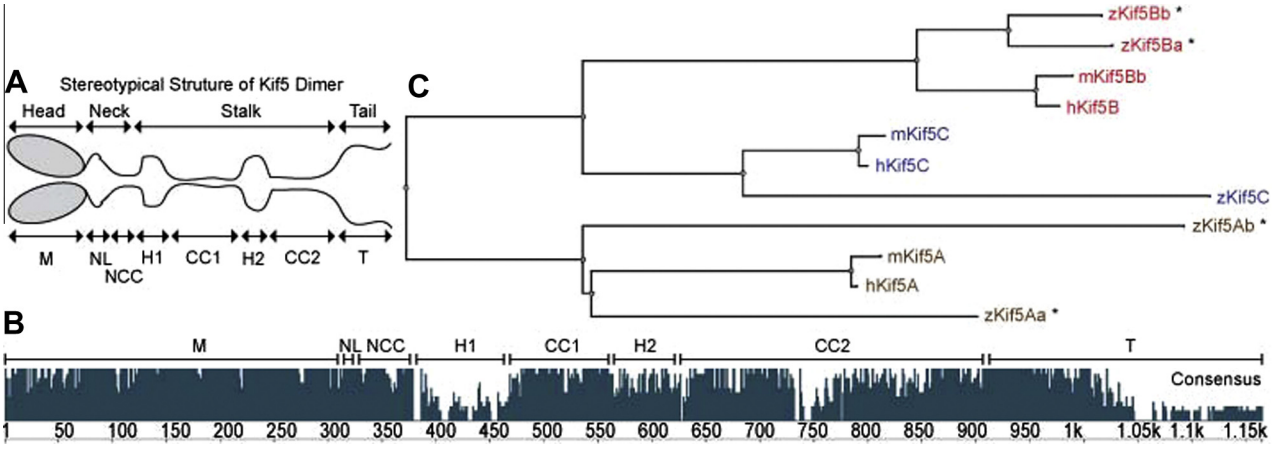

Fig. 1. Vertebrate Kif5 protein comparison. (A) Schematic of the protein domains of a Kif5 dimer. (B) Conservation of amino acid sequence between zebrafish, mouse, and human Kif5s. (C) Phylogenetic relationship between zebrafish, mouse, and human Kif5 proteins. Orthologs are shown in the same color (Kif5A, brown; Kif5B, red; Kif5C, blue) and paralogs are denoted with an asterisk (*). The length of the branches represents the degree of change between the sequences. M, motor domain; NL, neck linker; NCC, neck coiled-coil; H1, hinge 1; CC1, coiled-coil 1; H2, hinge 2; CC2, coiled-coil 2; T, tail.