Xenopus laevis FoxE1 is primarily expressed in the developing pituitary and thyroid

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ABSTRACT The members of the FoxE subfamily of Fox (forkhead) genes are expressed in the developing pituitary, thyroid and lens. Mammalian Foxe1 is expressed primarily in the developing pituitary and thyroid gland, Foxe3 is expressed in the developing lens, while Xenopus Foxe4 is expressed in the developing lens and thyroid. Here we report the identification of Xenopus FoxE1, a gene that is primarily expressed in the developing pituitary and thyroid.

KEY WORDS: forkhead, FoxE1, pituitary, thyroid, TTF-2, Xenopus

Fox proteins (also known as forkhead, or winged helix proteins) comprise a family of transcription factors containing the winged helix DNA binding motif. Many of these gene products are involved in regulation of gene transcription during development and several developmental disorders in humans are caused by mutations in Fox genes (Carlsson and Mahlapuu, 2002, Hromas and Costa, 1995, Kaufmann and Knochel, 1996). Members of the FoxE subfamily of Fox genes are expressed in the developing anterior ectoderm and endoderm (Blixt et al., 2000, Brownell et al., 2000, Dathan et al., 2002, Kenyon et al., 1999, Yu et al., 2002, Zannini et al., 1997). This subfamily includes mammalian Foxe1 (thyroid transcription factor 2 or TTF-2), Foxe3 and Xenopus Foxe4 (Xlens1). Foxe1 is expressed in several developing ectodermal and endodermal derivatives of the head including the thyroid, Rathke’s pouch, tongue, esophagus, epiglottis, pharynx, whiskers and nasal choanae (Dathan et al., 2002, Zannini et al., 1997). Foxe3 is primarily expressed in the developing lens ectoderm (Blixt et al., 2000, Brownell et al., 2000). In Xenopus, Foxe4 is expressed in the lens ectoderm and the developing thyroid (Kenyon et al., 1999). A recently identified Amphioxus Foxe4 ortholog, Anph/foxe4 is expressed in a region of the pharyngeal endoderm called the club-shaped gland and not in the endoderm, the Amphioxus thyroid homologue (Yu et al., 2002). We isolated a cDNA encoding the Xenopus laevis Foxe1 protein by degenerate PCR (Fig. 1). The most similar mammalian homologue is Foxe1 (45.8% identity), also known as thyroid transcription factor 2 (TTF2) (Zannini et al., 1997). However, there are notable differences between Xenopus Foxe1 and mouse Foxe1 (Fig. 1A). Notably, Foxe1 lacks the polyalanine repeats found in Foxe1, associated with transcriptional repression and mutated in patients with thyroid dysgenesis (Hishinuma et al., 2001), indicating that Foxe1 may be functionally distinct from Foxe1. Foxe1 is also closely related to Xenopus laevis Foxe4. Although Foxe1 and Foxe4 have highly similar forkhead domains, there are many differences outside of the forkhead domain (44.9% identity), confirming that they represent distinct gene products. To investigate the spatio-temporal expression pattern of Foxe1, whole mount in situ hybridizations were performed using Xenopus laevis embryos spanning a variety of embryonic stages. Foxe1 expression is first observed at late neural tube and early tailbud stages in a discrete area on the anterior face of the embryo, corresponding to the position of the hypophyseal placode (Fig. 2A-D). Foxe1 expression continues in the developing pituitary at late tailbud stages (Fig. 2E,F). At these stages, the expression of Foxe1 is very similar to the expression pattern of Xanf2 (Mathers et al., 1995) and POMC (Holling et al., 2000) (Fig. 2H,I). Expression of Foxe1 precedes expression of POMC and is preceded by Xanf2 expression in the anterior neural. As development progresses, Foxe1 is expressed in the mesoderm of the branchial arches (Fig. 2E,F,N). Foxe1 is expressed in the developing thyroid at st 38 (Fig. 2J,M), as is Nkx2.1 (also known as thyroid transcription factor 1) (Holleman and Pieler, 2000, Small et al., 2000) (Fig. 2K,O) and Foxe4 (Kenyon, Moody et al., 1999) (Fig. 2L). Foxe1 is also expressed in the pharyngeal

Abbreviations used in this paper: TTF, thyroid transcription factor.
Fig. 1. Comparison of amino acid sequences of FoxE1 and other related forkhead gene products. (A) Predicted amino acid sequence of FoxE1 aligned with mouse Foxe1. Forkhead domain is underlined. Dots indicate identical amino acids. Dashes indicate spaces inserted into sequence to aid spacing during alignment. (B) Alignment of forkhead boxes (B) of FoxE1 and related genes Foxe1 (NP_899121), Foxe3 (AAF15997), Xenopus laevis FoxE4 (AAF20385) and AmphiFoxE4 (AAK85731). (C) Phylogenetic analysis of Xenopus laevis FoxE1 and Foxe3, AmphiFoxE4, Ciona FoxE (BAC57420) and Xenopus laevis FoxD3a (AAK85731) was performed from ClustalW alignment using the MegAlign program (DNAStar, Inc). (D) Pairwise percent amino acid identities of Xenopus laevis FoxE1 and FoxE4, mouse Foxe1 and Foxe3, AmphiFoxE4, CifoxE and FoxD3a were calculated from ClustalW alignment using the MegAlign program (DNAStar, Inc).
Fig. 2. Expression of FoxE1 in Xenopus embryos. (A-F) Lateral (A,C-F) and anterior (B) views of whole mount in situ hybridized with antisense probe for FoxE1. (A,B) Stage 18, (C) Stage 24, (D) Stage 27, (E) Stage 33/34 and (F) Stage 35/36. (G-L) Comparison of FoxE1 expression with markers of the pituitary development (H,I) and thyroid development (K,L). (G) Anterior view of a st 35/36 embryo hybridized with FoxE1. (H,I) Anterior views of tailbud stage embryos hybridized with antisense riboprobes for Xanf2 and POMC, respectively. (J,K,L) Lateral view of tailbud stage embryos hybridized with antisense riboprobes for FoxE1, Nkx2.1 and FoxE4 (Xlens1), respectively. Arrowheads denote the position of thyroid expression. (M-O) Section in situ hybridization of st 38 embryos hybridized with antisense probes for FoxE1 (M, O) or Nkx2.1 (N). (M) Expression of FoxE1 in the thyroid (arrowhead) and pharyngeal endoderm. No expression is observed in the lens (open arrowhead). (N) Expression of FoxE1 in branchial arch mesoderm (arrow) and pharyngeal endoderm. (O) Expression of Nkx2.1 in the thyroid (arrowhead). B, brain; E, eye; H, heart; N, notochord; OV, otic vesicle; P, pharynx.

endoderm (Fig. 2M,N). Unlike FoxE4, FoxE1 is not expressed in the lens (Fig. 2E-G, M). Thus, even though Foxe1, Foxe3, FoxE1 and FoxE4 are expressed in distinct patterns, the composite expression patterns of the FoxE1/FoxE4 and Foxe1/Foxe3 gene pairs are similar.

Experimental Procedures

Isolation of FoxE1 cDNA

The forkhead domain of FoxE1 was amplified from stage 37 Xenopus laevis embryo head cDNA (prepared using the SMART cDNA Amplification Kit, Clontech) with degenerate primers encoding the amino acids GKPPYSYIA and (D/E)CF(I/V)K(I/V)P. Two sets of primers encoding GKPPYSYIA were mixed together to include all 6 alanine codons. The amplified product was subcloned (TA Cloning Kit, Invitrogen) and sequenced. The sequence was used to design primers for one-armed PCR (OA-PCR; Macrae and Brenner, 1994) using nested FoxE1 primers and 5’- or 3’-SMART primers (SMART cDNA Amplification Kit, Clontech). The sequences of the OA-PCR products were used to design primers to amplify the coding region from the same cDNA. The cDNA sequence was submitted to GenBank (Accession number AY509892). Sequences were aligned using the ClustalW program from the Baylor SearchLauncher Web page: (URL: http://searchlauncher.bcm.tmc.edu).

In situ hybridization

Gene expression was analyzed by in situ hybridization using whole embryos (Sive et al., 2000, Turner and Weintraub, 1994) or paraffin sections (Shimamura et al., 1994, Viczian et al., 2003) using digoxigenin-labeled antisense riboprobes.

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