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Distinct expression of two *foxg1* paralogues in zebrafish

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ABSTRACT

The forkhead proteins (Fox) act as transcription factors in many biological processes in a wide range of species. One member of this superfamily, Foxg1, has essential roles in the development of eyes, telencephalon, ears and olfactory system. Zebrafish *foxg1* has been reported to have similar roles as the mouse orthologue *Foxg1*. However, no data has been reported about possible zebrafish *foxg1* paralogues. In this study we identified one zebrafish *foxg1* paralogue by enhancer trapping, which we designate *foxg1b*. A more diverged paralogue, *foxg1c*, was identified by homology searches. Sequence comparisons indicate that both *foxg1b* and *foxg1c* are less related to mouse than the previously characterized *foxg1*. We report that *foxg1b* is expressed in a regionally restricted pattern within the developing eye, mainly in the dorsal–nasal retina, which is similar to the retinal expression of mouse *Foxg1*. By contrast, *foxg1c* is only expressed transiently in the eyes and forebrain between 14 and 20 h post-fertilization, while expression was detected exclusively in the developing inner ear at later stages. Our results suggest that *foxg1b* and *foxg1c* have undergone expression pattern divergence during evolution that has resulted in functional specialization.

1. Results and discussion

The forkhead box (Fox) proteins share a characteristic forkhead DNA binding domain, and constitute a family of transcription factors with a total of 17 different subclasses. Over 100 fox genes have been identified in species ranging from yeast to humans. The fox genes have essential roles in a wide spectrum of biological processes and are associated with human diseases. Notably, mutations in *FOXC1, FOXC2, FOXE3* and *FOXL2* cause ocular phenotypes, and *Foxi1* mutant mice resemble the human congenital inner ear malformations (Lehmann et al., 2003; Wijchers et al., 2006). Foxg1 has been extensively studied, and shown to act in proliferation, differentiation, patterning and neurogenesis (Huh et al., 1999; Regad et al., 2007; Seoane et al., 2004; Xuan et al., 1995). In mouse, *Foxg1* is expressed in the dorsal–nasal retina and is required for correct growth of axons from retinal ganglion cells (Tian et al., 2008). Loss of *Foxg1* also causes dorsal–ventral patterning defects in the eye (Huh et al., 1999), and severe defects in olfactory development (Duggan et al., 2008). In addition, *Foxg1* function is required for normal growth of the ventral telencephalon and development of the inner ear (Martynoga et al., 2005; Pauley et al., 2006; Xuan et al., 1995).

In zebrafish, *foxg1* is expressed in the nasal retina, telencephalon, otic vesicles and olfactory placodes (Duggan et al., 2008; Jeong et al., 2007; Nakayama et al., 2008; Picker and Brand, 2005; Toresson et al., 1998). Knockdown of *foxg1* by antisense RNA results in a reduction of the number of olfactory neurons (Duggan et al., 2008). However, it has remained unknown if there exist any zebrafish *foxg1* paralogues. Using a transposon enhancer trap vector in zebrafish (Davison et al., 2007), we identified one transgenic line displaying regionally restricted expression in the retina that identified a novel paralogue, *foxg1b*. By homology searches, we also identified a less conserved paralogue, *foxg1c*, which shows expression in the developing ear. Our results imply that the zebrafish paralogues *foxg1b* and *foxg1c* have become highly specialized as a result of sequence divergence and loss of gene subfunctions during teleost evolution.

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1.1. Enhancer trapping and identification of two foxg1 paralogues

Enhancer/gene trapping is a powerful approach to identify new genes with cell and tissue specific expression patterns in zebrafish (Ellingsen et al., 2005; Kawakami et al., 2004). Recent developments have combined enhancer/gene trapping with the Gal4-UAS system in zebrafish (Asakawa et al., 2008; Davison et al., 2007; Scott et al., 2007), and in a small scale enhancer trap screen performed in our laboratory, we identified the transgenic enhancer trap line, *Tg*(*Gal4-VP16, UAS:eGFP*)^{cp120}, further referred to as *cp120*. Analysis of its expression at 1 day post-fertilization (dpf), showed strong eGFP labeling in the whole retina, with slightly lower levels of fluorescence in the ventral part. Expression was also detected in the optic stalks and ventral telencephalon (Fig. 1A). Investigations of earlier stages detected eGFP initially in the anterior forebrain and eye primordia at 14 h post-fertilization (hpf), and stronger expression was observed in these tissues at 16 hpf (Fig. S1). The expression of eGFP continued in the retina at 2 dpf, with the ventral-posterior expression appearing less intensive (Fig. 1B insert). The projecting optic nerve and chiasm were also labeled with eGFP, while the eGFP labeled cells in the forebrain showed reduced intensity at 2 dpf compared to 1 dpf (Fig. 1B). The eGFP expression became more restricted to the dorsal–nasal retina by 3 dpf, while the projections to the tectum were visible (Fig. 1C). By this stage, eGFP labeled cells were no longer detected in the forebrain (data not shown). eGFP expression at 5 dpf showed a similar pattern as at 3 dpf (data not shown). The optic nerve and tectum labeled with eGFP were clearly visible (Fig. 1E).

The retinal expression pattern of eGFP in 5 dpf larvae was analyzed in detail by confocal microscopy of cross-sections (Fig. 1F–H). eGFP was expressed throughout the layers of the dorsal retina (restricted ventrally by the optic nerve), with higher levels of fluorescence in the outer nuclear layer (ONL), outer part of inner nuclear layer (INL) and ciliary marginal zone (CMZ) (Fig. 1F). The eGFP labeled optic nerve showed that ganglion cells in the dorsal retina mainly contributed to the retinotectal projection (Fig. 1F). Ventrally, the CMZ displayed strong eGFP expression and adjacent cells were also labeled. The region between the ventral CMZ and the optic nerve showed little or no eGFP expression (Fig. 1F–H).

To investigate if eGFP reporter expression was Gal4-VP16 dependent, *cp120* was crossed to *UAS:RFP* transgenic fish. Compound heterozygous larvae from this cross showed similar, co-localized patterns of eGFP and RFP expression (Fig. 1C, D, and F–H). Overall, the unique pattern of co-expression of eGFP and Gal4-VP16 implies that the enhancer trap line will be valuable for investigations of retinal regionalization, retinotectal projection and regeneration after damage.

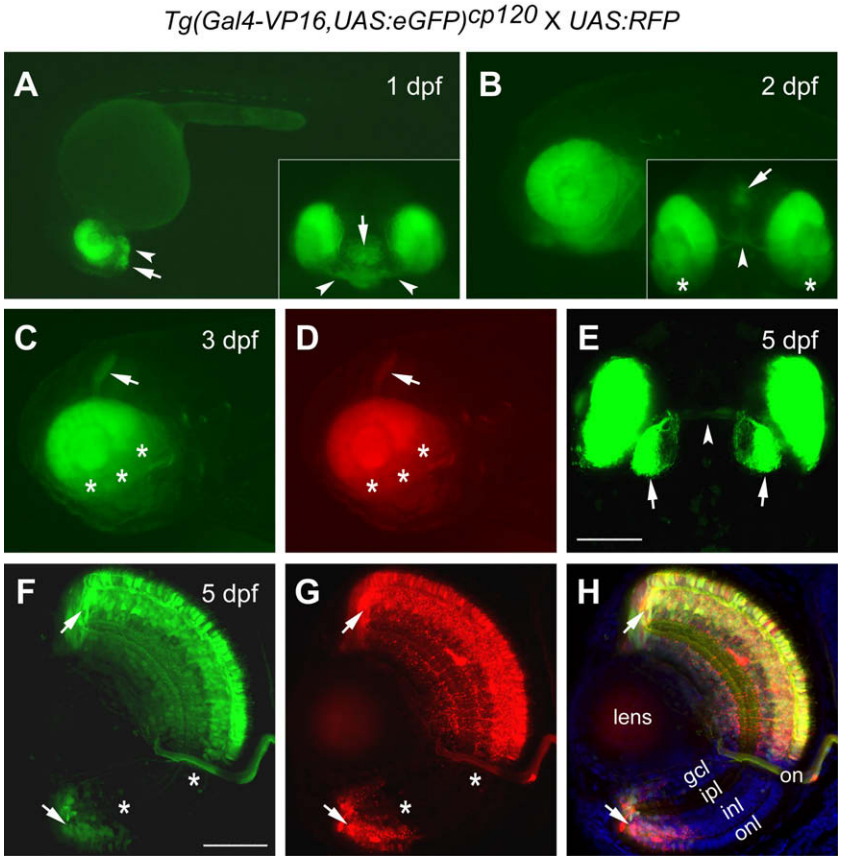

Fig. 1. Fluorescence expression profile of the enhancer trap line *Tg*(*Gal4-VP16,UAS:eGFP*)^{cp120} and its ability to induce expression of a *UAS:RFP* transgene. Expression of eGFP and RFP was analyzed in offspring from a cross between the enhancer trap line and transgenic *UAS:RFP* fish. (A) A lateral view at 1 dpf shows eGFP expression mainly in retina, optic stalk (arrowhead) and ventral telencephalon (arrow). The inset is a front view of the same embryo. (B) Expression of eGFP is maintained in the retina at a later stage (2 dpf). The ventral view in the inset shows eGFP labeling of the optic nerve (arrowhead), restricted area of the forebrain (arrow), and lower level in the posterior retina (stars). (C and D) Close up views of the fluorescence detected in dorsal-nasal retina and tectum (arrow) at 3 dpf. Ventral and posterior retina show no expression (stars). RFP is transactivated under control of the UAS element and shows the same pattern as eGFP. (E) Confocal image, viewed from the dorsal side, showing eGFP labeling in the tectum (arrows) and optic nerve (arrowhead). (F–H) Confocal images of a cryosection (dorsal at the top) show the co-localization of eGFP and RFP. Both the dorsal and ventral CMZ were labeled with fluorescence (arrows). An area in the ventral retina showing no fluorescence is marked (stars). Merged image of (F) and (G), along with DAPI stained nuclei is shown in (H). The different stages are indicated. The orientation is with anterior to the left (A–D). Abbreviations: dpf, days post-fertilization; gcl, ganglion cell layer; ipl, inner plexiform layer; inl, inner nuclear layer; onl, outer nuclear layer; on, optic nerve. Scale bars: 100 μm (E); 50 μm (F–H).