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EXPRESSION NOTE

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Expression of *Brachyury*-like T-box transcription factor, *Xbra3* in *Xenopus* embryo

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Abstract The *Xenopus Brachyury*-like *Xbra3* gene is a novel T-box gene that is closely associated with *Xenopus Brachyury*. The expression pattern of *Xbra3* during development is similar to that of *Xbra*. During gastrulation *Xbra3* is expressed in the marginal zone, with a gradient of increasing expression from ventral to dorsal. In the early neurula stage *Xbra3* is expressed in the notochord and posterior mesoderm, but by the tailbud stage its expression is restricted to the forming tailbud and the posterior portion of the notochord.

Key words T-box gene · *Brachyury* · Early mesoderm · Notochord tailbud

In early *Xenopus* development T-box transcription factor genes, including *Xbrachyury* (*Xbra*), *Eomesodermin* (*Eomes*), and *VegT*, function in early mesoderm or endoderm formation (Cunliffe and Smith 1992; Ryan et al. 1996; Zhang and King 1996). *Xbra*, the *Xenopus* homologue of mouse *Brachyury* (*T*), is expressed in presumptive mesodermal cells around the blastopore in the gastrula stage and is required for notochord and posterior development (Conlon et al. 1996; Smith et al. 1991). *Eomes* is expressed in mesodermal cells in a ventral-to-dorsal gradient of increasing concentrations and is required for mesoderm determination and differentiation (Ryan et al. 1996). *VegT* is localized to the vegetal hemisphere of the egg and expressed in mesoderm in the late gastrula stage. It induces ventral mesoderm, dorsal mesoderm, and endoderm in a concentration-dependent manner (Zhang and King 1996). Recently it has been shown that reduction in maternal *VegT* RNA changes animal cell fate from epidermis and nervous system to epidermis alone, equatorial cell fate from mesoderm to ectoderm, and vegetal cell fate from endoderm to mesoderm and ectoderm (Zhang et al. 1998). These T-box genes function downstream of the Vg1/activin-like signaling pathway. Activin is a member of the transforming growth factor-β superfamily and can induce a variety of mesoderm derivatives and endoderm in animal cap explants in a concentration-dependent fashion in vitro (Asashima et al. 1990; Henry et al. 1996). Therefore genes that act downstream of the Vg1/activin-like signaling pathway are thought to be important in mesoderm or endoderm.

We used a degenerate PCR strategy to identify novel T-box genes. By comparing the amino acid sequences of known T-box genes, such as *Xbra*, *VegT* and *Eomes*, we identified the consensus sequences "GRRMFP" and "VTAYQN" in the T-domain and constructed degenerate PCR primers. A fragment containing the T-box was PCR-amplified from the cDNA of animal cap cells treated with human recombinant activin A. We identified two novel T-box genes, *Xbra3* (accession number AB022680) and the *Xenopus* homologue of *Tbx2* (accession number AB 023815). *Tbx2* will be reported separately. A full-length cDNA clone was isolated from a gastrula stage cDNA library using the PCR fragment as a probe. DNA sequence analysis revealed that the *Xbra3* cDNA clone consisted of 1818 bp and contained an open reading frame of 434 amino acids that was closely related to *Xbra*. The overall amino acid sequence of *Xbra3* had 73% identity to *Xbra* and 90% identity within the T-domain. Recently the results of determination of the crystallographic structure of the T-domain-DNA complex suggested that *Xbra* dimerizes to bind palindromic

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The nucleotide sequence data reported in this paper have been submitted to the DDBJ, EMBL, and GenBank Data Libraries (accession no. AB 022680).

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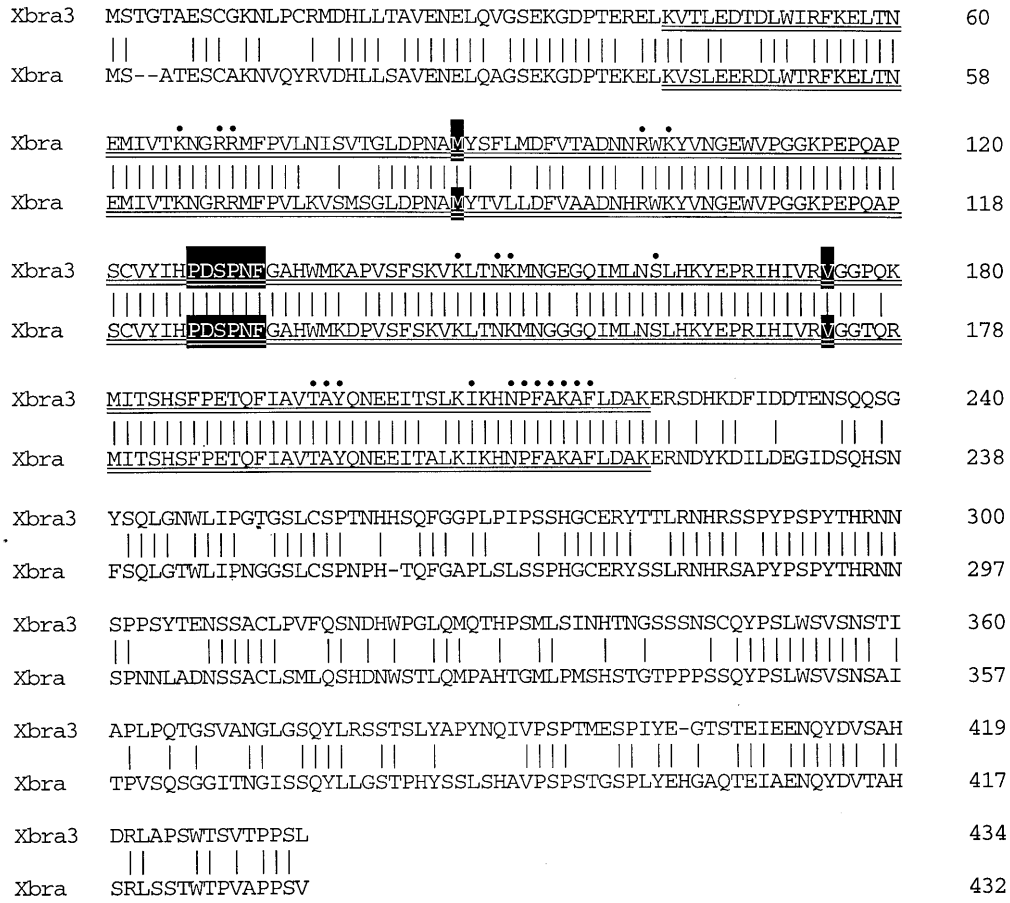

Fig. 1 Comparison of the amino acid sequences of *Xbra3* and *Xbra*. *Double-underlined* T-box domain; *vertical lines* identical amino acids; spaces (*dash*) have been added to maximize homology; *black circles* residues involved in DNA binding; *black background* residues involved in dimerization

DNA duplex (Müller and Herrmann 1997). All the amino acid residues involved in dimerization and DNA binding were conserved in the T-domain of *Xbra3* (Fig. 1). These findings suggest that *Xbra3* has similar DNA-binding specificity to *Xbra*, and also indicate that *Xbra3* may not only dimerize with itself but can also heterodimerize with *Xbra*. *Xbra3* possesses 59% identity with the carboxy-terminal portion of *Xbra*, which has transcriptional activity (Conlon et al. 1996), suggesting that *Xbra3* also has transcriptional activity. Because of the pseudotetraploidy of *Xenopus* (Graf and Kobel 1991) it is possible that *Xbra3* is only a pseudoallele of *Xbra*. However, the nucleotide sequences of the 5' and 3' untranslated regions of *Xbra3* have no significant similarity to those of *Xbra* (data not shown), and we suggest that *Xbra3* is a novel gene, not a pseudoallele of *Xbra* such as *Xbra*2 (Latinkic et al. 1997).

Whole-mount in situ hybridization was performed to determine the spatial expression profile of *Xbra3* (Fig. 2) and compare it with *Xbra*. In the gastrula stage *Xbra3* expression seemed to be lower than that of *Xbra*. Low levels of *Xbra3* expression were specifically detected in the marginal zone in the early gastrula stage (stage 10.5; Fig. 2A), and there was a concentration gradient of expression from the dorsal to the ventral side. *Xbra* was expressed widely throughout the marginal zone (Fig. 2D), but *Xbra3* was expressed in a more restricted fashion. In the mid-gastrula stage (stage 11), *Xbra3* was expressed in axial mesoderm the same as *Xbra* (arrowhead in Fig. 2B, E). In the early neurula stage (stage 14), *Xbra3* expression in the axial mesoderm (future notochord) was similar to that of *Xbra*, but *Xbra3* expression in axial mesoderm seemed more intense (Fig. 2C, F). *Xbra3* expression in the posterior mesoderm was observed in a more restricted fashion than *Xbra* expression. By the tailbud stage (stage 31), although most *Xbra* expression in the notochord had disappeared, *Xbra3* expression persisted in the most posterior part of the notochord (Fig. 2G, H). *Xbra* was expressed broadly in the forming tailbud, but *Xbra3* expression in this region was stronger and more posterior.

In conclusion, we describe the pattern of expression of a novel *Xenopus* T-box gene, *Xbra3*. However, further functional analysis is required to determine the developmental role of *Brachyury* genes in early *Xenopus* development. It would also be of interest to know whether *Brachyury*-like genes (except the Tbx family) are present in other vertebrates.

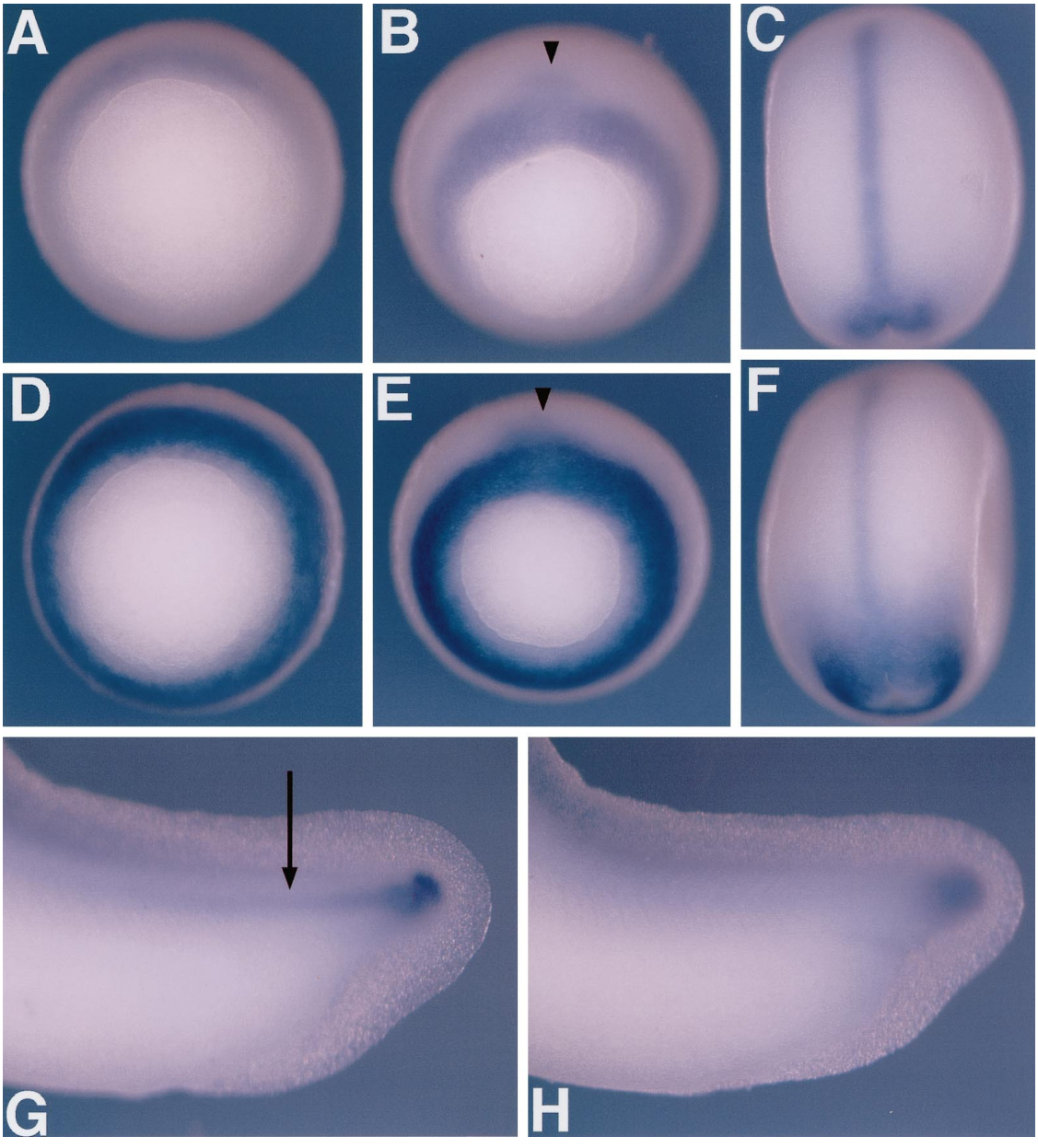

Fig. 2A–H Comparison of the spatial patterns of expression of *Xbra3* and *Xbra* by whole-mount in situ hybridization. **A**–**C**,**G** *Xbra3* expression. **D**–**F**,**H** *Xbra* expression. **A**,**D** Vegetal views of expression of *Xbra3* and *Xbra* at stage 10.5; *top* dorsal. **B**,**E** Vegetal views of expression of *Xbra3* and *Xbra* at stage11; *top* dorsal; *arrowheads* expression of axial mesoderm. **C**,**F** Dorsal views of neurula embryos (stage 14). **G**,**H** Enlarged view of the tailbud of tailbud stage embryos (stage 31); *arrow Xbra3* expression in the notochord. Whole mount in situ hybridization was performed as described previously (Harland 1991). Full-length *Xbra3* antisense probe was prepared by digesting p*Xbra3* with *Bgl*II and transcribing with T7 RNA polymerase. *Xbra3* sense probe was prepared by digesting p*Xbra3* with *Xho*I and transcribing with T3 RNA polymerase. Sense probe gave no background signals. Full-length antisense *Xbra* probe was prepared by digesting pXT1 (a gift from J. C. Smith) with *Eco*RV and transcribing with T7 RNA polymerase. We also used small probes containing only 3' UTR of both genes. The same results was obtained, except that the UTR probes gave fainter signals (data not shown)

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