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EXPRESSION NOTE

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Expression of *Xenopus* T-box transcription factor, *Tbx2* in *Xenopus* embryo

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Abstract We report here the cloning and expression of the *Xenopus* orthologue of the T-box transcription factor gene *Tbx2* (*optomotor-blind* in *Drosophila*). *Tbx2* is first detected in the ventral mesodermal cells just above the yolk plug at late gastrula. At the neurula stage it is strongly expressed in the cement gland, dorsal root ganglia, and otic vesicle region. At the tailbud stage strong *Tbx2* expression is observed in the dorsal part of the optic cup and trigeminal ganglia, and it is also expressed in the branchial arches, heart anlage, nasal pit, proctodeum, and the region around the pronephros.

Key words *Tbx2* · *Optomotor-blind* · *Xenopus* · Cement gland · Dorsal root ganglia

In *Drosophila* the *optomotor-blind* (*omb*) T-box gene is expressed in the brain region that develops into optic lobes and, less strongly, in the thoracic part of the ventral ganglion. Flies homozygous for *omb* mutations show defects in optic lobes, reduction in wing size, and increased abdominal pigmentation (Pflugfelder et al. 1992). *omb* homologues, known as *Tbx2* in vertebrates, have been isolated in human, mouse, and chick (Campbell et al. 1995; Chapman et al. 1996; Gibson-Brown et al. 1998a). In mouse development it is expressed in the otic and optic vesicles on day 9.5 and in the trigeminal ganglia, facial regions, retina, and limb bud mesenchyme on day 12.5. A similar pattern of expression is seen in the chick embryo, suggesting that the expression pattern of *Tbx2* is generally conserved during evolution. During chick limb specification it has been suggested that *Tbx2* may be a direct, short-range target of sonic hedgehog in the formation of the zone of polarizing activity (Gibson-Brown et al. 1998b). In *Drosophila* wing development *omb* expression is controlled by *decapentaplegic* and *wingless* and is required for distal wing development. Ectopic expression of *omb* also induces additional wings (Grimm and Pflugfelder 1996). Recently it was shown that *Tbx2* acts as a transcriptional repressor of the melanocyte-specific tyrosinase-related protein 1 promoter (Carreira et al. 1998).

During our screening for T-box genes involved in mesoderm or endoderm formation in *Xenopus* we have obtained a PCR fragment closely related to *Tbx2* and have isolated a full-length clone from a gastrula stage cDNA library. We thus named this clone *Xenopus Tbx2* (*XTbx2*). *XTbx2* has similarities in sequence and expression pattern with other vertebrate *Tbx2* genes and *Drosophila omb* gene.

The *XTbx2* cDNA contains a single open reading frame coding for 688 amino acids and includes a T-box. The deduced amino acid sequences and alignment with mouse *Tbx2* (*MTbx2*) are shown in Fig. 1. The overall amino acid identity of *XTbx2* with *MTbx2* is 69%, and *XTbx2* has an additional 10 amino acid sequence at the amino-terminus compared with *MTbx2*. Chick *Tbx2* (*CTbx2*) has also this N-terminal sequence (accession no. AF 033668). In *XTbx2*, however, since there is a consensus Kozak sequence (Kozak 1984) or *Nco*I restriction site at the second methionine (amino acid 11), translation of *XTbx2* may be initiated at the second methionine. There are three alanine-rich regions in *MTbx2*, residues 50–61, 571–577, and 585–593, but *XTbx2* has only the second alanine-rich region. *MTbx2* also has a glycine-rich region (507–517), but *XTbx2* lacks this. It is unclear, however, whether the absence of these regions affects the transcriptional character of *Tbx2*. Within the T-domain *XTbx2* has strong similarities with *Tbx2* homo-

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The nucleotide sequence data reported in this paper have been deposited in the DDBJ, EMBL, and GenBank data bases (accession no. AB 023815).

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XTbx2  MRDPAFPGAAMAYHPFHAPRAADFPMSAFLAAAQPSFFPALTLPPAALGKPLSDASLAGA   60
                 |||||||||| |||||||||||||||||||| ||| |||||| |  ||||
MTbx2  ----------MAYHPFHAPRPADFPMSAFLAAAQPSFFPALALPPGALGKPLPDPGLAGA   50

XTbx2  ----------AEAGLHLSALGHHHQAAHLRSLKSLEPEEEVEDDPKVNLEAKELWDQFHK  110
                 |||||| |||| |  ||||||||||||| |||||||| ||||||||||||
MTbx2  AAAAAAAAAAAEAGLHVSALGPHPPAAHLRSLKSLEPEDEVEDDPKVTLEAKELWDQFHK  110

XTbx2  IGTEMVITKSGRRMFPPFKVRVSGLDKKAKYILLMDIVAADDCRYKFHNSRWMVAGKADP  170
        |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
MTbx2  LGTEMVITKSGRRMFPPFKVRVSGLDKKAKYILLMDIVAADDCRYKFHNSRWMVAGKADP  170

XTbx2  EMPKRMYIHPDSPATGEQWMAKPVAFHKLKLTNNISDKHGFTILNSMHKYHARFHIVRAN  230
       ||||||||||||||||||||||||||||||||||||||||||||||||||  ||||||||
MTbx2  EMPKRMYIHPDSPATGEQWMAKPVAFHKLKLTNNISDKHGFTILNSMHKYQPRFHIVRAN  230

XTbx2  DILKLPYSTFRTYVFPETDFIAVTAYQNDKITQLKIDNNPFAKGFRDTGNGRREKRKQLS  290
       |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
MTbx2  DILKLPYSTFRTYVFPETDFIAVTAYQNDKITQLKIDNNPFAKGFRDTGNGRREKRKQLT  290

XTbx2  LPSLRMYEEQCKADRDGAESDASSCDPAPGRDSLHSPLSAEPSPLRLHRNNREEKFG-AD  349
       || || ||| ||  ||||||||||||| | |    || |  ||||||||   ||| | ||
MTbx2  LPTLRLYEEHCKPERDGAESDASSCDPPPAREPPPSP-SAAPSPLRLHRARAEEKPGAAD  349

XTbx2  SDQELDRREIRSARSHSPVGHRSPPSSPRLEDRGKDKSTPEKKSDSPESRKDGSDSLFSS  409
       || |  |                  |  ||               | |    | |  | |
MTbx2  SDPEPERTGEERSAAPLCRSPSRDASPARLTEPERSRERRSPERCSKEPTEGGGDGPF-S  408

XTbx2  IRSLEKDKAESRRKEDSKSDPECGSLSKETFAPLMVQTDSPPHLSASHLQSLALSG-LHG  468
        |||||   | |||     |   |       ||| |||||   | | ||  || |  |||
MTbx2  LRSLEKERPEARRKDEGRKDV--GEGKEPSLAPLVVQTDSASPLGAGHLPGLAFSSHLHG  466

XTbx2  QQFFNPLNAGQPLFIHPGQFTMGPGAFSAMGMGHLLASMTG------------AGALDNG  516
       |||| || | |||| ||||| |||||||||||||||||  |            |  || |
MTbx2  QQFFGPLGACQPLFLHPGQFAMGPGAFSAMGMGHLLASVAGGSGSSGGAGPGTAAGLDAG  526

XTbx2  SLSSVQGATGAANPFPFHFSQHMLASQGIPMPAFGGLFPYPYTYMAAAAAAASAMPATSA  576
        |     |   | ||||| ||||||||||||| ||||||||||||||||||||| |||||
MTbx2  GLGPAASAASTAAPFPFHLSQHMLASQGIPMPTFGGLFPYPYTYMAAAAAAASALPATSA  586

XTbx2  AT-------TMPRNPFLSSTRPRLRFNPYQIPVGIPPSTNLLTTGLPGSLNPGSESSKPG  629
       |           | ||| | |||||| |||||| ||||| ||||||        | || |
MTbx2  AAAAAAAAGSLSRSPFLGSARPRLRFSPYQIPVTIPPSTSLLTTGL------AAEGSKGG  640

XTbx2  SSRESSPIPDTPVPKRSHSN--SLSPKASMKDSINELQNIQRLVSGLESQREISPGRETP  687
        ||| || |     |        |||  | |    ||| ||||||||||||  ||||| |
MTbx2  NSREPSPLPELALRKVGGPSRGALSPSGSAKEAASELQRIQRLVSGLESQRALSPGRESP  700

XTbx2  K                                                             688
       |
MTbx2  K                                                             701
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Fig. 1 Comparison of amino acid sequences between *Xenopus Tbx2* and mouse *Tbx2*. *Vertical lines* identical residues; *double-underlined*, T-domain; *highlighted*, three alanine-rich regions and a glycine-rich region

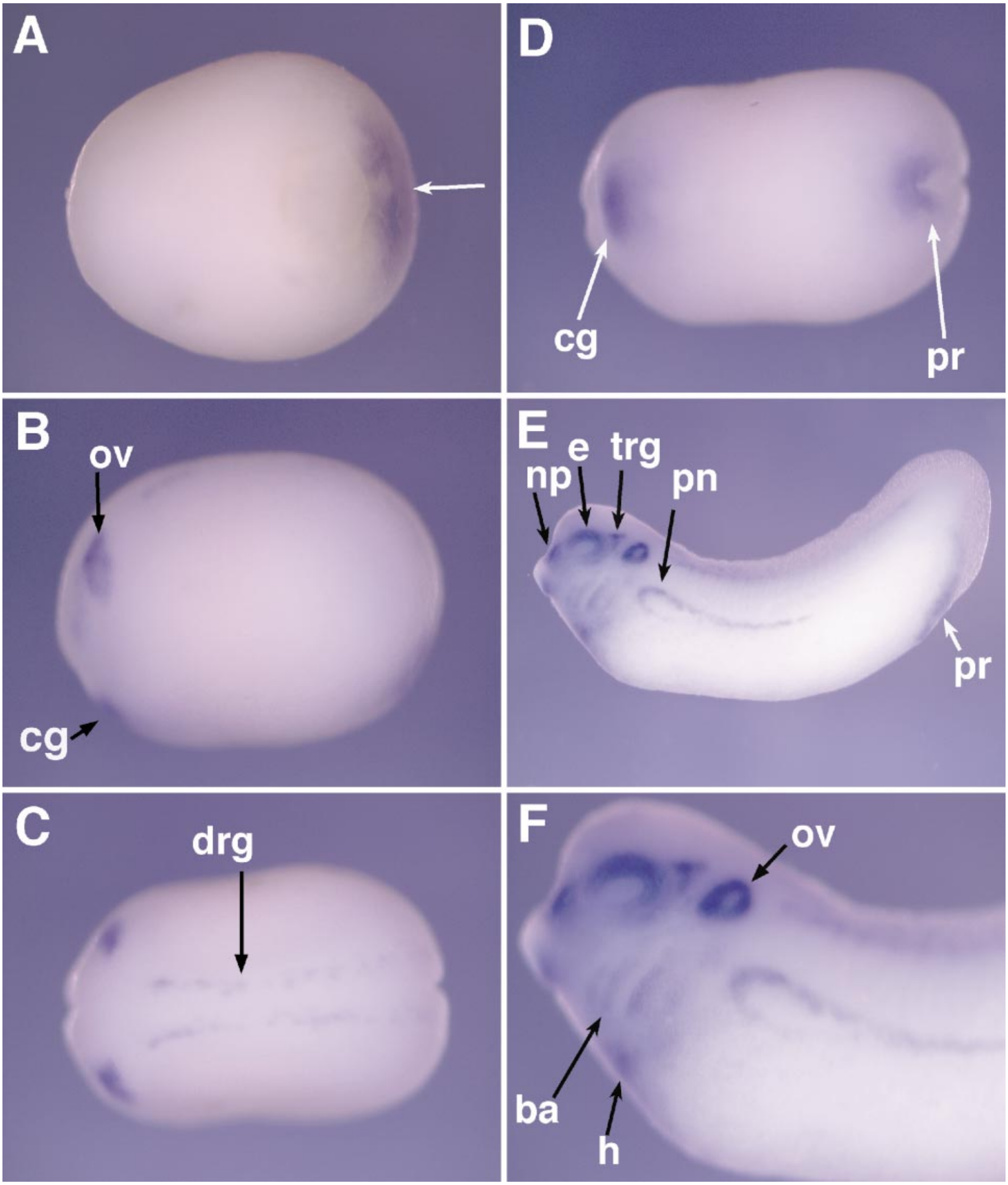

Fig. 2A–F Expression pattern of *Xenopus Tbx2* by whole-mount in situ hybridization. **A** Posteriodorsal view of late gastrula stage embryo (stage 12). Dorsal is *left*. *Arrow* staining of ventral mesodermal cells. **B** Lateral view of neurula stage embryo (stage 19). **C** Dorsal view of the same embryo as in **B**. **D** Ventral view of the same embryo as in **B**. **E** Lateral view of early tailbud stage embryo (stage 31). **F** Enlarged view of the same embryo as in **E**. Embryos that hybridized a sense probe show no background signals. Full-length *XTbx2* antisense probe was prepared by digesting p*XTbx2* with *Bgl*II and transcribing with T7 RNA polymerase. *XTbx2* sense probe was prepared by digesting p*XTbx2* with *Xho*I and transcribing with T3 RNA polymerase. *ba* Branchial arches; *cg* cement gland; *drg* dorsal root ganglia; *e* eye; *h* heart; *np* nasal pit; *pn* pronephros; *pr* proctodeum; *ov* otic vesicle; *trg* trigeminal ganglia logues, showing 98% identity with mouse and 86% identity with *Drosophila* (data not shown). These high similarities suggest that the DNA-binding specificity of *Tbx2* is conserved during evolution.

To determine the spatial expression pattern of *XTbx2* whole-mount in situ hybridization was performed (Fig. 2A–F). As shown in Fig. 2A, low levels of *XTbx2* expression were first detected in ventral mesodermal cells just above the yolk plug at the late gastrula stage (stage 12), which is different from *MTbx2*, which is not expressed in mouse embryos during gastrulation (Chapman et al. 1996). The expression in this domain was continued in future proctodeum throughout this developmental stage (Fig. 2D, E). From the neurula stage *XTbx2* expression was detected in the cement gland, otic vesicles, and dorsal root ganglia (Fig. 2B, C). By the tailbud stage the ventral part of cement gland is positive (Fig. 2E, F). From the tailbud stage (stage 31) strong *XTbx2* expression was observed in the dorsal part of optic cup and trigeminal ganglia, and there was weak expression in nasal pit, branchial arches, heart anlage, and the region around the pronephros (Fig. 2E, F).

Expression of *Tbx2* in *Xenopus* was generally similar to that in other vertebrates and *Drosophila*. However, *XTbx2* expression in the cement gland is unique to *Xenopus* because there is no homologous organ in other vertebrates. *Tbx2* expression in the proctodeum is also unique to *Xenopus*. *Tbx2* expression in the optic cup is homologous to that of *omb* in the optic lobes and of chick and mouse *Tbx2*. This region of expression is conserved between *Drosophila* and vertebrates, suggesting an important role for *Tbx2* in eye formation. It is interesting to note that *XTbx2* is expressed at the most anterior region (cement gland) and in the posterior region (proctodeum) in *Xenopus* embryo. *omb* is involved in *Drosophila* wing development, and chick *Tbx2* is involved in limb bud specification, but its function in other organs is not yet known. Further analysis should focus on its role in organogenesis, especially that of the nervous system and kidney.

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