Gene Expression Patterns 29 (2018) 82–87

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Developmental expression of the zebrafish Arf-like small GTPase paralogs *arl13a* and *arl13b*

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ARTICLE INFO

Keywords:
Photoreceptor
Cilia
Joubert syndrome
Zebrafish

ABSTRACT

Members of the Arf-like (Arl) family of small GTP-binding proteins regulate a number of cellular functions and play important roles in cilia structure and signaling. The small GTPase Arl13a is a close paralog to Arl13b, a small GTPase required for normal cilia formation that causes Joubert Syndrome when mutated. As mutation of *arl13b* causes a slow retinal degeneration in zebrafish (Song et al., 2016), we hypothesized that expression of *arl13a* may provide functional redundancy. We determined the expression domains of *arl13a* and *arl13b* during zebrafish development and examined subcellular localization by expression of fluorescence fusion proteins. Both genes are widely expressed during early cell division and gastrulation and Arl13a and Arl13b both localize to microtubules in ciliated and dividing cells of the early zebrafish embryo. Between 2 and 5 days post fertilization (dpf), *arl13b* is expressed in neural tissues while expression of *arl13a* is downregulated by 2 dpf and restricted to craniofacial structures. These results indicate that *arl13a* and *arl13b* have evolved different roles and that *arl13a* does not function in the zebrafish retina.

1. Introduction

Cilia are microtubule-based organelles that protrude from the surface of most eukaryotic cells (Satir and Christensen, 2007) and can be classified as motile cilia or non-motile (primary) cilia. Motile cilia function to drive locomotion in single-celled eukaryotes and gametes, or to propel fluid across the cell surface (Roy, 2009). Primary cilia function as sensory organelles and monitor the extracellular environment by concentrating receptors within the ciliary membrane (Malicki and Johnson, 2017). It is now well established that cilia are necessary for detecting a diverse range of signals, including light, hormones, neurotransmitters, morphogens, and growth factors (Ishikawa and Marshall, 2011; Hilgendorf et al., 2016). Given the importance of ciliary function for normal development and physiology, it is not surprising that defects in cilia can result in a number of pleiotropic genetic diseases, termed ciliopathies (Sharma et al., 2008). Joubert Syndrome (JBTS), Leber Congenital Amaurosis (LCA), Meckel Syndrome (MKS) Bardet-Biedl Syndrome (BBS), and Nephronophthisis (NPHP) are ciliopathies with overlapping clinical features, including retinal degeneration, obesity, polydactyly, skeletal abnormalities, and defects in hepatic, respiratory, and renal function (Sharma et al., 2008).

Three members of the ADP-ribosylation-factor-like (Arl) family of small G-proteins (Arl6, Arl3, and Arl13b) play important roles in cilia biogenesis (Li et al., 2010, 2012). Arl6 was the first member of the Arl family shown to cause disease when it was identified in a set of genes found to cause BBS (Chiang et al., 2004; Fan et al., 2004). Also known as Bbs3, Arl6 localized to the basal body in a ring-shaped pattern in hTERT cells (Wiens et al., 2010). Although not detected within the cilium of wild-type cells, it is thought that Arl6 facilitates ciliary exit of the BBSome, the large complex of BBS proteins required for ciliary membrane biogenesis (Nachury et al., 2007; Liew et al., 2014). A role for Arl3 in cilia function was first identified in the protozoan *Leishmania donovani* (Cuvillier et al., 2000). Subsequent work found that Arl3 directly binds with RP2 (retinitis pigmentosa protein 2), which functions as the GAP (GTPase activating protein) for Arl3 (Veltel et al., 2008). Both RP2 and Arl3 localize to the connecting cilium of photoreceptors (Grayson et al., 2002). Arl3 has been linked to the trafficking of lipidated membrane-associated proteins (Hanke-Gogokhia et al., 2016a, 2016b). Defects Arl3-mediated trafficking of prenylated proteins in rod photoreceptors led to cell death and retinal degeneration (Wright et al., 2016) Although human mutations in *ARL3* have not yet been identified, the *Arl3*$^{-/-}$ knockout mice exhibit phenotypes consistent with ciliary defects (Schrick et al., 2006). Unlike Arl6 and Arl3, which function both inside and outside the cilium, localization of Arl13b is limited to primary cilia. Arl13b was first linked to cilia in a forward genetic screen for kidney cysts in zebrafish (Sun et al., 2004). Shortly thereafter, the mouse *Arl13b*^{hnn} mutant was described as having defects in neural tube patterning, shortened cilia, and polydactyly, which were phenotypes

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https://doi.org/10.1016/j.gep.2018.07.002
Received 22 May 2018; Received in revised form 10 July 2018; Accepted 12 July 2018

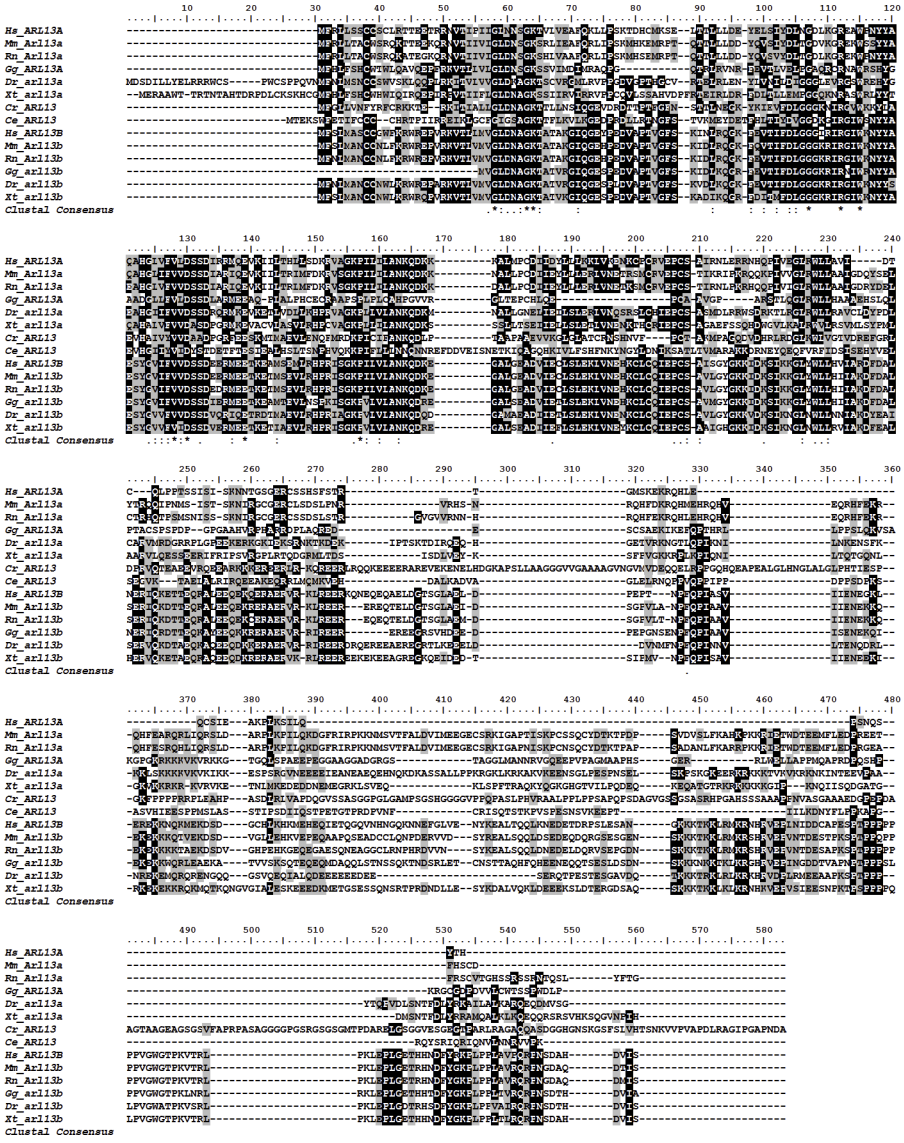

Fig. 1. Sequence alignment of Arl13a and Arl13b shows strong similarity in the N-terminal region of the protein. Using ClustalW, amino acid sequences for Arl13a homologs from human (Hs; NP_001155963.1), mouse (Mm; NP_083223.1), rat (Rn; NP_001019537.1), chicken (Gg; XP_015134146.1), zebrafish (Dr; NP_957112.1), *Xenopus tropicalus* (Xt; XP_004916818.1), *Xenopus laevis* (Xl; AAH99310.1), were aligned against amino acid sequences of Arl13b homologs from human (NP_001167621.1), mouse (NP_080853.3), rat (NP_001100571.1), chicken (XP_004938370.1), zebrafish (NP_775379.1), *Xenopus tropicalus* (NP_001184084.1), and Arl13 amino acid sequences from worm (Ce; NP_001032986.1), and algae (Cr; XP_001691430.1). Identical residues are noted in black and similar residues (minimum 50%) highlighted in gray. The nucleotide binding site (P loop) is underlined.

consistent with cilia defects (Caspary et al., 2007). Mutations in *ARL13*B were subsequently identified in families with Joubert Syndrome (Cantagrel et al., 2008). Arl13b has been proposed to function at the guanine nucleotide exchange factor (GEF) for Arl3, thereby spatially restricting Arl3 activation and cargo release to the cilium (Gotthardt et al., 2015).

Mutations disrupting the structure or function of cilia in zebrafish lead to a common set of phenotypes that include rapid photoreceptor cell death, kidney cysts, left-right asymmetry, and a curly body axis (Sun et al., 2004; Tsujikawa and Malicki, 2004; Pathak et al., 2007; Omori et al., 2008). Zebrafish lacking *arl13*b exhibit many of these phenotypes (Sun et al., 2004). We recently reported, however, that zebrafish *arl13*b$^{-/-}$ mutants undergo a slow, progressive photoreceptor degeneration (Song et al., 2016). While the *arl13*b$^{-/-}$ mutants develop kidney cysts during larval stages and exhibit phenotypes seen in other zebrafish cilia mutants, the mild retinal phenotype was unusual. One possible explanation for these results is functional redundancy with a closely related protein. Here, we explored the hypothesis that Arl13a may compensate for Arl13b in zebrafish photoreceptor function. Cellular localization studies revealed that Arl13a colocalized to microtubules in both ciliated and dividing cells of early zebrafish embryos. We report, however, that while *arl13a* expression overlaps with *arl13*b during early zebrafish development, the expression patterns differed at later stages during neural differentiation. We conclude that Arl13a likely does not compensate for Arl13b in the zebrafish retina.

2. Methods

2.1. Zebrafish care and maintenance

Zebrafish were maintained in a 14:10 h light-dark cycle on Aquatic Habitats recirculating water systems (Pentair; Apopka, FL). Animals were maintained in accordance with protocols approved by the Cleveland Clinic Institutional Animal Care and Use Committee (IACUC). To image and localize basal bodies, we used the transgenic line *Tg (5actb2:cetn2-GFP)^{cu6}*, which expresses a centrin2-GFP fusion protein from the β-actin promoter (Randlett et al., 2011). The *arl13*b *(sco)^{hi459}* line was previously described (Sun et al., 2004; Song et al., 2016).

2.2. In situ hybridization and immunocytochemistry

Localization of mRNA by *in situ* hybridization was done using digoxigenin- and fluorescein-labeled riboprobes as described (Jowett, 2001). The full-length zebrafish *arl13a* cDNA clone (NCBI Reference Sequence: NM_200818.1) was purchased from ATCC (ID 10167628).