Expression Cloning of Xenopus Os4, an Evolutionarily Conserved Gene, which Induces Mesoderm and Dorsal Axis

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Multiple factors, including members of the FGF, TGFβ, and Wnt family of proteins, are important mediators in the regulation of dorsal–ventral pattern formation during vertebrate development. By using an expression cloning approach to identify novel factors that could regulate dorsal–ventral patterning in the Xenopus embryo, we isolated the Xenopus homologue of the human Os4 gene by virtue of its ability to induce a secondary dorsal axis. While Os4 homologues have been identified in a variety of species, and human Os4 is overexpressed in human tumors, the biological function of Os4 is unknown. To explore the mechanism by which Xenopus Os4 (XOs4) induces a secondary dorsal axis, we used Xenopus explant and whole-embryo assays. The secondary axis induced by XOs4 is distinct from that induced by activation of Wnt or FGF pathways but similar to that induced by inhibition of BMP signaling or activation of an Activin pathway. However, XOs4 did not inhibit BMP signaling in dissociated animal cap explants, indicating that XOs4 does not inhibit BMP signaling. Similar to activation of an Activin-like pathway, expression of XOs4 induces molecular markers for mesoderm in animal cap explants, although expression of gastrula-stage mesodermal markers was very weak and substantially delayed. Yet, XOs4 does not require activity of the Activin signal-transduction pathway for mesoderm induction as dominant-negative components of the Activin/Nodal/Vg1 pathway did not prevent XOs4-mediated induction of mesodermal derivatives. Finally, like Activin/Nodal/Vg1 pathways, XOs4 requires FGF signaling for expression of mesoderm markers. Results presented in this study demonstrate that XOs4 can induce mesoderm and dorsalize ventral mesoderm resulting in ectopic dorsal axis formation, suggesting a role for this large evolutionarily conserved gene family in early development. © 2001 Academic Press

Key Words: **Xenopus; Os4; mesoderm.**

INTRODUCTION

The elucidation of the molecular mechanisms regulating establishment of the vertebrate body axis has been a subject of extensive research. By using the *Xenopus laevis* developmental model system, many groups have focused on the events that lead to induction and patterning of the early embryonic axis. In particular, the mechanism of induction and patterning of the dorsal mesodermal derivatives has received significant attention. Mesoderm induction occurs through interactions between the vegetal and animal regions of the embryo (reviewed in Harland and Gerhart, 1997). Establishment of the dorsal–ventral axis is initiated shortly after fertilization, when cortical rotation results in

the localization of dorsal determinants to the dorsal–vegetal region of the embryo (reviewed in Harland and Gerhart, 1997). These dorsal determinants are components of the Wnt signal transduction pathway and are involved in induction of a Nieuwkoop center. By late blastula stages, interaction of the Nieuwkoop center with cells in the dorsal marginal zone results in the establishment of the Spemann's Organizer. The Organizer patterns the embryo along the dorsal–ventral axis, dorsalizing ventral mesoderm and ectoderm. Furthermore, the organizer can induce a secondary dorsal axis when transplanted to the ventral region of the embryo (reviewed in Elinson and Holowacz, 1995). The molecular signals that mediate the dorsalizing behavior of the organizer have recently begun to be elucidated. Many of the secreted factors originating from the organizer (e.g., Chordin, Noggin, Follistatin, Cerberus, and Xnr3) act to inhibit BMP signaling in the mesoderm and

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ectoderm resulting in dorsalization of mesoderm and neural tissue, respectively (reviewed in Harland, 2000; Kimelman and Griffin, 2000; Weinstein and Hemmati-Brivanlou, 1999). Furthermore, Nodal-related TGFβ family members are expressed in the organizer and have the ability to dorsalize ventral mesoderm (Jones *et al.,* 1995; Joseph and Melton, 1997; Smith *et al.,* 1995).

TGFβ family members activate a signal transduction cascade resulting in changes in gene transcription (reviewed in Massague, 1998). Extracellular ligands bind to Type II receptors that phosphorylate and activate the Type I receptors. Receptor Smads (e.g., Smad2 and -3 in the case of Activin/Nodal/Vg1 and Smad1, -5, and -9 in the case of BMP) are then recruited and phosphorylated by the activated Type I receptor. Upon phosphorylation, the receptor Smad associates with a common Smad (Smad4), and the complex is translocated to the nucleus where it associates with transcription factors (e.g., FAST1 for Activin/Nodal/Vg1 and OAZ for BMP) to regulate transcription. In addition, the inhibitory Smads, Smad6 and Smad7, block activation of TGFβ pathways by blocking receptor-induced phosphorylation of receptor Smads (Ishisaki *et al.,* 1999; Souchelnytskyi *et al.,* 1998), whereas Smad6 also blocks Smad4 from interacting with Smad1 (Hata *et al.,* 1998).

Experiments where naive ectodermal explants are exposed to various extracellular ligands have demonstrated that members of the TGFβ family of ligands such as Activin, Nodal-related family members, and Vg1 (Activin/Nodal/Vg1) can mimic the mesodermal-inducing activities of the vegetal cells (reviewed in Whitman, 1998). These types of experiments have also demonstrated a role for FGF family members in the maintenance of mesodermal cell fates (Isaacs *et al.,* 1994; Schulte-Merker and Smith, 1995). Furthermore, FGF ligands are more likely involved in specifying ventral–posterior mesoderm rather than dorsal cell fates as observed with Activin/Nodal/Vg1 ligands (reviewed in Isaacs, 1997). TGFβ family of ligands act as morphogens, specifying multiple tissue types as a response to different concentrations of ligand. For example, incubation of ectodermal explants with Activin results in induction of lateral mesoderm at low doses and more dorsal mesoderm at higher doses (Gurdon *et al.,* 1994, 1996; and see McDowell and Gurdon, 1999 for review). TGFβ members in the BMP subfamily, on the other hand, have ventralizing effects in the embryo (reviewed in Dale and Jones, 1999; Dale and Wardle, 1999). BMPs will ventralize the ectoderm to adopt an epidermal rather than neural fate, and the mesoderm to adopt a ventral rather than dorsal mesodermal fate (reviewed in Harland, 1994; Weinstein and Hemmati-Brivanlou, 1997; Wilson and Hemmati-Brivanlou, 1997). Finally, dorsalization of the ventral mesoderm by either activation of Activin/Nodal/Vg1 or inhibition of BMP signaling results in dorsal mesoderm formation and induction of a secondary dorsal axis containing trunk but no head structures (reviewed in Lemaire and Kodjabachian, 1996).

In an attempt to isolate novel factors that can regulate embryonic patterning along the dorsal–ventral axis, we used an expression cloning approach. Novel molecules were isolated from a gastrula stage expression library that could induce a secondary dorsal axis when expressed in ventral mesoderm. From this screen, the Xenopus homologue of the human Os4 gene (XOs4) was isolated. The human Os4 gene was originally isolated from a region of chromosome 12q13–q15, which is frequently amplified in human tumors (Su *et al.,* 1997). Additionally, Os4 homologues have been identified in numerous species as a result of genome-sequencing projects. However, the function of this large gene family remains unknown. Our results suggest that, like Activin/Nodal/Vg1, XOs4 induces mesoderm in animal cap explants and induces dorsal axis that lack anterior structures. However, dominant-negative components in the Activin signal-transduction pathway did not prevent induction of mesoderm in animal cap explants, suggesting that XOs4 may activate pathways resulting in mesoderm induction either downstream from the Activin/Nodal/Vg1 pathways or independent of these pathways.

MATERIALS AND METHODS

Embryo Manipulations

Xenopus embryos were obtained by *in vitro* fertilization and staged according to Nieuwkoop and Faber (1967). Microinjections and dissections were performed as described (Brivanlou and Harland, 1989). Briefly, for animal cap assays, Xenopus embryos were injected at the two-cell stage with the indicated amounts of capped RNA. Animal cap explants were isolated at the blastula stage and cultured in 0.5× MMR until the indicated stage. Cell dissociation assays were performed as described (Wilson and Melton, 1994). Briefly, animal caps were incubated in Ca^{2+}/Mg^{2+}-free medium, reaggregated after 4 h of dispersal and cultured until control sibling embryos reached late neurula stages.

Construction of Plasmids

Construction of the Xenopus gastrula stage cDNA library was described previously (Weinstein *et al.,* 1998). pCs2-H4 was the full-length cDNA isolated from the screen. pCs2-XOs4 (ORF), pCs2-FLAG-XOs4 was generated by PCR using the following primers and ligated into the *Eco*RI/*Sal*I site of pCs2. U(ORF): 5′-CGC GGA ATT CCG CCA TGG AAA GCA GCT CTA TAA TCG CC-3′; U(FLAG): 5′-CGC GGA ATT CCG CCA TGG ACT ACA AGG ACG ACG ATG ACA ACC TTG AAA GCA GCT CTA TAA TCG CC-3′; D: 5′-CGC GAT GTC GAC TCA TCA AGG GGC CTT CAG CTG CCC-3′). pCs2-XOs4 (1–920) and pBS-XOs4 (920–2335) were generated by subcloning the *Sal*I/*Xba*I fragment and the *Xba*I fragment from pCs2-H4 into pCs2 (Baker and Harland, 1996; Turner and Weintraub, 1994) and pBS (Invitrogen), respectively. The Xwnt-8, tAR, Smad2, Smad7, FLAG-Ldb1, pCMV-FLAG-DPC4, pCMV-ActRIB(T-D), XFR, Ras(17N), and v-HA-Ras constructs have been described previously (Agulnick *et al.,* 1996; Amaya *et al.,* 1991; Casellas and Brivanlou, 1998; Hemmati-Brivanlou and Melton, 1992; Lagna *et al.,* 1996, 1999; Whitman and Melton, 1992). A3 and Gsc-Luc plasmids have been described elsewhere (Huang *et al.,* 1995; Watabe *et al.,* 1995). Recombinant human BMP-2 was obtained from R&D Systems and bFGF from Boehringer Mannheim. Activinβb proteins were generated in oocytes as described (Chang *et al.,* 1997).

A
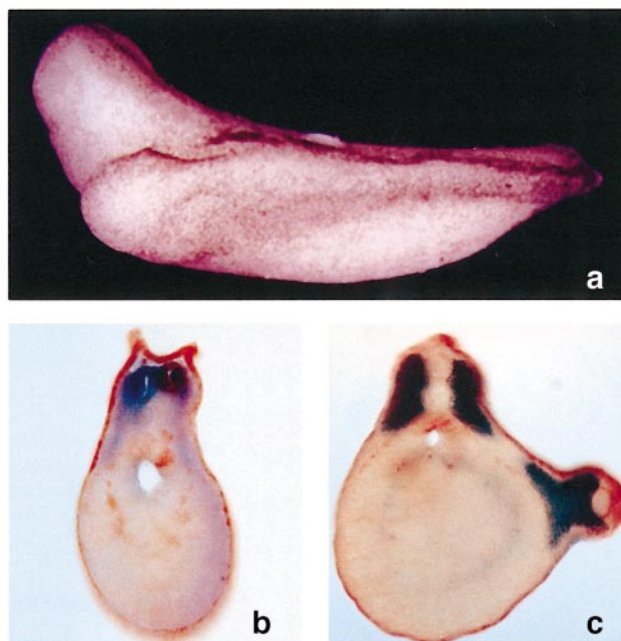
B

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1     ATG GAA AGC AGC TCT ATA ATC GCC CAA GTG CAC AGA GAA GAG GCG CTT GTG CAT TCA AAG CCA GGA CTG GTG TCC AAG
1      M   E   S   S   S   I   I   A   Q   V   H   R   E   E   A   L   V   H   S   K   P   G   L   V   S   K

79    TCC TCT CCA AAG AAG CCT CGA AGT CGG AGC ATA TTC AAG GCG CTC TTC TGT TGC CTC AGT GCA CAG AAT GTC AGC CGG
27     S   S   P   K   K   P   R   S   R   S   I   F   K   A   L   F   C   C   L   S   A   Q   N   V   S   R

157   CCA GGG GGC TCC AGT GAG TCA CCA ATA CAG AAG GAG GAG ACG AAT GCA ACC CCA AAG TCG GAT TTA CTC CAG TGT CTT
53     P   G   G   S   S   E   S   P   I   Q   K   E   E   T   N   A   T   P   K   S   D   L   L   Q   C   L

235   CAG TAT CAG TTT TAC CAG ATC CCA GGT ACC TCT CTG CTT CCT GAG GTA GCA CCA AAA GAC AAA GGA AAA ATC TGC ATG
79     Q   Y   Q   F   Y   Q   I   P   G   T   S   L   L   P   E   V   A   P   K   D   K   G   K   I   C   M

313   GTG ATT GAT TTG GAT GAA ACC CTG GTT CAT AGC TCA TTT AAG CCC ATC AGT AAT GCA GAT TTT ATA GTT CCA GTG GAG
105    V   I   D   L   D   E   T   L   V   H   S   S   F   K   P   I   S   N   A   D   F   I   V   P   V   E

391   ATA GAA GGA ACT ACA CAC CAG GTG TAT GTT CTA AAG AGG CCT TAT GTG GAT GAG TTC CTG GAG AGA ATG GGC CAA CTT
131    I   E   G   T   T   H   Q   V   Y   V   L   K   R   P   Y   V   D   E   F   L   E   R   M   G   Q   L

469   TAT GAA TGT GTA CTG TTC ACA GCC AGT CTG GCC AAG TAT GCC GAC CCA GTG ACA GAC CTT CTA GAC AAG TCA GGA GTC
157    Y   E   C   V   L   F   T   A   S   L   A   K   Y   A   D   P   V   T   D   L   L   D   K   S   G   V

547   TTC CGC TCA CGA CTT TTT AGG GAA GCC TGC GTG TTT CAC CAG GGC TGC TAT GTA AAG GAC CTT AGT CGT CTG GGT AGA
183    F   R   S   R   L   F   R   E   A   C   V   F   H   Q   G   C   Y   V   K   D   L   S   R   L   G   R

625   GAT CTG AAG AAA ACT GTT ATT TTG GAC AAC TCC CCT GCA TCC TAC ATC TTC CAT CCA GAA AAT GCA GTT CCG GTG CAG
209    D   L   K   K   T   V   I   L   D   N   S   P   A   S   Y   I   F   H   P   E   N   A   V   P   V   Q

703   TCC TGG TTT GAT GAC ATG TCT GAC ACG GAG CTC CTG AGC CTA ATC CCA ATC TTC GAG GAG TTC AGT TAC TCA GAG GAC
235    S   W   F   D   D   M   S   D   T   E   L   L   S   L   I   P   I   F   E   E   F   S   Y   S   E   D

781   ATT TAT ACA AGT CTC GGG CAG CTG AAG GCC CCT TGA
261    I   Y   T   S   L   G   Q   L   K   A   P   .
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FIG. 1. (A) Characterization of the secondary axis induced by ventral expression of XOs4. (a) XOs4 induces a secondary dorsal axis when injected into the ventral–marginal zone of four- to eight-cell stage embryos. (b) Transverse section of embryo probed with a neural-specific antibody (6F-11). (c) Transverse section of an embryo probed with somite-specific (12/101) antibodies. (B) Nucleotide and predicted amino acid sequence of XOs4. Conserved domain is underlined in red. Conserved phosphatase motif is boxed in blue.
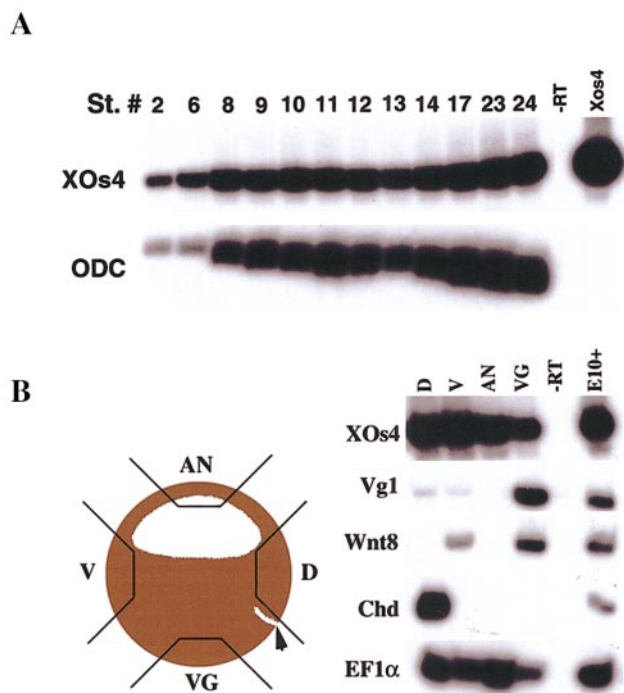
FIG. 2. (A) Expression of XOs4 gene during early Xenopus embryogenesis. Expression of XOs4 was detected by RT-PCR. ODC is a ubiquitously expressed gene and represents a loading control. XOs4 is expressed maternally and expression continues through tadpole stage. The cDNA clone encoding XOs4 was used in the last lane as a positive control. (B) XOs4 is ubiquitously expressed at gastrula stage. Stage-10+ embryos were dissected as diagrammed into dorsal marginal zone (D), ventral–marginal zone (V), animal pole (AN), and vegetal pole (VG) explants and subjected to RT-PCR analysis using primers that amplify XOs4 and various markers to control for proper dissection of explants.

Whole-Mount in Situ Hybridization, Antibody Staining, and Lineage Tracing

Xenopus embryos were fixed at the indicated stages in MEMFA and whole-mount *in situ* hybridization was performed as described (Harland, 1991) by using BM purple (Boehringer Mannheim) as the substrate for the chromogenic reaction. XOs4 antisense probe was synthesized following linearization of the following constructs with the indicated restriction enzymes and transcribed by using the indicated promoters: pCs2-H4 (Sal1/T3), pCs2-XOs4 (1–920) (Sal1/T3), pBS-XOs4 (920–2335) (*Eco*RI/T7), and pCs2-XOs4(ORF) (*Eco*RI/T3). All four *in situ* probes revealed identical expression patterns for XOs4. β-Galactosidase (βGal) staining was carried out as described (Smith and Harland, 1991). Whole-mount antibody staining was performed as described by Brivanlou and Harland (1989). The antibodies used were: 12/101 (1:500) for muscle (Kintner and Brockes, 1984); Tor70.1 (1:500) for notochord (Bolce *et al.,* 1992) anti-FLAG antibody (Sigma) and the neural antigen-specific antibody 6F11 (1:1) from hybridoma-conditioned medium (NIH). A goat anti-mouse horseradish peroxidase (1:500; Jackson Laboratories) was used as a secondary antibody. After staining, embryos

were refixed in MEMFA and embedded in 20% gelatin in PBS. Embedded embryos were refixed in 4% paraformaldehyde in PBS and sectioned (50- to 100-μm sections) by using a vibratome. Sections were cleared in 90% glycerol before being photographed.

RT-PCR Assay

The RT-PCR analysis of gene expression was performed as previously described (Chang *et al.,* 1997). The primers used were: XOs4 (U: 5'-GAT GGA AAG CAG CTC TAT AAT CG-3'; D: 5'-GGC TGA CAT TCT GTG CAT TGA GGC-3'); ODC (Bassez *et al.,* 1990); Vg1 (Weinstein *et al.,* 1996); Wnt8 (Smith and Harland, 1991); XBra (Smith *et al.,* 1991); Xnr3 (Smith *et al.,* 1995); Sia (Lemaire *et al.,* 1995); NCAM (Kintner and Melton, 1987); EK (Jonas *et al.,* 1985); XAG (Blitz and Cho, 1995); Col.II (Su *et al.,* 1991); CA (Stutz and Spohr, 1986); HoxB9/XIHBox-6 (Wright *et al.,* 1990); EF1α (Krieg *et al.,* 1989); Twist (Chang and Hemmati-Brivanlou, 1998); Chd (Sasai *et al.,* 1994), Lim1 (U: 5'-CCG CCC ATT CCT CTA AAA TCC C-3'; D: 5'-GGA GAT AAA CTG GGG TCA CTG CC-3', Xeomes (U: 5'-TGC GGC AAA GCG GAC AAC AAT A-3'; D: 5'-TCT CCG GGA AGG TAA AGG TCT GG-3'.

Cellular Localization in Cos1 Cells

Cos1 cells were transfected with the pCs2-FLAG-XOs4 or pCMV-FLAG-DPC4 plus activated Activin receptor (pCMV-ActRIB(T-D) by using lipofectamine according to the manufacturer's instructions. Thirty-six hours following transfection, cells were trypinsized and plated onto sterile coverslips. Forty-eight hours after transfection, cells were fixed in 4% paraformaldehyde and incubated overnight in blocking solution (PBS with 1% Triton X-100 and 3% BSA). Coverslips were incubated in blocking solution supplemented with anti-FLAG antibody (Sigma) for 1 h at room temperature (1:1000 dilution), followed by incubation in FITC-conjugated anti-mouse antibody (Jackson Laboratories) and treated with Vectashield (Vector Laboratories).

Luciferase Assays

Embryos were injected at the eight-cell stage in one animal blastomere with 1 ng of RNA encoding Smad2 or XOs4 and 100 pg of either A3-Luc or Gsc-Luc plasmids (Huang *et al.,* 1995; Watabe *et al.,* 1995). At stage 11.5, embryos were lysed and luciferase activity was measured by using Dual-Luciferase Reporter Assay system (Promega).

RESULTS

Cloning and Characterization of XOs4

To identify novel molecules involved in patterning of the vertebrate body axis, we used an expression cloning approach to identify factors that could induce a secondary dorsal axis when expressed in the ventral marginal zone of Xenopus embryos. For this screen, pools of 100 RNAs synthesized from a gastrula-stage cDNA library were injected in the ventral–marginal zone of eight-cell stage Xenopus embryos. Embryos were allowed to develop until tadpole stage, when secondary axis induction was examined. Individual clones that possessed secondary axis-

inducing activity were isolated by sib selection (Smith and Harland, 1991, 1992). We identified a single clone that, when injected at 0.5– 4 ng of RNA per embryo, induced secondary axis formation in 62% of embryos ($n = 130$; Fig. 1A-a). The secondary axis was incomplete and lacked head structures (Fig. 1A-a). Histological analysis showed that the secondary axis contained dorsal mesoderm derivatives including muscle (Fig. 1A-c), as well as neural tissue (Fig. 1A-b), and sometimes notochord (data not shown).

Sequence analysis revealed that the 3.5-kb insert contained an 816-bp open reading frame (ORF) encoding a putative 271-amino acid protein (Fig. 1B) with a predicted molecular weight of 30 kDa, which was confirmed by SDS–PAGE analysis (data not shown). Expression in the ventral marginal zone of 100 pg RNA synthesized from the predicted ORF resulted in induction of secondary dorsal axis, and this XOs4 (ORF) construct was used for further analysis. Sequence comparison with the databases revealed that the ORF corresponds to the Xenopus homologue of the human Os4 gene (Su *et al.,* 1997). Although no embryological activity has been assigned to the Os4 gene family, human Os4 (hOs4) was originally identified in a region of chromosome 12q13– q15, which is frequently amplified in human sarcomas and brain tumors (Su *et al.,* 1997). Another closely related family member, HYA22, is localized to human chromosome 3p22-21.3, which is frequently deleted in carcinomas of the lung, kidney, and other human tumors (Ishikawa *et al.,* 1997). Furthermore, genome-sequencing projects have identified Os4-related proteins in eukaryote species as divergent as *Dictyostelium, Oryza sativa* (rice), *Arabidopsis, Schizosaccharomyces pombe, Saccharomyces cerevisae, Caenorhabditis elegans, Drosophila melanogaster,* and *Homo sapiens.* Alignment of XOs4 with Os4 family members from a variety of species reveals a highly conserved domain of approximately 185 amino acids (underlined in Fig. 1B), which contains the conserved phosphatase DXDXT/V motif (boxed in Fig. 1B). No other motifs were detected that would suggest the function of XOs4.

XOs4 Transcripts Are Expressed throughout Early Xenopus Development

RT-PCR analysis using primers specific for XOs4 cDNA revealed that XOs4 was expressed maternally and expression continued through gastrula, neurula, and tadpole stages (Fig. 2A). At gastrula stage, XOs4 was expressed in dorsal, ventral, animal, and vegetal regions of the embryo (Fig. 2B). *In situ* hybridization analysis revealed high levels of XOs4 expression in the ectoderm and lower levels in the preinvoluting mesoderm in early gastrula stage (10+) embryos (Fig. 3A-a, and data not shown). At neurula stage, expression became enriched in the neural plate with lower levels observed in the epidermis (Fig. 3A-b). In tailbud- stage (20) embryos, high levels of XOs4 expression were detected in the skin and neural crest cell derivatives. Tadpole-stage embryos (Stage 28) exhibited high levels of XOs4 transcripts in the dorsal neural tube, including the roof plate and neural

crest (Fig. 3A-c). Expression of XOs4 transcripts continued in the epidermis and became enriched in the developing pronephros, the head, and neural crest cell derivatives, including the cephalic and trunk neural crest. XOs4 transcripts were also highly expressed in the eye and otic vessicle (Fig. 3A-d).

XOs4 Protein Is Present in the Nucleus and Cytoplasm

In order to determine the subcellular localization of XOs4, a FLAG epitope-tagged XOs4 construct was used. RNA encoding FLAG-XOs4 was equally efficient at inducing secondary axis formation as the untagged version when injected into the ventral marginal zone of eight-cell stage embryos (data not shown), indicating that addition of the epitope tag does not interfere with the function of the protein. RNA encoding FLAG-XOs4 was injected into two-cell stage embryos. Embryos were fixed at gastrula stage and expression of the FLAG-XOs4 fusion protein was detected by whole-mount immunocytochemistry with an anti-FLAG antibody. Visualization of FLAG staining revealed FLAG-XOs4 protein was localized to the cytoplasm and the plasma membrane regions of the cell (Fig. 3B-b). FLAG-XOs4 also exhibited staining typical of nuclear localized proteins, such as Ldb1 (Fig. 3B-a). A similar expression pattern was observed in Cos cells (Fig. 3B-d), where XOs4 protein was detected in the cytoplasm and higher levels in the nucleus.

XOs4 Induced a Secondary Axis Similar to That Induced by Activation of the Activin/Nodal/Vg1 Pathway or Inhibition of the BMP Pathway

In order to characterize the mechanism of secondary axis induction by XOs4, the morphology of XOs4-induced secondary axis was compared with secondary axis induced by other pathways. For these experiments, RNA encoding v-Ras (FGF signaling), Wnt-8, Smad-2 (Activin/Nodal/VG1 signaling), Smad7 (inhibition of BMP signaling), or XOs4 were injected into the ventral marginal zone of eight-cell stage embryos. Embryos were allowed to develop until tadpole stage where the morphology of the secondary axis was observed. Activation of the FGF pathway by expression of activated v-Ras (Fig. 4A-b), FGF, or laloo (Ruiz i Altaba and Melton, 1989; Weinstein *et al.,* 1998; Whitman and Melton, 1992) in the ventral marginal zone resulted in induction of ectopic tail-like rather than trunk-like structures. Activation of the Wnt pathway by expression in the ventral marginal zone of Wnt-8 resulted in ectopic dorsal axis that contained trunk structures with heads, cement glands, and eyes (Fig. 4A-c) (Sokol *et al.,* 1991). In contrast, activation of the Activin/Nodal/Vg1 pathway by expression of Smad2 (Fig. 4A-e) or inhibition of BMP signaling by expression of Smad7 (Fig. 4A-d) resulted in induction of secondary axis that was morphologically indistinguishable from those induced by XOs4 (Fig. 4A-f). The secondary axis

induced by Smad2, Smad7, and XOs4 contained trunk structures that lack heads, cement glands, or eyes. Therefore, the secondary axis induced by XOs4 is similar to that observed by activation of the Activin/Nodal/Vg1 pathway or inhibition of BMP signaling rather than activation of FGF or Wnt pathways. However, since low levels of Wnt pathway activation can induce secondary axis that lack head structures, the ability of XOs4 to activate the Wnt pathway was examined in more detail.

Activation of the Wnt signal-transduction pathway results in induction of a Nieuwkoop center. In order to determine whether XOs4 induces a Nieuwkoop center, lineage-tracing analysis was done to follow the fate of XOs4-injected cells. For these experiments, a D-teir ventral-vegetal blastomere of a 32-cell stage embryo (Fig. 4B) was coinjected with RNA encoding either: XOs4, Smad7, Smad2, or XWnt8 with βGal as a lineage tracer. Based on fate map analysis (Dale and Slack, 1987), this blastomere will primarily contribute to the endoderm (Figs. 4C-a and 4C-e). When coinjected with RNA encoding Wnt8, the βGal-expressing cells were not incorporated into the secondary axis but remained in the endoderm (Figs. 4C-b and 4C-f). In contrast, inhibition of TGFβ signaling (Smad7) or activation of the Activin/Nodal/Vg1 pathway (Smad2) (Figs. 4C-c and 4C-g, and data not shown) resulted in βGal-injected cells being incorporated into the secondary axis (Baker and Harland, 1996; Casellas and Brivanlou, 1998). Similar to overexpression of Smad7 or Smad2, injection of RNA encoding XOs4 resulted in localization of the βGal-expressing tissue to the secondary axis (Figs. 4C-d and 4C-h).

To further exclude activation of the Wnt pathway, the ability of XOs4 to induce expression of Wnt-responsive genes in an animal cap assay was determined. Expression of Wnt8 in the animal cap results in the induction of the molecular markers Xnr3 and Sia (Lemaire *et al.,* 1995; Smith *et al.,* 1995). In contrast, XOs4 failed to induce expression of Xnr3 or Sia (Fig. 4D), suggesting that XOs4 does not activate a Wnt8-like pathway. Taken together, these results suggest that it is unlikely that XOs4 induces a secondary axis by activation of Wnt or FGF pathways.

XOs4 Induces a Secondary Axis by Induction of Dorsal Mesoderm Rather Than Inhibition of BMP Signaling

Similar to inhibition of BMP signaling (Smad7, data not shown), or activation of the Activin/Nodal/Vg1 pathway (Smad2, Fig. 5A), expression of XOs4 in ventral mesoderm explants resulted in expression of dorsal mesodermal markers, such as Cardiac actin and Collagen II, when sibling control embryos reached tadpole stage (Fig. 5A). While both Smad7 and Smad2 dorsalize ventral marginal zone explants in this assay, they utilize different mechanisms. Activation of the Activin/Nodal/Vg1 pathway results in induction of mesoderm of a dorsal character, whereas inhibition of the ventralizing BMP signals results in dorsalization of ventral

mesoderm. In order to determine whether XOs4 inhibits BMP signaling, the ability of XOs4 to block the epidermal-inducing activity of BMP was assayed in dissociated animal cap cells. When animal cap explants are dissociated, endogenous BMP is diluted and, by default, neural tissue is induced (Hemmati-Brivanlou and Melton, 1992, 1994). Addition of BMP induces an epidermal cell fate as demonstrated by the induction of Epidermal Keratin (Wilson and Hemmati-Brivanlou, 1995). Expression of BMP inhibitors such as Smad7 blocks the ability of exogenous BMP to induce epidermal ectoderm in dissociated explants, resulting in neural induction (Casellas and Brivanlou, 1998). In this assay, we found that expression of XOs4 in dissociated animal caps did not inhibit BMP-mediated epidermal induction (Fig. 5B), suggesting that XOs4 does not inhibit the BMP signaling pathway.

Activation of Activin/Nodal/Vg1 signaling and inhibition of BMP signaling can further be distinguished by molecular marker analysis by using an animal cap assay. Activin/Vg1/Nodal causes mesoderm induction and secondary neural induction in animal cap explants (Chang *et al.,* 1997). In contrast, inhibition of endogenous BMP activity results in the induction of neural fate without induction of mesoderm (Suzuki *et al.,* 1997; Wilson and Hemmati-Brivanlou, 1995). To further elucidate the mechanism of secondary axis induction by XOs4, the ability of XOs4 to induce neural and mesodermal markers in animal cap explants was assayed. For these experiments, RNA encoding XOs4 was injected into the animal pole region of two-cell stage embryos and animal cap explants were dissected at blastula stage. Animal cap explants were assayed by RT-PCR for expression of neural and mesodermal markers at tadpole stage. XOs4-expressing animal caps exhibited a slightly elongated morphology (Fig. 6A). Upon examination of molecular markers at tadpole stage (Fig. 6B), XOs4 was found to induce expression of molecular markers for neural tissue and dorsal and lateral mesoderm. XOs4 induced expression of: Cardiac actin and Collagen II (dorsal mesodermal); HoxB9 (posterior lateral mesodermal and posterior spinal chord); Twist (lateral mesoderm and neural crest), NCAM (neural), and XAG (cement gland). These results further suggest that XOs4 induces a secondary axis in a manner similar to Smad2 rather than Smad7, since, like Smad2, XOs4 induces expression of molecular markers for dorsal and lateral mesoderm. Furthermore, since XOs4 did not inhibit BMP signaling in dissociated ectoderm explants, the neural tissue induced is likely due to secondary neural induction.

Activin/Nodal/Vg1 pathways induce expression of mesodermal markers in gastrula-stage animal cap explants. To further investigate the possibility that XOs4 activates the Activin/Nodal/Vg1-like pathway, the ability of XOs4 to induce mesoderm in gastrula-stage explants was assayed. For these experiments, Smad2- or XOs4-expressing animal cap explants were examined for expression of the pan-mesoderm markers XBra and Xeomes. XOs4 very weakly induced expression of Xeomes but not XBra in early gastrula-stage (10.5) animal caps (Fig. 7A). Since expression
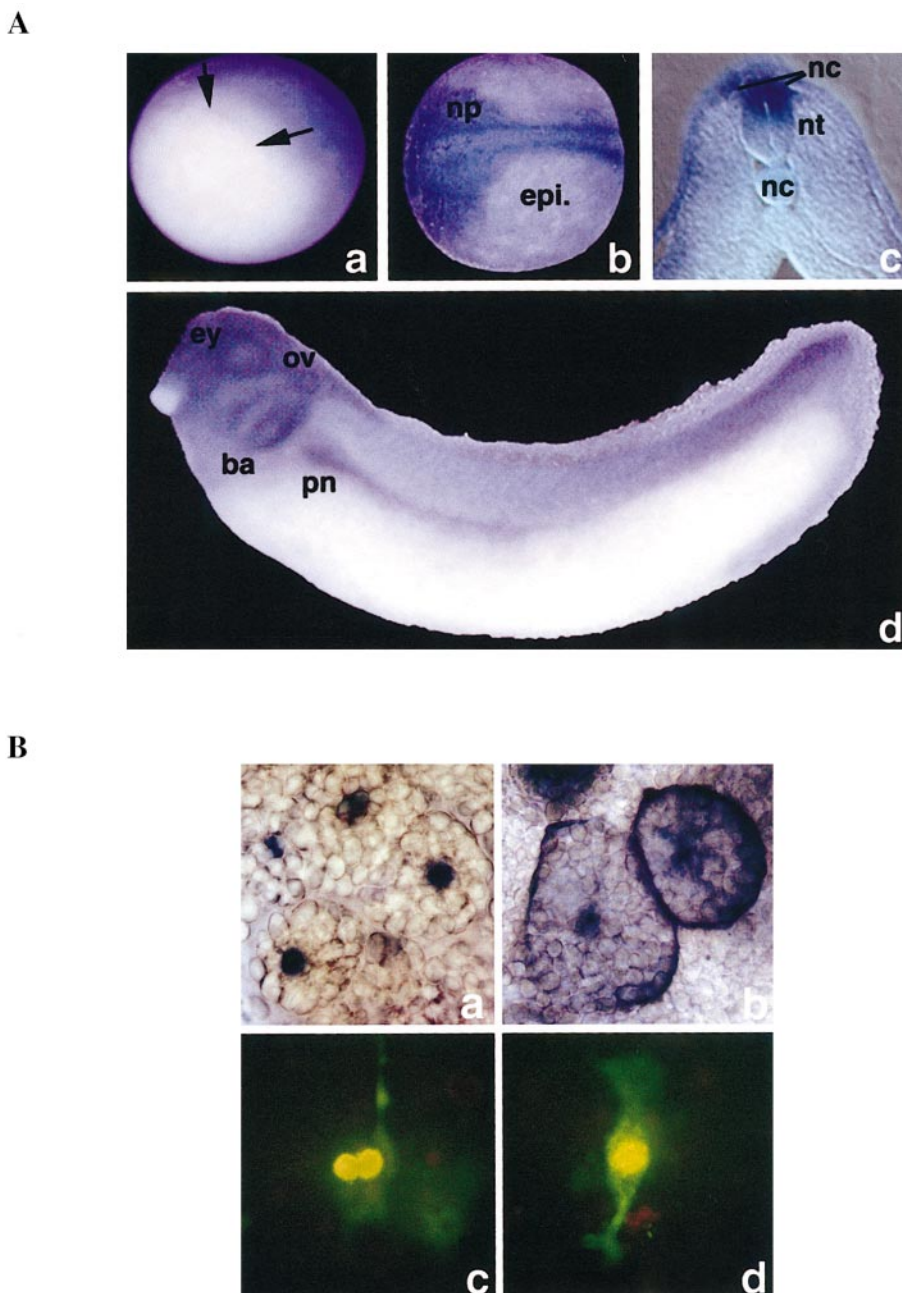
A

B

FIG. 3. (A) Spatial expression pattern of XOs4 in Xenopus embryos. (a) Vegetal view of a Stage-10+ embryo; dorsal is up; arrows mark dorsal blastopore lip. (b) Dorsal view of a Stage-15 embryo; anterior is to the left. (c) Transverse section of a Stage-28 embryo demonstrating staining in dorsal neural tube and neural crest. (d) Lateral view of a Stage-32 embryo; anterior is to the left. np, neural plate; epi, epidermis; nc, neural crest; nt, neural tube; nc, notochord; ey, eye; ov, otic vessicle; ba, branchial arches; pn, pronephros. (B) Intracellular localization of XOs4. (b) Ectopically expressed Flag-tagged XOs4 is localized in Stage-10+ Xenopus embryos to the cytoplasm and the plasma membrane and exhibits staining characteristic of nuclear localization. (a) FLAG-ldb1 is localized to the nucleus and exhibits characteristic staining. (d) XOs4 is localized to the cytoplasm and nucleus when expressed in Cos-1 cells. (c) pCMV-FLAG-DPC4 coexpressed with an activated Activin receptor (pCMV-ActRIB(T-D), FLAG-Smad4, is localized to the nucleus in Cos-1 cells.

of Xeomes transcripts are detected before XBra at Stage 9 rather than Stage 10 in Xenopus embryos (Ryan *et al.,* 1996), this raises the possibility that XOs4 is delayed in induction of mesoderm. Therefore, expression of early mesoderm markers was examined at later time points. At late gastrula stage (12), XOs4 very weakly induced expression of the
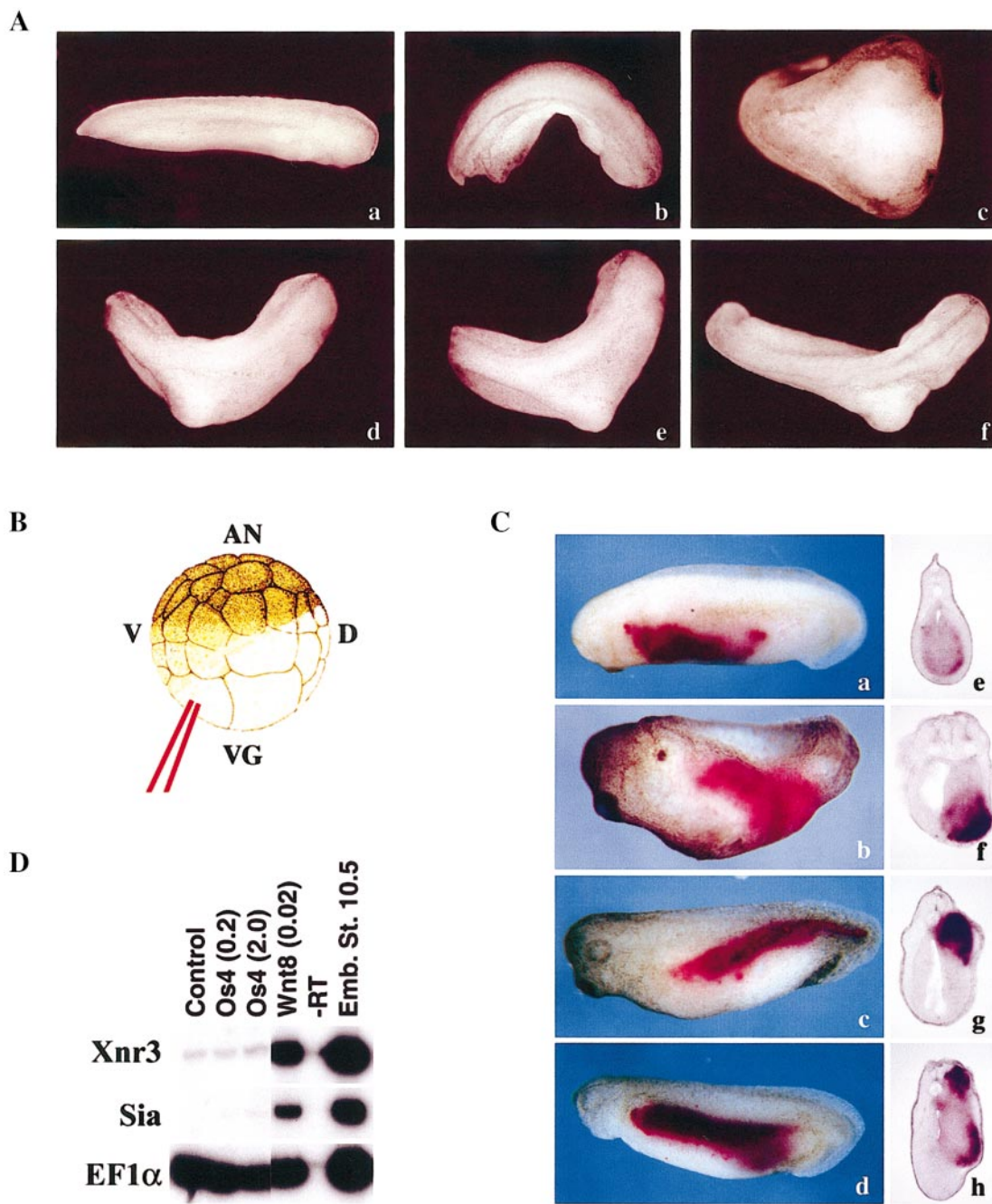
FIG. 4. (A) XOs4 induces a secondary axis distinct from that induced by activation of FGF or Wnt pathways but similar to activation of Activin or inhibition of BMP pathways. Uninjected (2) or RNA encoding v-Ras (25 pg, b), Wnt-8 (20 pg, c), Smad7 (100 pg, d), Smad2 (1 ng, e), or XOs4 (100 pg, f) was injected into the ventral marginal zone of eight-cell stage embryos. Embryos were allowed to develop until tadpole stage where the induced secondary axis was observed. (B, C) Lineage-tracing analysis reveals that XOs4 does not remain in the endoderm but participates in the formation of the secondary axis. One D-teir ventral–vegetal cell (B) of a 32-cell stage embryo was coinjected with RNA encoding βGal (C-a and C-e) or combined with RNA encoding Wnt8 (C-b and C-f), Smad7 (C-c and C-g), or XOs4 (C-d and C-h). (D) XOs4 is not able to induce the Wnt-responsive markers Xnr3 and Sia. The indicated amounts of RNA were injected into the animal pole regions of two-cell stage embryos and ectodermal explants were processed for RT-PCR analysis as described in Materials and Methods.
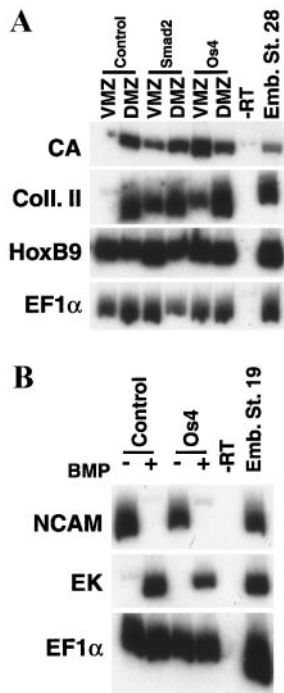
A

CA

Coll. II

HoxB9

EF1α

B

BMP

NCAM

EK

EF1α

FIG. 5. (A) XOs4 dorsalizes ventral–marginal zone explants. Four-cell stage embryos were injected in the marginal zone region in all four blastomeres with RNA (1 ng/embryo) encoding XOs4 or Smad2. Ventral or dorsal marginal zone explants were dissected at Stage 10+ and cultured until sibling control embryos reached tailbud stage (Stage 28). (B) XOs4 does not inhibit BMP signaling. Uninjected animal caps or animal caps expressing XOs4 (1 ng) were dissociated at late blastula stage and cultured in the presence or absence of BMP (10 ng/ml). At the end of neurulation, cells were reaggregated and cultured until control embryos reached late neurula stage (Stage 15) and processed for RT-PCR analysis for molecular markers for neural and epidermal tissue.

pan-mesoderm markers Xeomes and XBra and the ventral–lateral mesoderm marker Wnt-8 (Fig. 7B). In addition, unlike Smad2, XOs4 did not induce significant expression levels of organizer-specific markers such as Chordin and Lim-1.

To further address the possibility that XOs4 activates the Activin pathway, we assayed the ability of XOs4 to stimulate transcription from Activin-Responsive-Elements (AREs). The ability of XOs4 to activate transcription from the Gsc promoter and the ARE from the Mix.2 promoter were assayed by using Luciferase reporter constructs. Coinjection of XOs4 with the Gsc or Mix.2 transcriptional reporter constructs in one animal blastomere of eight-cell stage embryos resulted in little or no activation of the reporter gene construct as compared with Smad2 control injections at late gastrula stage (Fold activation of Gsc-Luc: control, 1 ± 0.28; Smad2, 13.6 ± 2.3; XOs4, 1.4 ± 0.1. Fold activation of A3-Luc: control, 1 ± 0.8; Smad2, 27.1 ± 5.7; XOs4, 1.5 ± 0.5. Data representative of three separate

experiments preformed in triplicate.). These results suggest that XOs4 may not activate the Activin/Nodal/Vg1 pathway.

Induction of Mesodermal Derivatives by XOs4 Does Not Require Activity of the Activin Signal-Transduction Pathway

To determine whether XOs4 requires activity of the Activin/Nodal/Vg1 pathway for induction of mesodermal derivatives, dominant-negative components of this pathway were coexpressed with XOs4 and the ability of these reagents to block XOs4-induced mesoderm was assayed. We first examined whether XOs4 required the activity of the Activin receptor to induce HoxB9 expression in tadpole-stage (28) animal cap explants, since XOs4 induces expression of HoxB9 at lower doses than more dorsal mesodermal markers such as Cardiac actin and Collagen Type II. For these experiments, XOs4 was coexpressed with a dominant-negative type II Activin receptor (tAR/DN-ActRIIB). While expression of tAR blocked induction of HoxB9 by Activin, expression of the tAR did not block XOs4 (Fig. 8A). Similarly, FAST1-EnR essentially eliminated induction of HoxB9 by Activin. In contrast, XOs4 was still able to induce HoxB9 expression in the presence of FAST1-EnR, suggesting that while the mesoderm-inducing activity of Activin is absolutely dependent on FAST1 activity, the mesoderm-inducing activity of XOs4 is, in part, FAST-independent. These results support the hypothesis that XOs4 may activate pathways either downstream from the Activin pathway, or independent of this pathway resulting in mesoderm induction.

Interestingly, like induction of mesoderm by Activin/Nodal/Vg1 pathway, induction of mesoderm by XOs4 was also dependent on FGF signaling. Coexpression of XOs4 with either the truncated FGF receptor (XFR) or dominant-negative Ras (Ras17N) resulted in inhibition of HoxB9 induction in tadpole (Stage 28)-stage animal cap (Fig. 8B). This was similar to the inhibition observed when Activin or Smad2 were coexpressed with these inhibitors (Fig. 8B, and Cornell and Kimelman, 1994; LaBonne and Whitman, 1994). These experiments cannot rule out a requirement for FGF signaling in the initial induction of mesoderm by XOs4. However, the observation that XOs4 induces a secondary axis distinct from that induced by activation of the FGF pathway (Fig. 4A), suggests that it is unlikely that XOs4 primarily activates this pathway for induction of a secondary axis.

DISCUSSION

Results presented in this study demonstrate that the Xenopus homologue of human Os4 can induce mesodermal derivatives in Xenopus embryos. XOs4 was identified by its ability to induce a secondary dorsal axis when injected into the ventral marginal zone of Xenopus embryos. XOs4

transcripts were expressed in the preinvoluting mesoderm in blastula- and gastrula-stage embryos, suggesting a role in induction and/or patterning of this germ layer. Later in development, XOs4 expression became enriched in the dorsal neural tube and neural crest. The secondary axis-inducing activity of XOs4 was distinct from other secondary axis-inducing pathways such as activation of the FGF or Wnt pathways or inhibition of the BMP pathway, and was similar, but not identical, to that of activation of the Activin/Nodal/Vg1 pathway. For example, while XOs4 induced expression of late mesodermal markers, XOs4 was delayed in a weak induction of gastrula-stage markers for mesoderm and XOs4 did not induce expression of organizer markers. Moreover, epistatic analysis suggests that mesoderm induction by XOs4 was partially independent of transcriptional activation of the Activin/Nodal/Vg1 pathway, since neither dominant-negative mutants of the Activin receptor or FAST1 prevented induction of lateral mesoderm by XOs4. Interestingly, like Activin-induced mesoderm, XOs4 required FGF signaling for expression of mesoderm markers in tadpole-stage animal cap explants. Therefore, our results suggest that XOs4 induces a secondary dorsal axis by activating a mesoderm-inducing pathway that, like the Activin/Nodal/Vg1 pathway, requires FGF signaling for expression of mesoderm markers.

XOs4 and the Induction and Patterning of Mesoderm

When overexpressed, XOs4 has the ability to induce dorsal and lateral mesoderm in animal cap explants. In addition, XOs4 is expressed in the preinvoluting mesoderm. Taken together, these data suggest a possible role for XOs4 in induction of mesodermal derivatives. However, at gastrula stages, XOs4 transcripts are ubiquitously expressed and we found no difference in the intracellular localization of ectopic expression of FLAG-tagged XOs4 (a possible indication of a change in activity) in dorsal vs. ventral mesoderm. While this ubiquitous expression pattern of XOs4 at gastrula stage does not immediately suggest a role in mesoderm induction, like many ubiquitously expressed signal-transducing molecules involved in mesoderm induction, the function of Os4 in mesoderm induction may be regulated at multiple steps posttranscriptionally. For example, transcripts for Smad2 are ubiquitously expressed, but phosphorylation and nuclear localization regulate the activity of Smad2 in the prospective mesoderm (Faure *et al.,* 2000). Alternatively, association with a temporally or spatially localized protein may regulate XOs4 activity. For example, XLdb1 is ubiquitously expressed at gastrula stages, but association with the organizer-specific Xlim1 regulates its activity (Agulnick *et al.,* 1996). While the results presented in this study do not demonstrate a requirement for XOs4 in mesoderm induction and patterning, they do suggest the possible involvement of this gene family in early patterning of the embryo. The demonstration of the requirement of XOs4 in the induction and/or patterning of endogenous mesoderm awaits further loss-of-function analysis.

Mechanism of Mesoderm and Secondary Axis Induction by XOs4

In order to determine the mechanism of secondary axis induction by XOs4, we compared the morphology of XOs4-induced secondary axis to those induced by other pathways. We concluded that it was unlikely that XOs4 induces a secondary axis by activation of FGF pathways since FGF-induced secondary axis consists of tail-like rather than trunk-like structures. However, we have not completely ruled out the possibility of XOs4 activating some FGF-dependent pathways; in fact, we found that mesoderm induction by XOs4 does required FGF signaling (see discussion below). Furthermore, we determined that XOs4 does not induce Nieuwkoop center-specific markers like Wnt pathways nor does XOs4 inhibit BMP signaling. From these observations, we concluded that XOs4 induces a secondary axis similar to that induced by ectopic activation of Activin/Nodal/Vg1 pathways.

Like activation of Activin/Nodal/Vg1 pathways, XOs4 induced mesoderm in tadpole-stage animal cap explants. However, unlike Activin, XOs4 did not activate AREs nor did XOs4 induce significant levels of organizer-specific markers in gastrula-stage animal cap explants. Additionally, XOs4 was substantially delayed in induction of gastrula-stage mesodermal markers and was very weak in its ability to induce expression of these markers. Furthermore, XOs4 induction of HoxB9 was independent of the activity of the Activin receptor. In contrast, we found that FAST1-EnR consistently reduced XOs4 induction of HoxB9 by approximately 50 percent. While this finding suggests that XOs4 is still able to induce mesoderm when this pathway is inhibited, this result does illustrate a partial requirement for TGFβ signaling via FAST1 in expression of mesoderm markers in XOs4-expressing animal caps. This does not reflect a general requirement for FAST1 in mesoderm induction and/or maintenance, as FGF induction of XBra was not blocked by an anti-FAST1 antibody, though FAST-EnR was not tested (Watanabe and Whitman, 1999). Furthermore, this inhibition could be a result of FAST1-binding sites in a promoter acting upstream from HoxB9 transcription. If FAST1 is binding this promoter, association with an Engrailed transcriptional repressor domain could inhibit transcription from the promoter even if XOs4 is not activating this promoter via FAST1. Nevertheless, these observations suggest that XOs4 is activating mesoderm and secondary axis induction at least partially independent of the Activin/Nodal/Vg1 pathway.

Another interesting observation was that XOs4 required FGF signaling for expression of mesoderm markers. This could indicate that XOs4 requires FGF for mesoderm induction and directly activates the pathway or alternatively, XOs4, like Activin, requires FGF pathway for maintenance of mesoderm (Cornell and Kimelman, 1994; LaBonne and
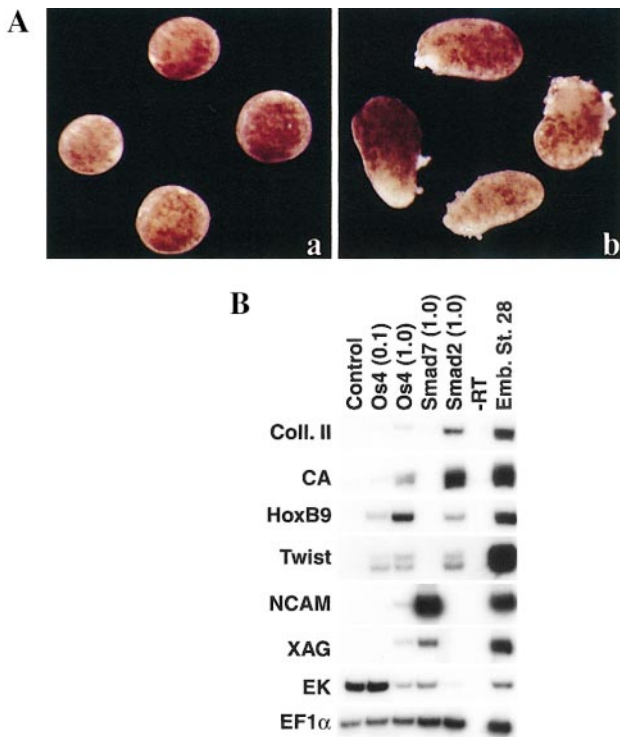
FIG. 6. XOs4 induces dorsal and lateral mesoderm in animal cap explants. (A) XOs4-expressing (1 ng) animal caps exhibit a partially elongated morphology indicated an inductive event. (a) Uninjected animal caps. (b) XOs4-injected animal caps at Stage 17. (B) XOs4-expressing animal caps express markers of posterior neural, neural crest, and dorsal and lateral mesoderm. Embryos were injected at the two-cell stage with the indicated RNAs. Animal caps explants were assayed by RT-PCR for expression of the indicated molecular markers at Stage 28.

Whitman, 1994). We favor the latter alternative because of the secondary axis phenotype induced by XOs4. While our results do not exclude involvement of FGF pathways in induction of mesoderm by XOs4, we find it unlikely that XOs4 primarily activates FGF pathways to induce a secondary axis and mesoderm, since, morphologically, the secondary axis induced by XOs4 is distinct from FGF but identical to that induced by Activin.

One interpretation of these combined observations is that XOs4 is acting downstream of a mesoderm-inducing pathway (such as the Activin pathway). For example, XBra can induce mesoderm in animal cap explants, and expression of XBra is induced by mesoderm-inducing pathways such as Activin and FGF (reviewed in Smith, 2001), thus XBra acts downstream of these mesoderm-inducing pathways. Conceivably, XOs4 may interact with pathways downstream of Xbra. The observation that XOs4 induces mesoderm later than activation of the Activin pathway supports this type of model. Furthermore, the observation that XOs4 requires FGF signaling for expression of mesoderm at tadpole stages

suggests that XOs4 may also require the same feedback loops of other mesoderm-inducing pathways. Alternatively, XOs4 may be contributing to these feedback loops, which could explain the delayed and weak mesoderm-inducing activity of XOs4. This model is purely speculative and elucidation of the pathway that XOs4 utilizes for mesoderm induction awaits further characterization of XOs4 function. For example, identification of XOs4-binding partners may allow for a better understanding of the mechanism by which XOs4 induces mesoderm and secondary dorsal axes.

The Os4 Gene Family

XOs4 is a member of a large evolutionary conserved family of proteins with members in all eukaryotes from species as divergent as *Dictyostelium*, *O. sativa* (rice), *Arabidopsis*, *S. pombe*, *S. cerevisae*, *C. elegans*, *D. melanogaster* and *H. sapiens.* They share a conserved C-terminal domain of approximately 200 amino acids and have little or no sequence homology in their N-terminal domains. Most family members have been identified through genome-
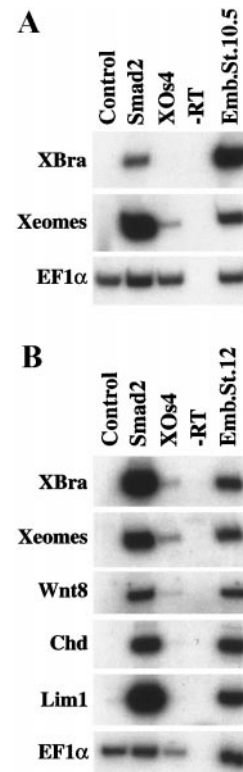

FIG. 7. XOs4 induces expression of mesoderm markers in animal cap explants by late gastrula stages. (A) RNA encoding Smad2 (1 ng) or XOs4 (1 ng) was injected into the animal pole region of two-cell stage embryos. Animal cap explants expressing Smad2 or XOs4 were assayed by RT-PCR for expression of the indicated mesoderm markers at early gastrula stage (10.5) (A) or late gastrula stage (12) (B).
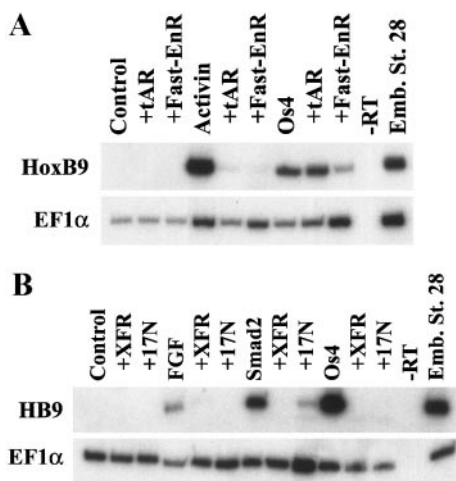
FIG. 8. (A) XOs4 does not require activity of the Activin/Nodal/Vg1 signal-transduction pathway upstream of FAST1 for induction of mesodermal derivatives. Embryos were injected with RNA (1 ng/embryo) encoding either tAR or Fast-EnR in the absence or presence of XOs4. Animal cap explants were dissected at blastula stage, incubated in the presence or absence of Activin as indicated, and cultured until control embryos reached tailbud stages (Stage 28). Animal cap explants were then processed for RT-PCR analysis for expression of HoxB9, which is strongly induced by both Activin and XOs4. (B) Like Smad2, XOs4 requires the FGF pathway for expression of mesoderm markers at tadpole stages. Embryos were injected with the indicated RNAs and animal cap explants were assayed by RT-PCR for HoxB9 expression at tadpole stage. XFR (1.5 ng/embryo), dominant-inhibitiory Ras(17N), Smad2, and XOs4 (1 ng/embryo). Basic FGF (FGF) was added to a final concentration of 50 mg/ml.

sequencing projects, and consequently little is known about the biological function of this gene family.

A distant relative, Fcp1 (the Xenopus homologue has 15% overall sequence identity to XOs4), is a RNA Polymerase II phosphatase (Kobor *et al.,* 1999). Fcp1 phosphatase activity is dependent on a novel DXDXT/V motif, which was initially identified in a family of phosphotransferases and phosphohydrolases (Collet *et al.,* 1998; Kobor *et al.,* 1999). This motif is conserved throughout the Os4 family. The *S. cerevisiae* Psr1p and Psr2p homologues contain the DXDXT/V phosphatase motif and exhibit phosphatase activity (Siniossoglou *et al.,* 2000). Furthermore, mutation of this motif to EXEXT/V results in a loss of ability to rescue the mutant phenotype. Accordingly, mutation of the DXDXT/V motif in XOs4 results in a loss of secondary axis-inducing activity (I.Z. and A.H-B., unpublished observations), suggesting that the conserved phosphatase domain may also be important for XOs4 function.

Nem1p is another more distantly related member of the family that is required for proper nuclear membrane morphology and sporulation in yeast (Siniossoglou *et al.,* 1998). Studies with this protein suggest that not all Os4 homo-

logues have the same function. Other yeast Os4 homologues could not rescue the nuclear envelope phenotype in Nem1p cells, nor could fusion proteins of the conserved Psr2p C-terminal domain with the N-terminal Nem1p domain (Siniossoglou *et al.,* 1998). However, this could be a result of an altered cellular localization, as this fusion protein was localized to the plasma membrane rather than the nuclear membrane. Nem1p also contains the conserved phosphatase motif; however, the requirement of this motif for Nem1p activity and the ability of Nem1p to function as a phosphatase have not yet been determined.

Role of hOs4 in Tumor Formation

XOs4 shares 79% sequence identity to hOs4. hOs4 is localized to a region of chromosome 12q13–q15, which is frequently amplified in human sarcomas and brain tumors (Su *et al.,* 1997). hOs4 was found to be amplified in a number of tumors examined including pleomorphic sarcomas, malignant fibrous histocytomas, osteosarcomas, and malignant Schwannomas. hOs4 was also amplified in tumor cell lines including NGP-127 (neuroblastoma), OsA-Cl (osteosarcoma), and RMS-13 (Rhabdomyosarcoma). This region of amplification contains two core segments separated by several megabases that can be amplified independently, but are most often coamplified. The first region contains MDM2 (a negative regulator of the tumor-suppressor p53) and CHOP (a transcription factor). The second region contains CDK4 (which promotes the G_1 to S transition in the cell cycle), SAS, Os9, and hOs4. Previously, the tumor-promoting effects of amplification of this region of DNA were attributed to the activity of overexpression of CDK4 and MDM2; however, results from this study suggest that Os4 may also contribute to tumor formation by regulating cell fate in these tumors. Interestingly, many of these tumors are derived from mesodermal cells and the possibility of XOs4 regulating mesoderm cell development further suggests a role in formation of these tumors.

This study is the first report of an embryological function for the large evolutionary conserved Os4 gene family. Two members of this family are mutated in human tumors, suggesting a role in human disease. Our data indicate that XOs4 can regulate changes in cell fate during Xenopus embryogenesis and may provide insight into the role of Os4 family members in human disease and normal cell function.

ACKNOWLEDGMENTS

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