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Gene expression pattern

Expression of the *Xvax2* gene demarcates presumptive ventral telencephalon and specific visual structures in *Xenopus laevis*

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Abstract

vax2 is a recently isolated homeobox gene, that plays an important role in controlling the dorso-ventral patterning of the retina. In this paper we present a thorough description of the *Xvax2* expression pattern all along *Xenopus* embryogenesis, and compare this pattern in detail to that shown by *Xvax1b* and *Xpax2*, two genes also involved in ventral eye development. At early neurula stages, while *Xpax2* starts to be expressed within the eye field, both *Xvax2* and *Xvax1b* are exclusively activated in the presumptive ventral telencephalon. Since midneurula stages, *Xvax2* and *Xvax1b* are also transcribed in the medial aspect of the eye field. At tailbud and tadpole stages, *Xvax2*, *Xvax1b* and *Xpax2* expression overlaps in the optic stalk and nerve and in the optic disk, while *Xvax2* and *Xvax1b* also display specific activation domains in the ventral retina as well as in the ventral telencephalon and diencephalon. Finally, during metamorphosis a high level of both *Xvax2* and *Xvax1b* transcription is maintained in the optic chiasm. In addition, *Xvax1b* is transcribed in the ventral hypothalamus and in the hypophysis, whereas a strong *Xvax2* expression is retained in the ventral portion of the mature retina.

Keywords: *vax* genes; *Xenopus* embryo; Anterior neural plate; Ventral telencephalon; Ventral retina; Optic stalk/nerve; Optic chiasm

1. Results

The recently identified *vax2* homeobox gene plays an important role in eye development. *vax2* is consistently expressed in the ventral portion of the developing *Xenopus*, mouse and human retina and its overexpression in frog and chick embryos ventralizes the eye (Barbieri et al., 1999; Ohsaki et al., 1999; Schulte et al., 1999). To achieve further insight into the function of the *Xenopus Xvax2* gene, we decided to map its expression domains throughout frog development in comparison with *Xvax1* (Hallonet et al., 1998) and *Xpax2* (Heller and Brändli, 1997), two genes also involved in ventral eye development. In the same screening leading to the identification of *Xvax2*, we also isolated a cDNA coding for a predicted protein highly homologous (94.8% aminoacid identity) to the putative Xvax1 protein. This cDNA was therefore named *Xvax1b* (accession number AJ271730). Since the expression pattern of *Xvax1* and *Xvax1b* appeared to be indistinguishable, we used *Xvax1b* as a marker for our comparative study.

After whole-mount in situ hybridization, expression of *Xvax2* and *Xvax1b* is first detected at early neurula (st. 13/14), in the rostralmost neural plate (Fig. 1A,G). At this stage, *Xpax2* is also activated in the anterior neural plate (Fig. 1M). To further define these expression domains, we compared them with those of *Xrx1* (an eye field marker; Casarosa et al., 1997) and *XBF-1* (an early telencephalic marker; Bourguignon et al., 1998) in double in situ hybridizations. Both *Xvax2* and *Xvax1b* expressing regions lay anteriorly to the *Xrx1* domain (Fig. 1D,J) and overlap with the *XBF-1* domain (Fig. 1F,L), while *Xpax2* expression is contained within that of *Xrx1* (Fig. 1P). Double hybridizations between either *Xvax2* or *Xvax1b* and *Xpax2* also show that the *Xpax2* and the *Xvax2* or *Xvax1b* domains are spatially distinct (Fig. 1R and data not shown). Since both *Xvax2* and *Xvax1b* also showed distinct domains with respect to the dorsal telencephalic marker *Xemx1* (Pannese et al., 1998; data not shown), we conclude that their first activation occurs in the presumptive ventral telencephalon. At midneurula (st. 15/16), both *Xvax2* and *Xvax1b* domains have expanded along the anteroposterior axis (Fig. 1B,H), thus overlapping with the medial aspect of the *Xrx1* positive territory (Fig. 1E,K), where *Xpax2* expression is also maintained (Fig. 1Q). By late neurula

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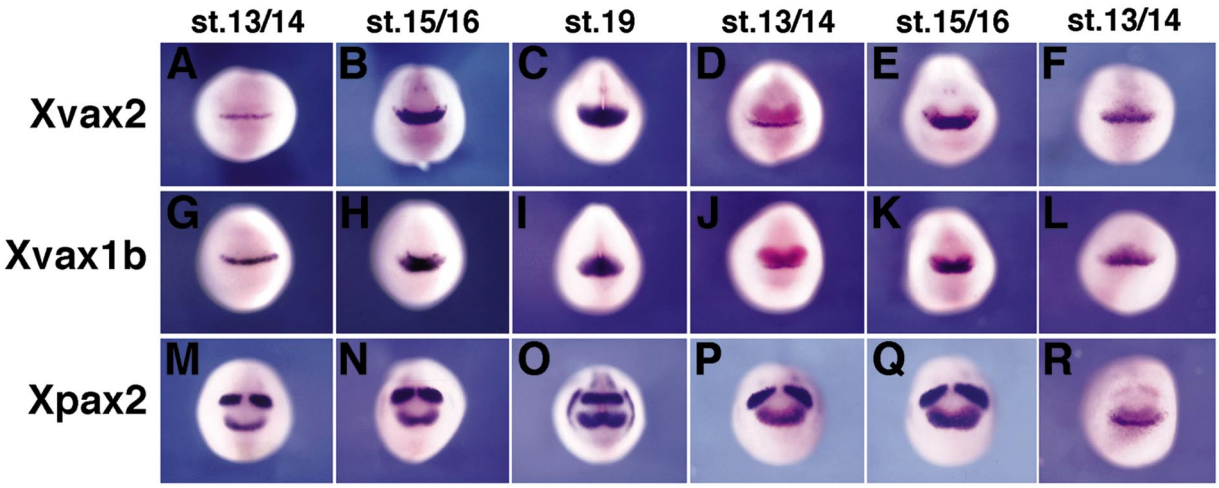

Fig. 1. Whole-mount single or double in situ hybridizations on stage 13/14 (A,D,F,G,J,L,M,P,R), stage 15/16 (B,E,H,K,N,Q) and stage 19 (C,I,O) *Xenopus* embryos, showing expression of *Xvax2* (A–F,R, blue staining) in comparison with *Xvax1b* (G–L, blue staining) and *Xpax2* (M–Q, blue staining; R, magenta staining). *Xrx1* (D,E,J,K,P,Q, magenta staining) and *XBF1* (F,L, magenta staining) are used as markers of eye field and telencephalon, respectively.

(st. 19), *Xvax1b* transcription is restricted to the prospective ventral forebrain and optic stalk (Fig. 1I), while *Xvax2* and *Xpax2* expression also embraces the ventral evaginating optic vesicle (Fig. 1C,O).

We subsequently mapped the *Xvax2*, *Xvax1b* and *Xpax2* domains on histological sections at different stages of larval development. At early tailbud stage (st. 23), the optic vesicles just evaginated from the forebrain. At this stage, at the level of the anterior optic vesicle *Xvax2* and *Xpax2* expression embraces similar anterior domains, including the ventral forebrain and optic vesicle (Fig. 2A,C). Instead, *Xvax1b* transcription is more medially restricted and does not extend into the eye bud (Fig. 2B). However, at the level of the posterior optic vesicle *Xpax2* expression appears to be similar to that of *Xvax1b* and more ventro-medially restricted than that of *Xvax2* (Fig. 2D–F). At late tailbud stage (st. 30), when invagination of the optic vesicle to form the optic cup just began, *Xvax2* and *Xvax1b* are similarly expressed in the ventral telencephalon, where *Xpax2* is not transcribed (Fig. 3A,E and data not shown). At the level of the diencephalon, *Xvax2*, *Xvax1b* and *Xpax2* display partially overlapping but distinct domains of expression in the eye and the brain. In particular, within the eye all three genes are expressed in the prospective optic stalk (Fig. 3B,C,F,G,I,J); *Xvax2* transcription also embraces the ventral half of the prospective retina (Fig. 3B–D), while *Xvax1b* and

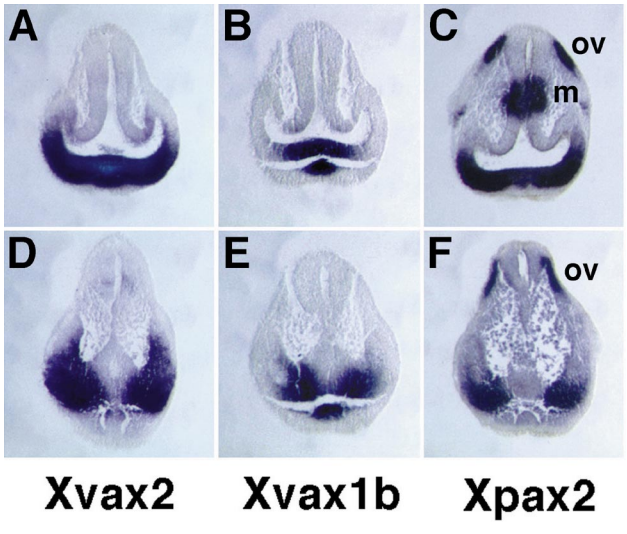

Fig. 2. Expression of *Xvax2* (A,D), *Xvax1b* (B,E) and *Xpax2* (C,F) as detected in transverse sections of stage 23 *Xenopus* embryos following whole-mount in situ hybridization, cut at the level of the anterior (A–C) or posterior (D–F) optic vesicle. m, mesencephalon; ov, otic vesicle.

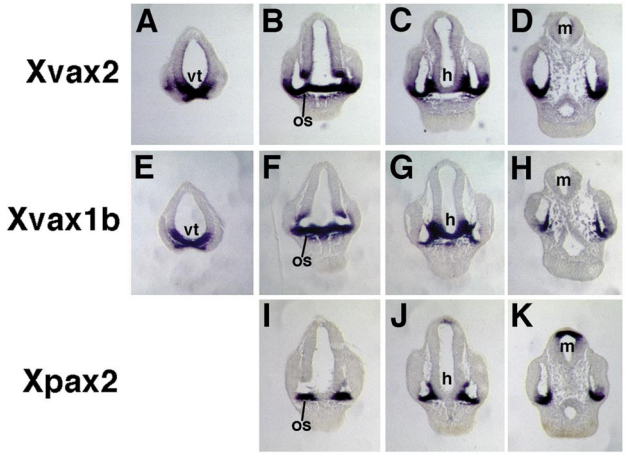

Fig. 3. Expression of *Xvax2* (A–D), *Xvax1b* (E–H), *Xpax2* (I–K) as detected in transversal sections of stage 30 *Xenopus* embryos following whole-mount in situ hybridization. Left to right sections show expression at progressively posterior levels. h, hypothalamus; m, mesencephalon; os, optic stalk; vt, ventral telencephalon.

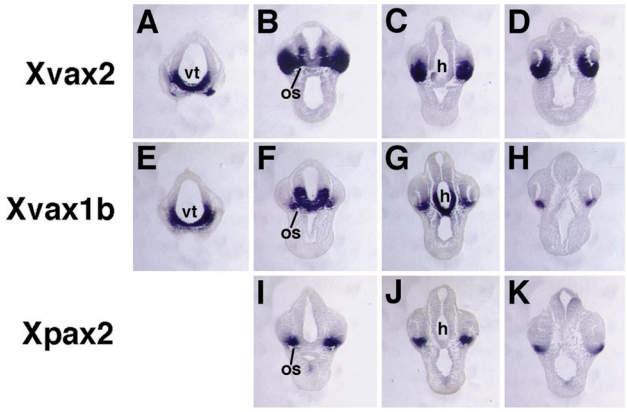

Fig. 4. Expression of *Xvax2* (A–D), *Xvax1b* (E–H), *Xpax2* (I–K) as detected in transversal sections of stage 33 *Xenopus* embryos following whole-mount in situ hybridization. Left to right sections show expression at progressively posterior levels. h, hypothalamus; os, optic stalk; vt, ventral telencephalon.

Xpax2 transcription is restricted to more ventro-proximal regions of the developing optic cup (Fig. 3H,K). In the diencephalon, both *Xvax2* and *Xvax1b* are expressed anteriorly in the prospective chiasmatic region (Fig. 3B,F), while *Xvax1b*, but not *Xvax2*, is also active more posteriorly in the prospective hypothalamus (Fig. 3C,G). *Xpax2* is not transcribed in the diencephalon at this stage (Fig. 3I,J). At early tadpole (st. 33), the optic cup has formed and is joint to the brain by the narrow optic stalk. Anteriorly, *Xvax2* and *Xvax1b* are transcribed in the ventral telencephalon (Fig. 4A,E). In the eye, *Xvax2*, *Xvax1b* and *Xpax2* are all strongly expressed in the optic stalk (Fig. 4B,F,I). Moreover, *Xvax2* is prominently expressed in the ventral half of the retina (Fig. 4B–D), while *Xvax1b* and *Xpax2* expression is confined to the optic disk (Fig. 4G,H,J,K). *Xvax1b* expression in the diencephalon clearly extends more posteriorly with respect to *Xvax2*, including the retrochiasmatic and the hypothalamic regions, with a sharp dorsal boundary possibly corresponding to the zona limitans intrathalamica (Fig. 4B,C,F,G). At late tadpole stages (st. 41/42), both the retina layers and the optic nerve have formed. In the forebrain, *Xvax2* and *Xvax1b* are still coexpressed in the ventral telencephalon (data not shown) and in the optic chiasm (Fig. 5A,D), while *Xvax1b* transcription is also detectable in the ventral hypothalamus and in the hypophysis (Fig. 5E,F). In the eye, *Xvax2* and *Xvax1b* are strongly coexpressed in the optic nerve (Fig. 5H,I); *Xvax2* is still highly transcribed in the ventral retina (Fig. 5B,C,G), while *Xvax1b* expression is only detectable in the optic disk (Fig. 5I). At this stage, in the forebrain region *Xpax2* is exclusively expressed in the optic nerve (data not shown).

We also analyzed *Xvax2* and *Xvax1b* expression during metamorphosis by whole-mount hybridization of dissected brains and retinas. Both at early (st. 48) and at mid-metamorphosis (st. 59/60), *Xvax2* and *Xvax1b* transcription is strongly maintained in the optic chiasm and, more weakly, in the ventral telencephalon; in addition, *Xvax1b* is expressed in the ventral hypothalamus and in the hypophysis (Fig. 6). A prominent *Xvax2* expression is retained in the ventral retina through these stages, with a stronger signal in the ventral ciliary marginal zone at st. 59/60 (Fig. 6G,H).

Altogether, these data suggest a dual role for *Xvax2* in neural development: an earlier function in the presumptive ventral telencephalon, and a later integrated function at the level of different visual structures, namely the ventral retina, the optic nerve and the optic chiasm.

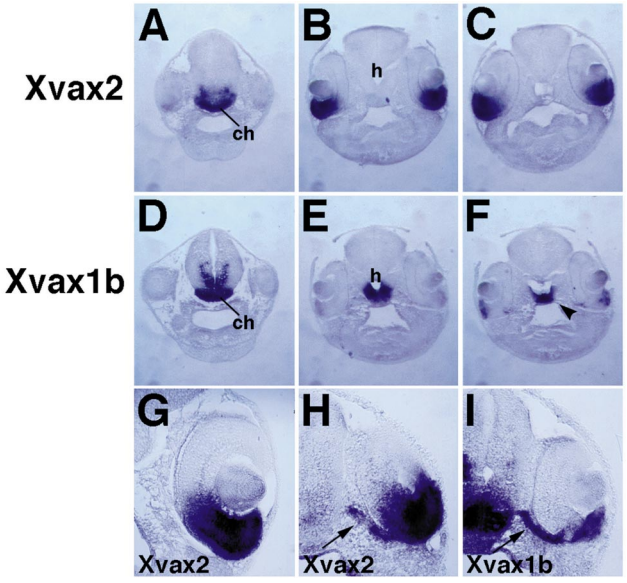

Fig. 5. Expression of *Xvax2* (A–C,G,H) and *Xvax1b* (D–F,I) as detected in transversal sections of stage 41/42 *Xenopus* embryos following whole-mount in situ hybridization. (A–F) Left to right sections show expression at progressively posterior levels; arrowhead points to the hypophysis. (G–I) Transversal sections under a higher magnification show the expression of *Xvax2* in the ventral part of the retina (G,H) and the coexpression of *Xvax2* (H) and *Xvax1b* (I) in the optic nerve (arrows). h, hypothalamus; ch, optic chiasm.

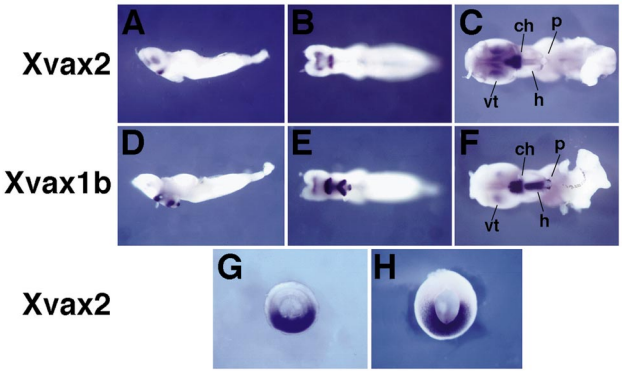

Fig. 6. Expression of *Xvax2* (A–C,G,H) and *Xvax1b* (D–F) as detected in dissected brains (A–F) and neural retinas (G,H) of stage 48 (A,B,D,E,G) and stage 59/60 (C,F,H) embryos by whole-mount in situ hybridization. (A,D) Lateral views; (B,C,E,F) Ventral views. Anterior is to the left for (A–F) and dorsal is up for (A,D,G,H). (A–F) From anterior to posterior, the staining locates at ventral telencephalon (vt), optic chiasm (ch), hypothalamus (h) and pituitary gland (p), respectively, as labeled in (C,F).

2. Methods

2.1. cDNA identification and sequence analysis

Xvax1b full-length cDNA was isolated by screening of a st. 28/30 head library with a murine *Vax2* probe. Plating, hybridization and washing conditions have been described (Franco et al., 1991). cDNA sequence analysis as well as nucleotide and protein database searches were performed as described (Banfi et al., 1996).

2.2. Xenopus laevis embryos

Induction of ovulation in females, in vitro fertilization, embryo culture and staging were carried out as described (Newport and Kirschner, 1982; Nieuwkoop and Faber, 1967). Frogs undertaking metamorphosis (st. 48–60) were anesthetized with MS222; brains and retinas were dissected out in PBS, fixed with 4% paraformaldehyde in PBS and stored in ethanol at −20°C before hybridization.

2.3. In situ hybridization

Whole-mount in situ hybridization on *Xenopus* embryos was performed as described (Harland, 1991). Double in situ hybridization experiments were done as in Andreazzoli et al. (1999). Histological examinations were performed as described in Pannese et al. (1998). Bleaching of pigmented embryos was done after color reaction as described (Mayor et al., 1995).

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References

Andreazzoli, M., Gestri, G., Angeloni, D., Menna, E., Barsacchi, G., 1999. Role of *Xrx1* in *Xenopus* eye and anterior brain development. Development 126, 2451–2460.

Banfi, S., Borsani, G., Rossi, E., Bernard, L., Guffanti, A., Rubboli, F., Marchitiello, A., Giglio, S., Coluccia, E., Zollo, M., Zuffardi, O., Ballabio, A., 1996. Identification and mapping of human cDNAs homologous to *Drosophila* mutant genes through EST database searching. Nat. Genet. 13, 167–174.

Barbieri, A.M., Lupo, G., Bulfone, A., Andreazzoli, M., Mariani, M., Fougerousse, F., Consalez, G.C., Borsani, G., Beckmann, J.S., Barsacchi, G., Ballabio, A., Banfi, S., 1999. A homeobox gene, *vax2*, controls the patterning of the eye dorsoventral axis. Proc. Natl. Acad. Sci. USA 96, 10729–10734.

Bourguignon, C., Li, J., Papalopulu, N., 1998. *XBF-1*, a winged helix transcription factor with dual activity, has a role in positioning neurogenesis in *Xenopus* competent ectoderm. Development 125, 4889–4900.

Casarosa, S., Andreazzoli, M., Simeone, A., Barsacchi, G., 1997. *Xrx1*, a novel *Xenopus* homeobox gene expressed during eye and pineal gland development. Mech. Dev. 61, 187–198.

Franco, B., Guioli, S., Pragliola, A., Incerti, B., Bardoni, B., Tonlorenzi, R., Carrozzo, R., Maestrini, E., Pieretti, M., Taillon-Miller, P., Brown, C.J., Willard, H.F., Lawrence, C., Persico, M.G., Camerimo, G., Ballabio, A., 1991. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 353, 529–536.

Hallonet, M., Hollemann, T., Wehr, R., Jenkins, N.A., Copeland, N.G., Pieler, T., Gruss, P., 1998. *Vax1* is a novel homeobox-containing gene expressed in the developing anterior ventral forebrain. Development 125, 2599–2610.

Harland, R.M., 1991. In situ hybridization: an improved whole-mount method for *Xenopus* embryos. Methods Cell Biol. 36, 685–695.

Heller, N., Brändli, A., 1997. *Xenopus Pax-2* displays multiple splice forms during embryogenesis and pronephric kidney development. Mech. Dev. 69, 83–104.

Mayor, R., Morgan, R., Sargent, M., 1995. Induction of the prospective neural crest of *Xenopus*. Development 121, 767–777.

Newport, J., Kirschner, M., 1982. A major developmental transition in early *Xenopus* embryos. Cell 30, 687–696.

Nieuwkoop, P.D., Faber, J., 1967. Normal Table of Development of *Xenopus laevis* (Daudin), Amsterdam, North Holland.

Ohsaki, K., Morimitsu, T., Ishida, Y., Kominami, Takahashi, N., 1999. Expression of the *Vax* family homeobox genes suggests multiple roles in eye development. Genes Cells 4, 267–276.

Pannese, M., Lupo, G., Kablar, B., Boncinelli, E., Barsacchi, G., Vignali, R., 1998. The *Xenopus Emx* genes identify presumptive dorsal telencephalon and are induced by head organizer signals. Mech. Dev. 73, 73–83.

Schulte, D., Furukawa, T., Peters, M.A., Kozak, C.A., Cepko, C.L., 1999. Misexpression of the *Emx*-related homeobox genes *cVax* and *mVax2* ventralizes the retina and perturbs the retinotectal map. Neuron 24, 541–553.