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Characterization of the overlapping expression patterns of the zebrafish *LIS1* orthologs

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ABSTRACT

Mutations in the *LIS1* (*Lissencephaly-1*) gene underlie classical lissencephaly. This neurodevelopmental disorder is characterized by a loss of cortical gyri and improper laminar formation of the brain due to impaired neuronal migration. Patients with type 1 lissecephaly present with mental retardation and an increased risk of developing other disorders resulting from abnormal neurodevelopment, such as epilepsy. LIS1 is a dynamic protein implicated in numerous cellular mechanisms important for brain development. We have cloned and characterized the orthologs of *LIS1* in the zebrafish. The zebrafish is a well-documented model organism for studies of brain development and offers many advantages including embryonic transparency, the ability to easily manipulate gene expression and also generate transgenic animals which can be used to track single, migrating neurons. In the zebrafish nervous system, the *LIS1* orthologs are expressed in overlapping temporal and partially overlapping spatial patterns. While *lis1a* is primarily expressed in the developing central nervous system and the eye, *lis1b* is highly expressed in the peripheral nervous system as well as the Rohon-beard neurons. Rohon-beard neurons are the early sensory system of the embryo. We postulate that understanding the functions of Lis1 in the whole embryo will provide better insight into the genetic and neurodevelopmental basis of lissencephaly. This will not only aid in the development of therapeutic interventions for diseases such as lissencephaly but will also contribute to the general understanding of brain development.

1. Results and discussion

Development of the vertebrate brain depends upon the coordinated action of numerous cellular and molecular processes. Progenitor proliferation at the ventricular zone, migration of precursors to form the cortical layers, and differentiation and process extension of the neurons and glia of the developing brain must occur in a spatially and temporally precise manner for the proper functioning of the mature organ (reviewed in (Cowan et al., 1997). One gene which has been shown to be necessary for proper morphological development and function of the brain is *LIS1* (*Lissencephaly-1*), also known as *platelet-activating factor acetylhydrolase, isoform 1b* (*PAFAH-1b*).

Though *LIS1* was first identified as a deactivator of platelet-activating factor, mutations in *LIS1* were subsequently shown to underlie risk of developing type 1, also known as classical, lissencephaly (Reiner et al., 1993; Lo Nigro et al., 1997). Lissencephaly is a disease characterized by abnormal migration of neurons into the developing cortical layers resulting in a dramatic loss of cortical convolutions and subsequent smooth appearance of the mature brain (Dobyns and Truwit, 1995). In addition to morphological abnormalities, patients with lissencephaly have an extremely high risk of mental retardation, epilepsy, and other mental illnesses (Reiner et al., 2006). *LIS1*'s action in cortical development appears to be unrelated to its function in modulating platelet-activating factor function as overexpression of LIS1 lacking the catalytic region does not perturb cortical development (Koizumi et al., 2003; Yan et al., 2003).

Recent *in vivo* studies of Lis1 loss of function point to a role for Lis1 in interkinetic nuclear oscillation, nucleokinesis, microtubule stability, mitotic spindle orientation, and progenitor maintenance (Shu et al., 2004; Tsai et al., 2005, 2007; Vallee and Tsai, 2006;

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Pawlisz et al., 2008), all necessary for neural development. Knockdown of Lis1 in cortical neuron progenitors results in loss of centrosome-nucleus coupling, which is necessary for nucleokinesis and migration of newborn neurons into the forming cortical layers (Shu et al., 2004). In zebrafish, Lis1a, one of the two LIS1 orthologs, was similarly shown to regulate nuclear localization in photoreceptor cells of the eye which indirectly affects cell survival (Tsujikawa et al., 2007). In addition, Lis1 has been shown to function together with Ndel1 to regulate the orientation of the mitotic spindle in neural progenitors in the developing brain. This regulates symmetry of cell division which, in turn, governs the maintenance of a neural stem cell population at the ventricular zone (Yingling et al., 2008). Interestingly, another study demonstrates a function for Lis1 and another binding partner, Nde1, in the regulation of fate of the cells born in the ventricular zone. Mice carrying mutations in both Lis1 and Nde1 have a dramatic increase in early born preplate and Cajal-Retzius neurons and consequent loss of neural progenitors. This early differentiation of an abnormally high number of neural progenitors results in a decreased pool of neuroblasts born and loss of cells to populate the cortical layers (Pawlisz et al., 2008). These data implicate Lis1 in numerous microtubule-associated processes necessary for neurodevelopment.

The zebrafish has become a very popular model system in which to study the development of numerous organ systems. Recently, there has been a surge in the characterization of the expression of zebrafish orthologs of genes shown to be necessary for proper neural development in other systems. The characterization of these expression patterns is the first step towards the investigation of the evolutionarily conserved function of these genes in the cellular and molecular processes necessary for proper development of the vertebrate brain. Here, we present the expression patterns of *lis1a* and *lis1b*, the two zebrafish orthologs of the *LIS1* gene. As is the case for many genes in the zebrafish, there are two orthologs of the *LIS1* gene likely due to a whole genome duplication event early in the teleost lineage (Jaillon et al., 2004). Unlike other genes with multiple orthologs, such as the *NDEL1* orthologs (Drerup et al., 2007), the expression patterns of the *LIS1* orthologs in the zebrafish partially overlap at all developmental stages, suggesting conservation of regulatory sequences and perhaps function of the Lis1 protein.

1.1. Cloning and bioinformatic analysis of the zebrafish Lis1 orthologs

To identify the zebrafish *LIS1* orthologs, the human and mouse *LIS1* sequences were compared to the zebrafish genomic database (www.ensembl.org) using BLAST (Basic Local Alignment Search Tool). These searches identified two genomic loci which contained regions of high similarity to the query sequences. In addition, the zebrafish information network database (www.zfin.org) was searched and the same corresponding sequences were identified. *lis1a*, also known as *pafah1b1b*, corresponds to **NM201346** in GenBank and lies on chromosome 21 in the zebrafish. *lis1b*, i.e. *pafah1-b1a*, corresponds to **NM201345** in GenBank and resides on chromosome 15. Primers were designed to amplify both *LIS1* orthologs from an oligo dT primed cDNA library. These PCR products were ligated into the pCR4-TOPO vector (Invitrogen) and subsequently sequenced to confirm the sequence of the zebrafish *LIS1* orthologs.

Lis1 is highly conserved between vertebrates, but much less highly conserved in invertebrates (Fig. 1A). In addition, there is little divergence between the two zebrafish orthologs of this protein. Lis1a and Lis1b are 94% and 93% identical to the human LIS1 ortholog respectively. The two zebrafish variants are 93% identical to each other with an additional 4% of amino acids sharing strong similarities. In addition, both orthologs contain an amino terminal LisH domain and a carboxy terminal WD40 domain, both found in the LIS1 protein across species. The WD40 domain is present in all orthologs analyzed (see Fig. 1A) while the LisH domain is present in all orthologs except *aspergillus nidulans*.

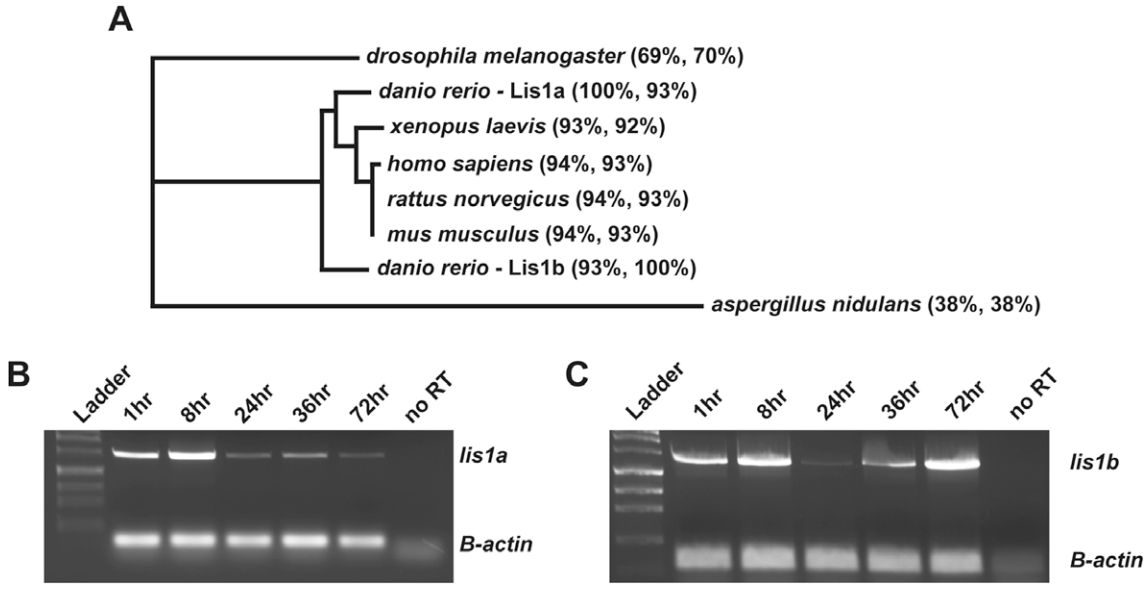

Fig. 1. LIS1 is highly conserved across species and is expressed in the developing zebrafish. (A) The LIS1 protein is highly conserved between vertebrate and invertebrate species. Two orthologs of LIS1 exist in the zebrafish, likely due to a whole genome duplication event. There is a 93% conservation of amino acid sequence between these two proteins and conservation between species is similar for each. Percent conserved amino acid identity between the listed species and zebrafish Lis1 orthologs are shown respectively in parentheses (Lis1a and Lis1b respectively). (B) Semi-quantitative RT-PCR analysis of *lis1a* expression in the developing zebrafish illustrates maternal expression of this transcript at 1hr. Expression levels decrease after 8 hpf and then reach a fairly steady-state level through early larval development. (C) Similarly, semi-quantitative RT-PCR analysis of *lis1b* demonstrates maternal expression of this transcript with a decline in expression after 8 hpf. However, unlike *lis1a*, *lis1b* expression increases from 24 hpf to 72 hpf, in the early stages of larval development. A portion of *β-actin* was amplified in a multiplexed PCR reaction for both transcripts to serve as an internal control. In addition, a no RT (reverse transcriptase) control (last lane) was done for each using 24 hpf total RNA.