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Developmental expression of FoxJ1.2, FoxJ2, and FoxQ1 in *Xenopus tropicalis*

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Abstract

Members of the Fox gene family exhibit remarkably restricted patterns of expression where they have interesting, required functions during development. We have analyzed the developmental expression patterns of three members of the Fox gene family, FoxJ1.2, FoxJ2, and FoxQ1, which have not been previously described in *Xenopus*. FoxJ1.2 is expressed in the otic vesicle during late neurula stages and is then also expressed in the presumptive nephrostomes of the pronephros during tailbud stages. FoxJ2 is expressed in the notochord and ventral portion of the neural tube. FoxQ1 is expressed specifically in the pharyngeal pouches as early as neurula stages and remains on in pharyngeal tissue throughout the tailbud stages. At later stages, FoxQ1 is also expressed in the anterior gut. FoxJ1.2, FoxJ2, and FoxQ1 may prove to be useful tissue-specific markers of these embryonic structures.

Keywords: *Xenopus tropicalis*; Fox transcription factors; Forkhead family; Otic vesicle; Pronephros; Nephrostomes; Notochord; Neural tube; Pharyngeal pouches; Tongue; Anterior intestine

1. Results and discussion

The Fox transcription factors are present in organisms ranging from yeast to humans and play extraordinarily diverse roles that are critical to the organism (Lehmann et al., 2003). In addition to functions in the adult, they have been implicated in a number of developmental processes including the establishment of body axes and the differentiation of tissues from all three germ layers (Erickson, 2001; Lehmann et al., 2003). The Fox gene family comprises transcription factors that share a common forkhead DNA binding domain (Pohl and Knochel, 2005). Previously, the expression of many of the Fox genes has been reported for *Xenopus laevis* (Pohl and Knochel, 2005). Using recently available genomic data in *Xenopus tropicalis* (Gilchrist et al., 2004; Klein et al., 2002), we have identified *X. tropicalis* Fox gene family members representing each of the 17 subclasses A–Q of the Fox genes (data not shown) (Kaestner et al., 2000). This gene set includes orthologues of all the previously reported *X. laevis* Fox genes as well as a number of novel ones (Pohl and Knochel, 2005).

Here, we present the expression patterns of three Fox genes from *X. tropicalis*, FoxJ1.2, FoxJ2, and FoxQ1, whose expression has not been reported for the *X. laevis* orthologue.

FoxJ1.2 is a novel Fox gene. We aligned protein sequences of FoxJ genes from various species as well as *X. tropicalis* FoxQ1 using ClustalX (Thompson et al., 1997) and generated a phylogenetic tree (Fig. 1) in PAUP* (Swofford, 2003). FoxQ1 was used as an outgroup to root the tree. This tree shows that FoxJ1.2 is indeed a member of the FoxJ subclass but not a FoxJ1 gene. However, it is more closely related to FoxJ1 than to FoxJ2 or FoxJ3; hence, we named it FoxJ1.2. We were unable to find an orthologue for FoxJ1.2 in the human, mouse or chicken genomes, but in the zebrafish, fugu, and tetraodon genomes, we did find genes that appeared to be quite similar to FoxJ1.2 but not FoxJ1 (Fig. 1). Presumably, FoxJ1.2 is ancestral to vertebrates but then lost in amniotes.

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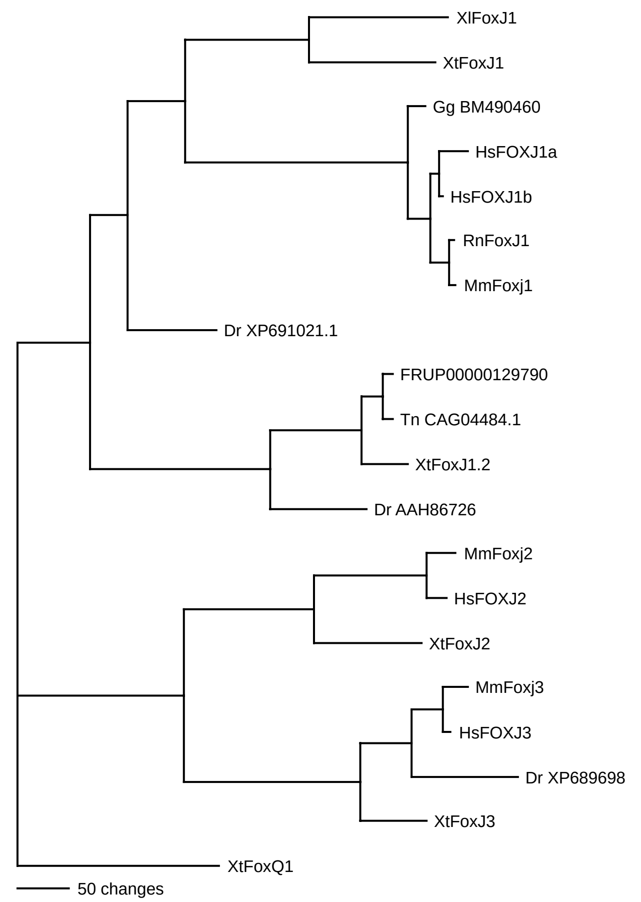

Fig. 1. Phylogenetic analysis of FoxJ gene family members. FoxJ sequences were obtained from Genbank or from the Joint Genome Institute (JGI). Previously annotated genes include: HsFOXJ1a (CAA67729), HsFOXJ1b (AAB09039), MmFoxj1 (AAA21689), MmFoxj2 (AAG30406), HsFOXJ2 (AAF65927), XtFoxJ2 (AAH74713), XlFoxJ1 (CAE76650), MmFoxj3 (AAH58231), HsFOXJ3 (NP_055762). We identified the following sequences in Genbank and have provided putative annotations (indicated with parentheses) based on our preliminary tree: RnFoxJ(1) (AAC37671), TnFoxJ(1) (CAG02275) DrFoxJ(1.2) (AAH86726), DrFoxJ(3) (XP_689698), TnFoxJ(1.2) (CAG04484), DrFoxJ(1) (XP_691021), GgFoxJ(1) (XP_425375). The DrFoxJ(3) sequence appears to be a chimera between two genes, and only the Fox portion of the sequence was used in this analysis. FoxQ1 (AL636071) was identified by alignments to other FoxQ genes (data not shown). The following two genes were identified by gene modeling in the fugu and *X. tropicalis* genomes (JGI) and are provided with gene model IDs: XtFoxJ(3) (e_gw1.257.123.1), FrFoxJ(1.2) (FRUP00000129790). Abbreviations: Hs (*Homo sapiens*), Mm (*Mus musculus*), Xt (*Xenopus tropicalis*), Xl (*Xenopus laevis*), Rn (*Rattus norvegicus*), Dr (*Danio rerio*), Tn (*Tetraodan nigrovidis*), Gg (*Gallus gallus*), Fr (*Fugu rubripes*).

FOXJ2, formerly known as FHX, has been cloned and characterized in humans where it is expressed in all tissues assayed (Perez-Sanchez et al., 2000). Foxj2 is also expressed in the mouse embryo as early as the eight-cell stage and is present in both layers of the blastocyst (Granadino et al., 2000). The expression pattern of FoxJ2 has not yet been described at the embryonic stages examined here.

FoxQ1, formerly Hfh-1L, is expressed in both developing and mature organisms. In *Amphioxus*, it is expressed in the developing endostyle, which is homologous to the vertebrate thyroid, and peripharyngeal bands (Mazet et al., 2005). In *Ciona*, FoxQ1 is also expressed in the endostyle, endodermal regions of the pharyngeal-gill, and esophagus of the young adult as well as dorsal regions of the adult endostyle (Ogasawara and Satou, 2003). In mice, Foxq1 mRNA is found in the metanephros during embryonic development and in hair follicles where it plays a role regulating hair differentiation (Frank and Zoll, 1998; Hong et al., 2001). FOXQ1 has been isolated and characterized in humans where it is strongly expressed in the stomach, trachea, bladder and salivary gland and weakly expressed in adult and fetal kidney and lung tissue. Additionally, it is overexpressed in colorectal adenocarcinoma and lung carcinoma cell lines (Bieller et al., 2001).

In order to identify the tissues expressing these Fox genes during *X. tropicalis* development, we performed whole mount in situ hybridization at various embryonic stages.

1.1. FoxJ1.2

During gastrula and neurula stages, FoxJ1.2 is expressed diffusely throughout much of the embryo (data not shown). At stage 23, FoxJ1.2 is expressed only in the otic vesicle (Fig. 2A,B). By stage 28, expression is also found in the pronephros (Fig. 2C–F,H). Staining in the pronephros is restricted to the region of the presumptive three nephrostomes, which drain coelomic fluid into the proximal kidney tubules (Carroll et al., 1999). Since expression fades rapidly in this region, it is difficult to be certain that expression predicts the definitive nephrostome. At stage 35, FoxJ1.2 is expressed broadly in the head but is strongly expressed in the developing gill structures (Fig. 2E,F). This unusual pattern of expression suggests that this gene may be associated with pronephric function, and indeed, no ortholog has been found in amniotes, which have non-functional, vestigial pronephroi. Relatives of FoxJ1.2 can be detected in fish genomic sequences, and may indicate a conserved role in pronephros development.

1.2. FoxJ2

FoxJ2 is expressed broadly prior to stage 23 (data not shown) but then begins to be restricted to dorsal tissues (Fig. 3A). By stage 28, expression is restricted to the notochord and floorplate (Fig. 3B). FoxJ2 is expressed almost exclusively in the most ventral part of the neural tube in anterior regions of the embryo. In progressively posterior regions, FoxJ2 is expressed more dorsally in the neural tube (Fig. 3D,E). At stage 35, FoxJ2 expression