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Expression of the forkhead transcription factor *FoxN4* in progenitor cells in the developing *Xenopus laevis* retina and brain

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Abstract

Forkhead proteins are involved in gene regulation in a large variety of developmental situations. Several forkhead gene products are expressed in the developing eye and brain. Here we characterize the expression of *FoxN4* during *Xenopus* development. We report that *FoxN4* is expressed in the eye from the earliest stages of specification through retinal maturation. *FoxN4* is also expressed in the pallium, optic tectum, isthmus, reticular formation, and in cells lining the ventricle of the tadpole brain.

Keywords: Forkhead; Transcription factor; *Xenopus laevis*; Retinal progenitor cells; Pallium; Optic tectum; Isthmus; Brain

The forkhead gene products comprise a large family of transcriptional regulators involved in controlling many aspects of embryonic development, including cell specification, differentiation, and cell cycle regulation (Carlsson and Mahlapuu, 2002; Hromas and Costa, 1995; Kaufmann and Knochel, 1996; Pohl and Knochel, 2005). Members of this gene family encode a 110-amino acid DNA binding domain, the forkhead box, related to that of the founding member of the family, *Drosophila forkhead* (Weigel and Jackle, 1990, 1989). Forkhead domains share a high degree of similarity among family members within and across species. Several forkhead genes are expressed in the developing *Xenopus* retina, including *FoxD1* (Mariani and Harland, 1998), *FoxK1* (Pohl and Knochel, 2004), *FoxM1* (Pohl et al., 2005), *FoxO3* (Pohl et al., 2004), and *FoxP1* (Pohl et al., 2005). Another forkhead transcription factor, *FoxN4*, is expressed in progenitor cells of the murine and zebrafish retina (Danilova et al., 2004; Gouge et al., 2001; Li et al., 2004). The *Xenopus* retina has been extensively studied as a model for the development and differentiation of retinal progenitor cells (Perron and Harris, 2000). Additionally, the *Xenopus* tadpole brain is increasingly being used as a model for studies in comparative brain anatomy and development (Bachy et al., 2002, 2001; Brox et al., 2002, 2004). Recently, *FoxN4* has been identified in *Xenopus*, where it is expressed in the developing retina (Schuff et al., 2006). Here, we report the expression of *FoxN4* in the developing *Xenopus* brain and further characterize its expression in the retina.

1. Results and discussion

1.1. Sequence comparisons

We isolated a cDNA encoding *Xenopus laevis FoxN4* (Fig. 1). The sequence we obtained was essentially identical to a *X. laevis FoxN4* sequence independently deposited in GenBank (Accession No. AM114796), differing at only 5 of 506 amino acid positions (99% identity). Overall, *X. laevis FoxN4* was similar to previously identified orthologs from other vertebrate species (Fig. 1E). Several domains

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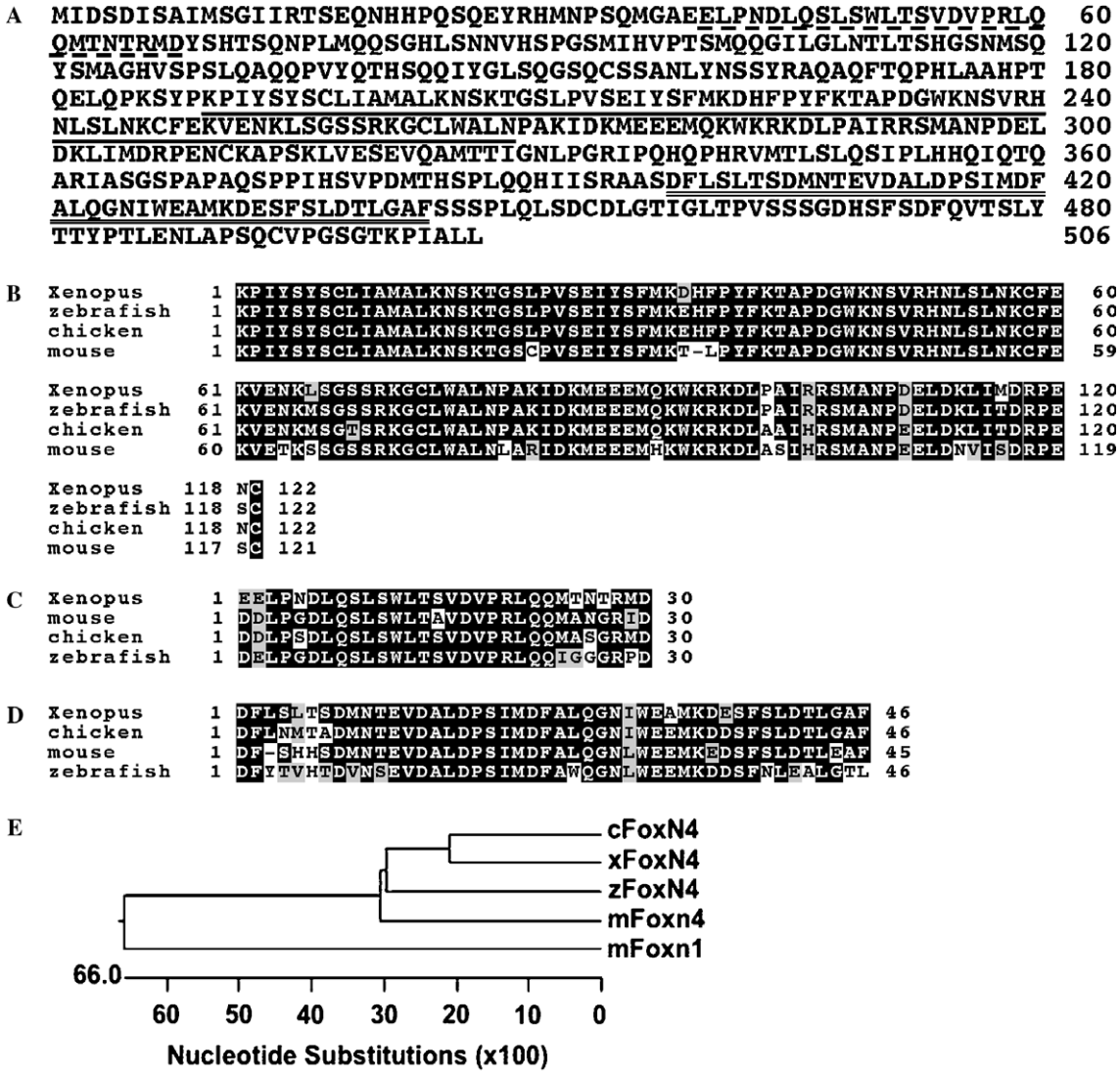

Fig. 1. *Xenopus laevis* FoxN4 is closely related to other vertebrate FoxN4 gene products. (A) Predicted amino acid sequence of the *X. laevis* FoxN4 gene product. The forkhead domain is underlined. The previously denoted FoxN4-specific sequence and a putative activation domain are marked by a dashed line and a double underline, respectively. (B–D) Alignment of forkhead (B), FoxN4-sepcific (C), and activation domains (D) from *X. laevis* and other vertebrate FoxN4 gene products sequences. Sequences were aligned using ClustalW 1.8 at the EMBL-EBI webpage (URL: http://www.ebi.ac.uk/clustalw/index.html) and then formatted using BOXSHADE at the EMBnet website (URL: http://www.ch.embnet.org/software/BOX_form.html). (E) Phylogenic analysis performed using the MegAlign program (part of the DNAStar/Lasergene suite of programs) from a ClustalW 1.8 alignment of the sequences shown. Accession numbers for sequences used are as follows: mouse Foxn1 – NP_032264; mouse Foxn4 – AAL06288; chicken FoxN4 – XP_415189; zebrafish FoxN4 – AAG27086.

identified in FoxN4 gene products from other vertebrates were clearly conserved, including the forkhead domain (Fig. 1B), a putative activation domain (Fig. 1C) and a FoxN4 domain (Fig. 1D). The FoxN4 domain was identified as a string of amino acids that is conserved among orthologs of FoxN4 but not the related gene product, FoxN1 (Danilova et al., 2004).

1.2. Expression in early embryos

We visualized *FoxN4* expression during *Xenopus* development by wholemount in situ hybridization (WISH; Fig. 2A–I). The *FoxN4* expression pattern (Fig. 2A–D) is similar to that of other eye field transcription factors (Zuber et al., 2003), including *Rx* (Fig. 2F–I). Expression is first detected as a single patch at the anterior edge of the neural plate (Fig. 2A), corresponding to the developing eye field. As development progresses, this field splits into two distinct regions (Fig. 2B). During tailbud stages, the expression of *FoxN4* becomes further restricted to the neural retina and is absent from the lens (Fig. 2D). *FoxN4* expression is also observed in several parts of the developing brain (Fig. 2B and D). Additionally, *FoxN4* is expressed in the pronephros at tailbud stages (Fig. 2E). The expression of *FoxN4* in the developing eye is strikingly similar to that of *Rx* (Fig. 2F–I) except for in the brain, where Rx is only expressed in the