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Developmental Expression Pattern

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, *AmphiFoxE4*, is expressed in a region of the pharyngeal endoderm called the club-shaped gland and not in the endostyle, 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) (Hollemann 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.

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A

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xFoxE1     1 MTAESQQSPTRATAAGAGLQQTSGFTMPVVKVEKDPAPEASMSNGGSEVDDTHKGRRRKR
mFoxe1     1 .....-----------.PPPPPQPE.LAA..E.RGE.AA.GAGVPAEAAGRGAG......

xFoxE1    61 PLQKGKPPYSYIALIAMSIANSADRKLTLGGIYKFITERFPFYRDNSKKWQNSIRHNLTL
mFoxe1    50 ...R.............A..HAPE.R.....................P.............

xFoxE1   121 NDCFIKIPREPGRPGKGNYWALDPNAEDMFDSGSFLRRRKRFKRTDLTTYPAYIHDTSMF
mFoxe1   110 ....L.....A...................E.............S..S.....M..AAAA

xFoxE1   181 SPLQVARATYPNTVYPNMTMSPNYSQQIAPHSSVYYPSSSPAFSSAQPRVFSINTLIGHS
mFoxe1   170 AAAAA.AIFPGAVPAARPAYPGAVYAGY..PLAAPP.VYY..A.PGPC...GLVPER---

xFoxE1   241 GSEHAQPPNRSISPEVNSTSSSSCNYGGSAYSSQAGSGTMLPRSTNPVPYSYSVPNSHLQ
mFoxe1   227 ------.LSPDLG.APSAAGG.CAFAAAAGAAGTGSFQPAVCTGAR..NPAAYAAAYAGP

xFoxE1   301 MNQSTYTHSNAQLFGSASRLPMPTSPPMN------SDTVDFYGRMSPGQYTSLAT--YNS
mFoxe1   281 DGAYPQGA.S.LFAAA.G..AG.A...AGGGSGGVEA.......T....FGAALGPC..P

xFoxE1   353 NGQLGGTNAYLRH----ATYSGNMERFVPAV
mFoxe1   341 G....AGGGGAY.SRHATA.P.AVD...S.M
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B

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FoxE1       1 GGRRRKRPLQKGKPPYSYIALIAMSIANSADRKLTLGGIYKFITERFPFYRDNSKKWQN
FoxE4       1 ........V.....................................M.......E.......
AmphiFoxE4  1 ........V.K...........S.....................MD...Y...RD.....
Foxe1       1 ............................HA...R.....................P.....
Foxe3       1 -.K..R...................L.HA.G.R...AA..R......A....SPR....

FoxE1      61 IRHNLTLNDCFIKIPREPGRPGKGNYWALDPNAEDMFDSGSFLRRRKRFKRTDLTTYP
FoxE4      61 ...................H................................I....
AmphiFoxE4 61 ...................................................A.SS..M
Foxe1      61 ...........L.....A.........A...N.....ES............S..S...
Foxe3      60 ............V.....N.............A.................AE.P---
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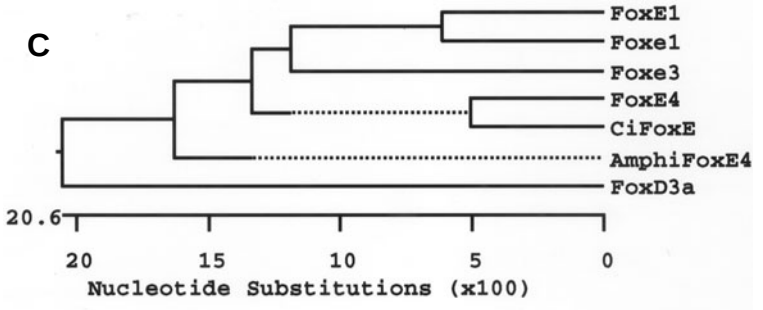

D

Percent Identity

1	2	3	4	5	6	7		
	44.9	45.8	42.0	36.1	29.6	36.8	1	FoxE1
		33.7	43.1	41.1	31.0	38.1	2	FoxE4
			49.3	34.2	24.8	33.7	3	Foxe1
				37.2	35.1	37.8	4	Foxe3
					27.5	34.1	5	AmphiFoxE4
						27.8	6	FoxD3a
							7	CiFoxE

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 Amphioxus AmphiFoxE4 (AAK85731).* **(C)** *Phylogenetic analysis of* Xenopus laevis *FoxE4 and FoxE5, mouse Foxe1 and Foxe3, AmphiFoxE4 (AAK85731),* Ciona *FoxE (BAC57420) and* Xenopus laevis *FoxD3a (BAA36334) 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).*

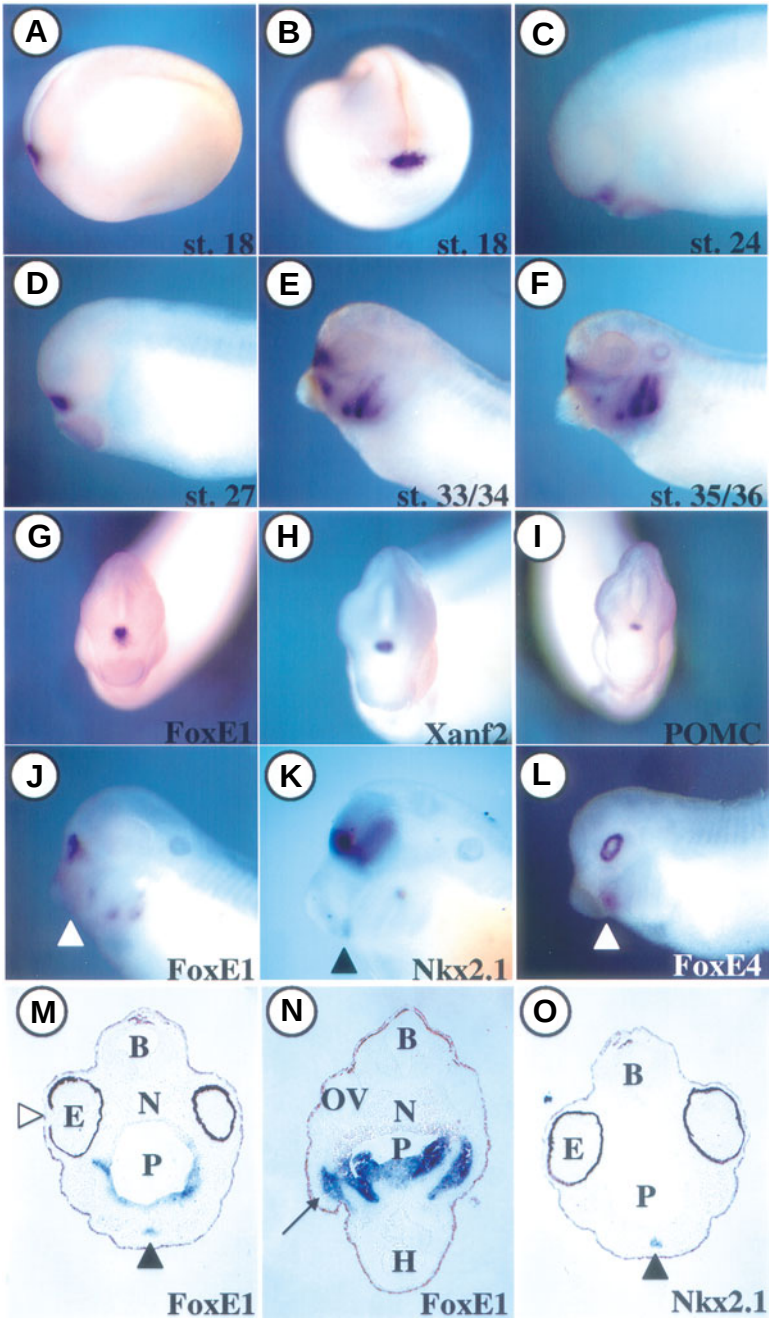

Fig. 2. Expression of *FoxE1* in *Xenopus* embryos. *(A-F) Lateral (A,C-F) and anterior (B) views of whole mount* in situ *hybridized with antisense probe for* FoxE1. **(A,B)** *Stage 18,* **(C)** *Stage 24,* **(D)** *Stage 27,* **(E)** *Stage 33/34 and* **(F)** *Stage 35/36. (G-L) Comparison of* FoxE1 *expression with markers of the pituitary* **(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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