Development 122, 4179-4188 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV1130

The *Xenopus* T-box gene, *Antipodean*, encodes a vegetally localised maternal mRNA and can trigger mesoderm formation

Fiona Stennard*, Gilles Carnac and J. B. Gurdon

Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, United kingdom and Department of Zoology, University of Cambridge, UK

*Author for correspondence

SUMMARY

We have used differential display to identify genes inducible by activin and isolated a novel member of the T-box gene family that includes the *Xenopus* genes *Xbrachyury* and *Eomesodermin*. Here we show that this novel gene is unique within the T-box family because it is maternally expressed at a high level. Furthermore, it belongs to a rare class of maternal mRNAs in *Xenopus* that are localised to the vegetal hemisphere of the egg and we have therefore named it *Antipodean*. We show here that low amounts of *Antipodean* injected into ectoderm (animal cap cells) strongly induce pan mesodermal genes such as *Xbrachyury* and ventral mesodermal genes such as *Xwnt-8*. Overexpression of *Antipodean* generates mesoderm of ventral character, and induces muscle only weakly. This property is consistent with the observed late zygotic *Antipodean* mRNA expression in the posterior paraxial mesoderm and ventral blastopore, and its exclusion from the most dorsal mesodermal structure, the notochord. *Antipodean* is induced by several molecules of the TGF-β class, but in contrast to *Xbrachyury*, not by bFGF. This result suggests that the expression of these T-box genes may be under the control of different regulatory pathways. Finally, we demonstrate that *Antipodean* and *Eomesodermin* induce each other and both are able to induce *Xbrachyury*. The early zygotic expression of *Antipodean* is not induced by *Xbrachyury*, though later it is to some extent. Considering its maternal content, *Antipodean* could initiate a cascade of T-box gene activations. The expression of these genes may, in turn, sustain each other's expression to define and maintain the mesoderm identity in *Xenopus*.

Key words: T-box, *Antipodean*, *Xenopus*, mesoderm induction, maternal determinant, localised mRNA, TGF-β, bFGF, *brachyury*, *Eomesodermin*, differential display, endoderm

INTRODUCTION

Two fundamental mechanisms are able to establish different regions within the developing embryo (reviewed in Gurdon, 1992). One involves the asymmetric distribution of determinants in the egg, whereby factors that are able to confer a specific developmental fate are inherited by only a subset of cells after the onset of cleavage. The other mechanism is that of embryonic induction, where an inducing cell is able to influence one or more nearby responding cells to change developmental fate. Mesoderm induction in *Xenopus* is a classical example of the latter, where a signal originating from the vegetal pole is believed to induce mesoderm in the equatorial region of the gastrula. As the initial stages of this induction occur well before the onset of zygotic transcription (Jones and Woodland, 1987), it therefore appears that mesoderm induction initially involves factors that are maternally inherited.

We have been interested in tracing the formation of mesoderm back through the sequential series of inductions arising from the earliest signal. We have chosen to search for novel genes that are activated by the TGF-β molecule, activin, whose likely developmental importance is indicated because: (1) activin very efficiently induces a wide variety of mesoderm derivatives in *Xenopus* ectodermal (animal cap) cells in vitro (Green et al., 1992); (2) the maternally provided activin protein is required for mesoderm and axis formation in fish in vivo (Wittbrodt and Rosa, 1994) and (3) a dominant negative activin receptor that abolishes TGF-β signalling blocks mesoderm induction (Hemmati-Brivanlou and Melton, 1992).

Our cloning strategy used the PCR-based technique known as differential display (Liang and Pardee, 1992) to compare the genes expressed in untreated or activin-treated animal cap cells. We have consequently cloned a novel gene belonging to the *Xenopus* T-box gene family that also includes *Xbra*chyury (*Xbra*) (Smith et al., 1991) and, most recently, *Eomes*odermin (*Eomes*).

Our results indicate that *Apod* is unique in this T-box gene family in that it is maternally expressed at a high level in addition to being zygotically expressed throughout the mesoderm. Most importantly, maternal transcripts are localised to the vegetal hemisphere from very early in oogenesis and we have therefore named it *Antipodean* (*Apod*). Since the vegetal pole gives rise to cells that are directly involved with normal mesoderm induction, *Apod* may act as an asymmetrically distributed determinant contributing to this process. Overexpression of *Apod* mRNA in ectoderm (animal cap) cells activates

mesoderm formation as well as the expression of *Xbra* and *Eomes*, two key genes in mesoderm cell differentiation (Smith et al., 1991; Ryan et al., 1996). We suggest that Apod expression from maternal and zygotic *Apod* mRNA may make an important contribution to mesoderm formation in *Xenopus*.

MATERIALS AND METHODS

Xenopus oocytes, eggs and embryos

Eggs were in vitro fertilised then dejellied and reared in 0.1× Modified Barth Saline (MBS) as described previously (Gurdon et al., 1985a). Embryos were staged according to Nieuwkoop and Faber (1967). Dissection and culturing of explants was performed in 1× MBS. Oocytes were defolliculated in 0.2% collagenase in 0.1 M sodium phosphate buffer at room temperature for approximately 2 hours.

Embryos for microinjection were transferred to 1× MBS; 4% Ficoll at the 2-cell stage and injected with a Drummond Nanoject variable microinjector. The injected volume was between 10 and 20 nl. At stage 7, embryos were transferred to 50% (0.1× MBS):50% (1× MBS, 4% Ficoll), then 30 minutes later transferred to 0.1× MBS.

Differential display

2-cell stage *Xenopus* embryos were injected in the animal pole region with 20 pg activin mRNA. Animal caps were cut at stage 9 and cultured until sibling embryos had reached stage 10.25. Total RNA was extracted from activin mRNA-injected and uninjected animal caps by the NETS method.

Unless otherwise indicated, reagants used for differential display were components of an RNAimage kit (GenHunter). Total RNA (1 μg) was treated with 1 U DNase (Gibco BRL) in a volume of 10 μl according to the manufacturer's instructions. An aliquot (0.2 μg) was reverse transcribed in a 20 μl reaction (25 mM Tris-Cl, pH 8.3, 37.6 mM KCl, 1.5 mM $MgCl_2$ and 5 mM DTT) with 20 μM dNTPs, 0.2 μM anchored oligo(dT) primer (5′-AAGCTTTTTTTTTTTM-3′ where M is A, G or C) in a thermocycler: 65°C, 5 minutes; 37°C, 60 minutes; 75°C, 5 minutes. After 10 minutes at 37°C, 100 U MMLV Reverse Transcriptase was added to the reaction. Either 2 or 4 μl of the reverse transcription reaction was transferred to a 20 μl PCR reaction (10 mM Tris-Cl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.001% gelatin) containing 1 U AmpliTaq DNA polymerase (Perkin-Elmer) and 0.25 μl α-[^{33}P]dATP (2,000 Ci/mmole) (DuPont-NEN), 20 μM dNTPs, 0.2 μM of the same anchored oligo(dT) primer used in the reverse transcription and 0.2 μM of a 10-mer of arbitrary sequence. Tubes were placed in a thermocycler: 94°C, 30 seconds; 40°C, 2 minutes; 72°C, 30 seconds for 40 cycles then 72°C for 5 minutes. Different combinations of primers were used to examine the entire mRNA population. An aliquot from the PCR reaction (3.5 μl) was electrophoresed through 6% denaturing polyacrylamide gels. The gel was blotted onto 3MM paper (Whatman) and dried at 80°C for 1 hour under vacuum. Gels were exposed to X-ray film at –80°C for 24 to 48 hours. Gels and autoradiographs were aligned and differentially expressed bands cut from the gel and the DNA eluted and reamplified. Fragments were gel purified before further use.

Nucleotide sequencing

The nucleotide sequence of *Apod* was determined by Sanger dideoxy sequencing of overlapping double-stranded clones in RN3 using designed oligo primers and Sequenase version 2 (USB), according to the manufacturer's instructions. Both strands of the *Apod* open reading frame were sequenced.

RNase protection assays

RNase protections were performed as described previously (Gurdon et al., 1985a). An *Apod* RNase protection probe was prepared by cloning a PCR amplified 280 bp fragment of the *Apod* 3′-UTR into *Sma*I restricted BlueScript. This construct (pApodRP/BS) was linearised with *Bam*HI and transcribed with T7 RNA polymerase to produce a 360 bp unprotected probe that protects a 280 bp fragment of *Apod* mRNA. All other probes were as described previously: *Xbra*, *gsc*, *FGFr*, *Xwnt-8* (Lemaire and Gurdon, 1994), *Mix.1* (Lemaire et al., 1995), *Siamois* (Carnac et al., 1996) and *Eomes* (Ryan et al., 1996).

Synthetic mRNA

Synthetic capped mRNA was prepared as described in Lemaire et al. (1995). Synthetic *Apod* mRNA was prepared by cloning the 2562 bp *Apod* cDNA, containing the open reading frame and untranslated regions, into *Eco*RI/*Not*I-restricted pBluescript-RN3 (Lemaire et al., 1995). This plasmid (pApodR/RN3) was linearised with *Sfi*I and transcribed with T3 RNA polymerase.

Activin mRNA was prepared from pSP64T Activin βB (a gift from Doug Melton) as previously described (Thomsen et al., 1990). *BMP-4* mRNA was prepared from plasmid pSP64T-XBMP4II (a gift from Leslie Dale) as described by Dale et al. (1992). *bFGF* mRNA was made from plasmid containing *bFGF* in 64T(gift from Betsy Pownall) as described by Thompson and Slack (1992). *Xnr-2* mRNA was prepared from plasmid *Xnr-2* (a gift from Jim Smith) as described by Jones et al. (1995). *bVg1* mRNA was prepared from plasmid pSP64TBVg1 (a gift from Doug Melton) as described by Thomsen and Melton (1993). *Xwnt-8* mRNA was prepared from an RN3-based vector as described by Lemaire et al. (1995). *Xbra* mRNA was prepared from a construct called pSP64RN3XbraR (Lemaire unpublished), which was constructed with the coding region of *Xbra* from pSP73Xbra (a gift from J. Smith), cloned into pBluescipt-RN3 (Lemaire et al., 1995). *Eomes* was prepared from pEomes/RN3-3 as described in Ryan et al. (1996). XFD mRNA was prepared from the construct XFD/Xss (a gift from Enrique Amaya) as described in Amaya et al. (1991).

Immunostaining and in situ hybridisation

All samples were fixed in MEMFA (Hemmati-Brivanlou and Harland, 1989) for 2 hours, 30 minutes in methanol, then stored at –20°C. DIG-labelled riboprobes were synthesized essentially as described by Harland (1991). The construct pApodI/BS, consisting of a 1.3 kb *Pst*I-*Eco*RI fragment covering the 3′-UTR and coding sequence of the C-terminal region of *Apod* cloned into Bluescribe, was cut with *Eco*RI and transcribed with T3 RNA polymerase to make antisense probes, or cut with *Hin*dIII and transcribed with T7 RNA polymerase to make sense probes. The *Xbra* probe was synthesised as described in Lemaire and Gurdon (1994). In situ hybridisation to sectioned material was performed as described previously (Lemaire and Gurdon, 1994), but without hydrolysing probes and using Boehringer BM purple substrate (Cat. No. 1442074) to visualise DIG-labelled hybrids. Whole-mount in situ hybridisation was performed essentially as described previously (Hemmati-Brivanlou et al., 1990) with the same method of visualisation as the whole-mount procedure. In some cases embryos were fixed overnight in Bouin's fixative then bleached (70% methanol:30% hydrogen peroxide) to remove pigment.

Immunohistochemistry was performed as previously described (Carnac et al., 1996).

RESULTS

Differential display cloning of *Apod* cDNA and sequence analysis

Partial cDNA clones from the 3′ ends of activin-induced mRNAs were identified using differential display and sequenced. Clones showing no similarity to sequences in the database were radioactively labelled and hybridised to northerns containing RNA from different developmental stages of *Xenopus* embryos. Of these, a 600 bp partial cDNA clone hybridised to an approximately 2.7 kb transcript that was

highly expressed in both the egg and gastrula (data not shown). This was used to extract full-length clones from a stage 10 *Xenopus* embryo cDNA library. A 2562 bp cDNA with stops in all reading frames except one was isolated and sequenced (EMBL Accession number: X99905). Conceptual translation of the 1.3 kb open reading frame gave a 435 amino acid sequence, shown in Fig. 1A. The clone contained 17 bp of the 5′- and 1.24 kb of the 3′-untranslated regions.

A comparison of the deduced amino acid sequence of Apod with other proteins using Blast program analysis (Altschul et al., 1990) shows a region in the Apod N-terminal domain homologous to a T-box sequence motif (Bollag et al., 1994). Recently, mouse *brachyury* has been shown to act as a tissue-specific transcription factor with the T-box defining a DNA-binding domain (Kispert et al., 1995). Thus, the conservation of this motif suggests that the role of Apod in the embryo may be that of a transcription factor controlling the expression of developmentally regulated genes. There is no sequence homology at the amino acid or nucleotide level outside the T-box; *Apod* therefore encodes a new member of the T-box family of genes, that within *Xenopus*, includes *Xbra* (Smith et al., 1991) and most recently *Eomes* (Ryan et al., 1996).

An amino acid sequence comparison between the Apod T-box and related T-boxes is shown in Fig. 1B. Within the T-box, Apod is most similar to the human and mouse homologs of Tbx2 (Bollag et al., 1994; Campbell et al., 1995) and to *Drosophila* optomotor-blind (omb) (Pflugfelder et al., 1992). It shows a weaker similarity to *Xenopus* Xbra (Smith et al., 1991) and to Eomes (Ryan et al., 1996). A diagrammatical representation of these proteins is shown in Fig. 1C, and demonstrates that the T-box's position in Apod is most like that in Xbra.

Temporal expression in oogenesis and in early development

Initial screening for activin response genes had shown that *Apod* mRNA was present at high levels in both the egg and gastrula (data not shown). To further characterise the temporal expression of this gene, we examined *Apod* mRNA levels throughout oogenesis and early embryonic development by RNase protection analysis. In parallel, we compared the *Apod* expression profile to that of *Xbra*, the expression of which has been previously analysed by Smith et al. (1991).

As shown in Fig. 2, *Apod* mRNA accumulates during stage 1 of oogenesis. It is then present during oogenesis from stage 2 and thereafter at similar levels. After fertilisation, levels of *Apod* mRNA again remain relatively constant until there is an increase at the onset of zygotic transcription. The peak of *Apod* expression starts between stages 9 and 10, just before the onset of gastrulation. This is much earlier than the peak of *Xbra* expression, which is beginning to reach maximal levels between stages 10.5 and 12. Similar to *Xbra*, *Apod* mRNA levels decline in the stage 13, late gastrula embryo. In stage 16 neurula embryos, expression of *Apod* and *Xbra* mRNA is noticeably reduced and is barely

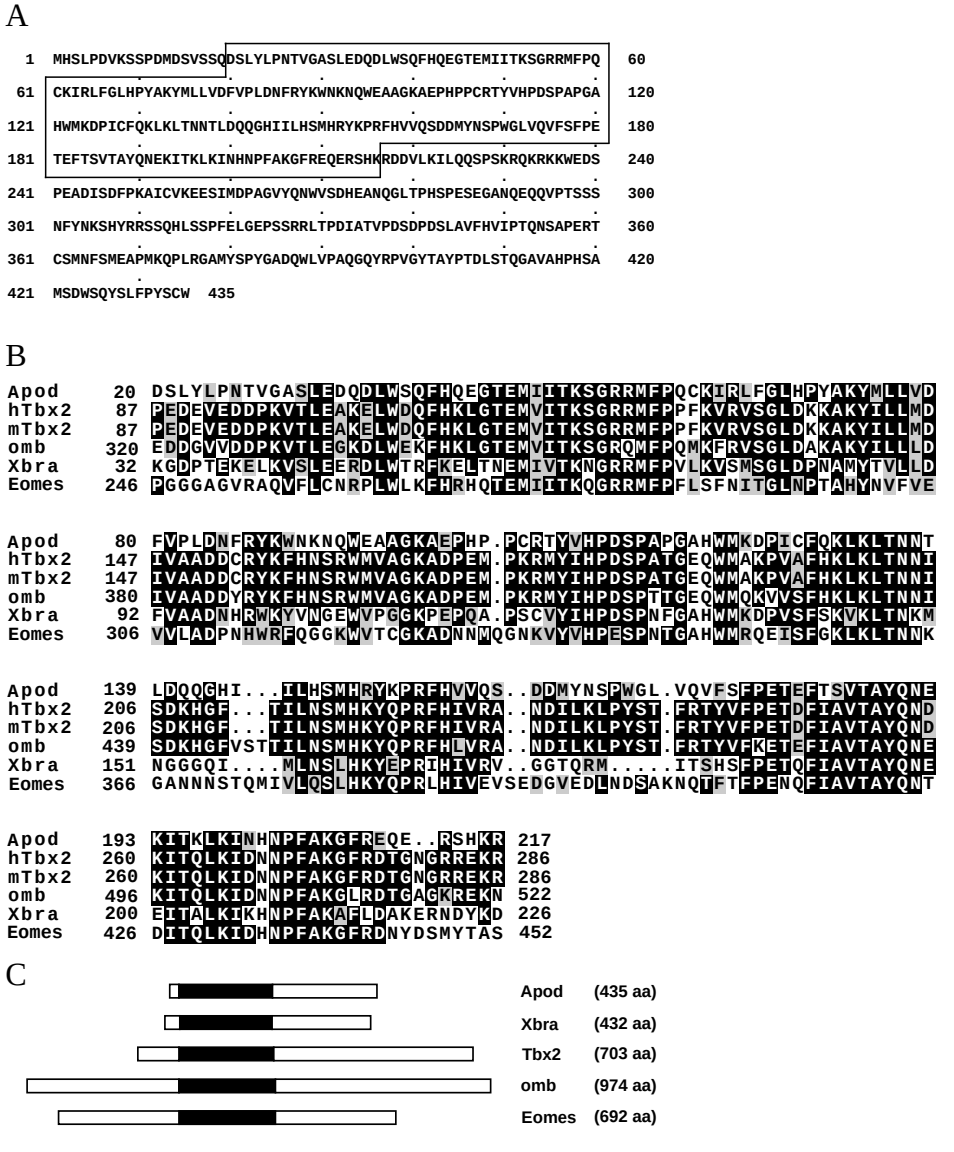

Fig.1. (A) Deduced amino acid sequence of Apod. The T-box in the N-terminal domain, as determined by sequence analogy to other T-box proteins, is boxed. (B) Amino acid sequence comparison of the Apod T-box and other related T-box proteins. Residues that are identical (dark shading) or functionally conserved (light shading) in at least 80% of these sequences have been boxed. (C) Diagrammatic representation, drawn to scale, of proteins closely related to Apod over the T-box motif. The dark shaded region corresponds to the boxed region in A and the area of comparison in B.

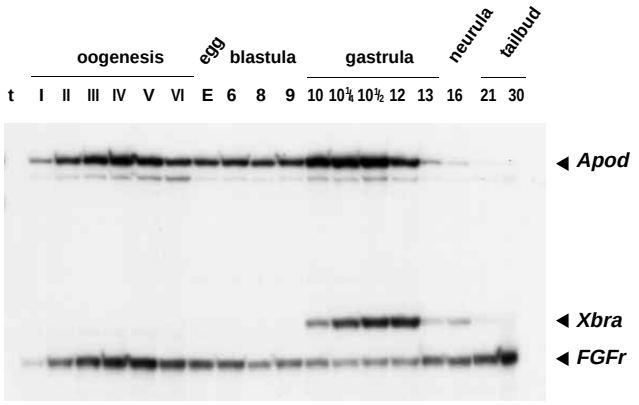

Fig. 2. Temporal expression of *Apod* throughout oogenesis and embryonic development. RNA was extracted from two defolliculated oocytes, eggs or embryos at the indicated stage of development and analysed by RNase protection for expression of *Apod* and *Xbra* mRNA. Probes for *fibroblast growth factor receptor* (*FGFr*) mRNA were also included to indicate the mRNA levels of a gene expressed at relatively constants levels throughout development. (t, tRNA control)

detectable in the tailbud embryo. *Apod* transcripts are therefore present continuously from the egg to late gastrula stages. *Apod* is expressed prior to, and during, the onset of *Xbra* mRNA expression. The expression of *Apod* mRNA overlaps with that of *Eomes* mRNA, which is also first expressed soon after the onset of zygotic transcription and prior to the onset of *Xbra* expression (Ryan et al., 1996).

Maternal *Apod* mRNA is vegetally localised

In situ hybridisation with antisense DIG-labelled riboprobes was used to determine the localisation of *Apod* mRNA during oogenesis. Whole-mount in situ hybridisation reveals that maternal *Apod* mRNA is distributed throughout the stage 1 oocyte and begins its localisation during stage 2 of oogenesis (data not shown). Hybridisation to sectioned stage 3 oocytes (Fig. 3A) clearly demonstrates that *Apod* mRNA is deposited at both the vegetal cortex and in the vegetal yolk mass. In stage 4 oocytes (Fig. 3B), *Apod* mRNA has a predominantly vegetal localisation, and is still found at the vegetal cortex as well as in the vegetal yolk mass. Staining for *Apod* mRNA in these earliest stages is very granular in appearance, suggesting that the mRNA is aggregated into clumps. In mature stage 6 oocytes (Fig. 3C), there is a significantly reduced distribution of *Apod* mRNA at the cortex. It is predominantly in the vegetal yolk mass and is still particulate in its appearance. By this stage, we have noted that mRNA particles have moved towards the equator and are now in a more subequatorial position.

In situ hybridisation to egg sections shows that *Apod* mRNA has a similar distribution to that observed in the mature oocyte; however, the pattern of staining is diffuse rather than particulate (Fig. 3D). Probes consisting of a labelled sense RNA strand which are unable to bind mRNA were used to detect non-specific staining in our protocol. Hybridisation with a sense *Apod* probe (Fig. 3E) to sectioned eggs shows no non-specific staining in the vegetal pole. A distribution of *Apod* mRNA similar to that found in the egg is maintained after fertilisation and is mainly confined to vegetal cells upon formation of the horizontal cleavage plane that forms at the 8-cell stage (data not shown).

Regional expression of *Apod* in embryos

The distribution of *Apod* throughout the embryo after the onset of zygotic transcription was determined by in situ hybridisation to either whole-mount or sectioned material. Both types of in situ hybridisation techniques were used so that any problems that might arise from poor penetration of the probe into yolky endodermal cells associated with whole-mount staining would be detected by use of sectioned material. Whole-mount and section in situ hybridisations with *Apod* antisense DIG-labelled riboprobes to stage 10.5 gastrulae are shown in Fig. 4A,C. These reveal mesodermal *Apod* mRNA expression throughout the equatorial region and a greater area of *Apod* mRNA-expressing cells on the dorsal side, compared to the ventral side, at this stage of development. Interestingly, *Apod* mRNA is expressed right up to the edge of the dorsal lip (Fig. 4C). Its expression may also extend into some of the future dorsal and ventral endoderm. In contrast, the whole-mount and sectioned material stained for *Xbra* mRNA (Fig. 4B,D, respectively) shows a less widespread distribution. The domain of *Xbra*

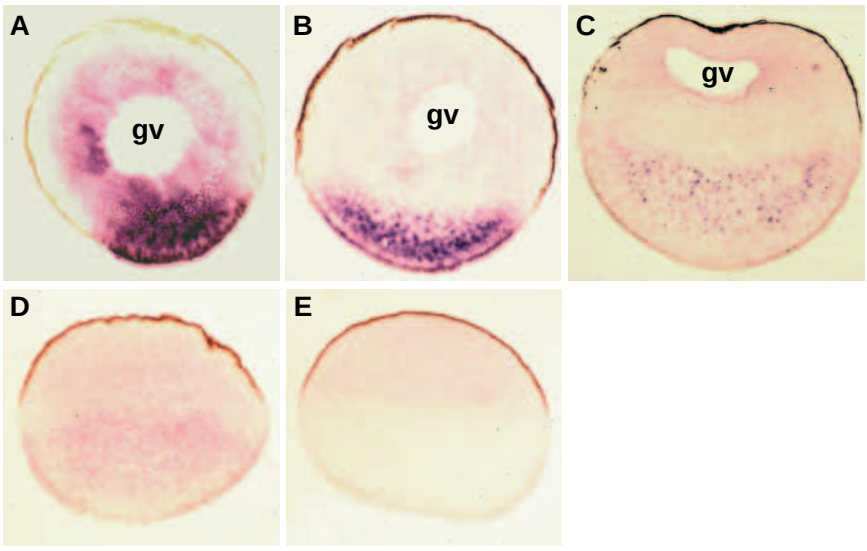

Fig. 3. Localisation of maternal *Apod* mRNA throughout oogenesis and early cleavage stages in *Xenopus*. *Apod* mRNA was visualised by in situ hybridisation to previously sectioned material using DIG-labelled antisense riboprobes (A-D) or sense probes as negative controls (E). Cells are orientated such that the animal pole is uppermost. (A) Stage 3 oocyte showing presence of *Apod* mRNA in both the vegetal cortex and yolk mass. (B) Stage 4 oocyte. (C) Mature stage 6 oocyte showing the particulate localisation of *Apod* mRNA in the vegetal yolk mass. The mRNA is now localised in a more subequatorial position. (D) Egg showing more diffuse localisation of *Apod* mRNA, still distributed in the vegetal yolk mass. (E) Egg hybridised with a sense probe showing very faint non-specific staining throughout the animal hemisphere only. A and B are shown at a greater magnification than C-E for convenient comparison. (gv, germinal vesicle).

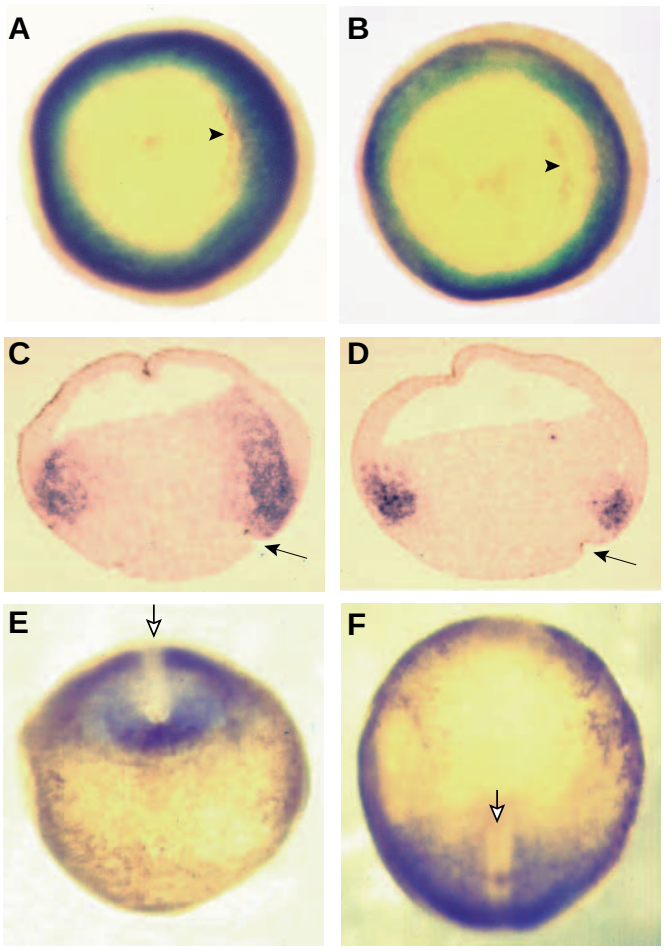

Fig. 4. *Apod* mRNA shows a more widespread distribution than *Xbra* mRNA in the gastrula. In situ hybridisation was performed on whole-mount (A,B,E,F) or sectioned (C,D) embryos. The dorsal side is to the right in A-D, and the position of the dorsal lip is indicated with a solid arrow. Late stage gastrulae were also hybridised with probes for *Apod* mRNA as whole mounts (E,F) and show its presence in the posterior paraxial mesoderm and ventral blastopore. *Apod* mRNA is notably excluded from the notochord (indicated with an open arrow). (A,C) *Apod* probe, stage 10.5. (B,D) *Xbra* probe, stage 10.5. (E) Posterior view, dorsal side at top; *Apod* probe stage 13. (F) Dorsal view, anterior end at top; *Apod* probe, stage 13.

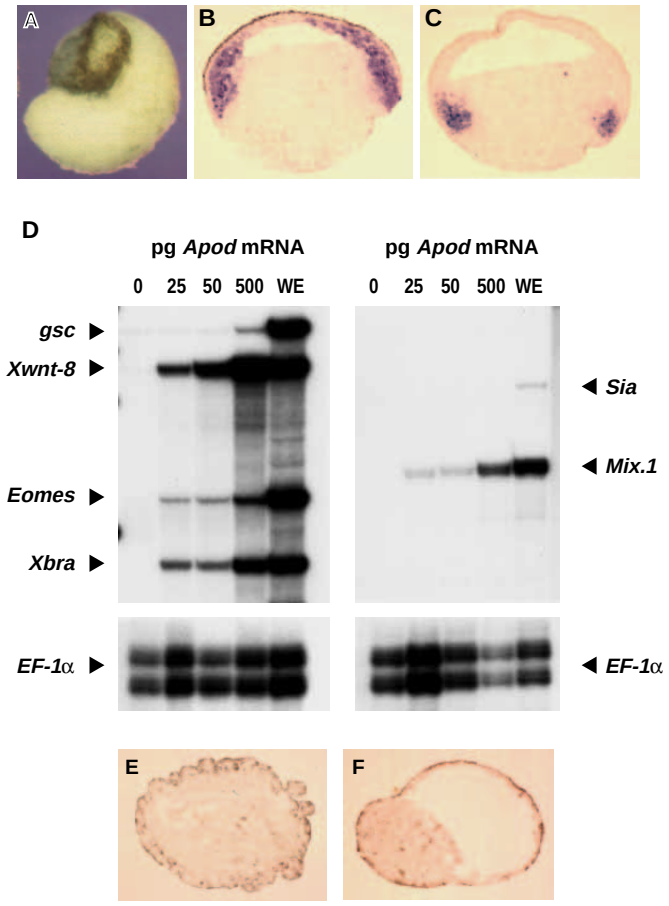

Fig. 5. *Apod* mRNA can induce mesoderm. *Apod* mRNA (500 pg) was ectopically expressed in the whole embryo by injection of synthetic mRNA into the animal pole region of two-cell embryos. These were reared until uninjected siblings had reached stage 26. Embryos ectopically expressing *Apod* failed to gastrulate and developed as exogastrulae (A). *Apod*-injected and uninjected embryos were sectioned and then analysed for the expression of *Xbra* by in situ hybridisation at stage 10.25 (B,C, respectively). The ectopic expression of mesodermal markers in the animal cap region of the embryo suggests that *Apod* is able to induce mesoderm. To further characterise *Apod*'s mesoderm-inducing properties, the indicated amounts of *Apod* mRNA were injected into the animal pole region of embryos at the 2-cell stage. At stage 9, animal caps were isolated and cultured as explants until sibling embryos had reached stage 10.25. Total RNA was extracted from the equivalent of two embryos and analysed for the expression of mesodermal markers by RNase protection (D). *Apod* mRNA is able to strongly induce *Xwnt-8*, *Xbrachyury* (*Xbra*), *Eomesodermin* (*Eomes*) and *Mix.1*. At high *Apod* mRNA concentrations there is a weak activation of *goosecoid* (*gsc*). *Siamois* (*Sia*) is not activated. Histological examination shows that whereas uninjected controls form atypical epidermis (E), *Apod*-injected animal caps form mesoderm of ventral character as judged by the presence of mesenchyme and vesicles (F). Immunostaining of explants in E and F with the muscle-specific 12/101 antibody showed no muscle (see also Table 1).

mRNA expression is separated by several cell diameters from the dorsal lip and is entirely restricted to the mesoderm (Fig. 4D, see also Smith et al., 1991).

In late gastrula embryos, *Apod* mRNA is expressed predominantly around the ventral blastopore (Fig. 4E) and extends into the posterior paraxial mesoderm (Fig. 4F). A particularly interesting feature of *Apod* expression is that, whereas *Xbra* at this stage is expressed around the blastopore and in the notochord (Smith et al., 1991), expression of *Apod* mRNA is excluded from the notochord (Fig. 4E,F) and thus presents a reciprocal expression pattern to that of *Xbra*.

Overexpression of *Apod* mRNA induces mesoderm

In situ hybridisation shows that *Apod* mRNA is not expressed in the animal cap region of the embryo. To determine whether ectopic expression of *Apod* in the whole embryo can affect development, we injected *Apod* mRNA into the animal pole of 2-cell-stage embryos and allowed them to develop until sibling embryos had reached tailbud stage. Invagination failed to occur in these embryos after formation of the dorsal lip and they developed as exogastrulae (Fig. 5A). This defect also occurs upon injection of

Table 1. Induction of muscle by ectopic expression of *Apod* mRNA

pg Apod mRNA	muscle	*n*
0	0	17
25	0	5
50	0	19
100	0	24
1000	5*	27

Embryos were injected at the 2-cell stage with the indicated amounts of *Apod* mRNA

Animal caps were cut at stage 9 and cultured as conjugates until sibling embryos were at stage 26. These were then fixed, sectioned and immunohistochemistry performed with a 12/101 antibody which stains muscle. Doses higher than 1 ng were toxic.

*The area of 12/101 immunoreactivity observed was small – approximately 15-20% of the volume of the conjugate.

the mesoderm inducer, *Xbra*, into the animal pole region of the embryo (Cunliffe and Smith, 1992). To determine if injection of *Apod* mRNA into the animal pole of *Xenopus* embryos also induces ectopic mesoderm, we looked for expression of the mesodermal marker, *Xbra*, by in situ hybridisation. Expression of *Xbra* extends abnormally into the animal cap region of *Apod* mRNA-injected embryos (Fig. 5B) relative to controls (Fig. 5C), indicating that *Apod* mRNA is able to direct cells normally fated to become ectoderm towards a mesodermal fate.

To further characterise the ability of *Apod* mRNA to induce mesoderm, we injected different amounts of *Apod* mRNA into the animal pole region of 2-cell-stage embryos. Animal caps were dissected at stage 9, cultured until stage 10.25 and then analysed by RNase protection assay for the expression of mesodermal genes (see Fig. 5D). Injection of as little as 25 pg *Apod* mRNA is able to strongly induce ventral (*Xwnt-8*) (Christian et al., 1991) or pan mesodermal (*Xbra*, *Eomes*, *Mix.1*) genes (Smith et al., 1991; Ryan et al., 1996; Rosa, 1989). At higher concentrations of *Apod* mRNA, there is a weak activation of dorsal mesodermal markers such as *gsc* (see Dawid, 1994). Another dorsal gene, *Siamois* (Lemaire et al., 1995) is not activated at all.

Histological examination of animal cap explants injected with 25 pg *Apod* mRNA and cultured until stage 26 shows that, whereas uninjected controls form atypical epidermis (Fig. 5E), *Apod*-injected animal caps form mesoderm of ventral character as judged by presence of mesenchyme and vesicles (Fig. 5F). To further characterise the type of mesoderm induced by *Apod*, animal caps previously injected with increasing amounts of *Apod* mRNA were cultured until stage 26 and immunostained with the monoclonal antibody 12/101, which detects muscle cells. Doses from 25 to 500 pg of *Apod* mRNA do not induce muscle formation (Table 1). However, at a high dose (1 ng), there is a low percentage of explants (5 out of 27) that express the muscle marker in a small number of cells. Injections of higher concentrations of *Apod* mRNA are toxic.

Thus, *Apod* can induce ventral mesoderm and only at high doses can it weakly produce muscle at low frequencies. *Xbra* induces ventral-lateral mesoderm when expressed in animal caps, as characterised by vesicles, mesenchyme and muscle (Cunliffe and Smith, 1992). *Eomes* is able to induce more dorsal structures such as muscle and notochord (Ryan et al., 1996). These data demonstrate that different T-box genes may be involved with the production of different types of mesoderm.

Apod is induced by molecules of the TGF-β class, but not by bFGF

It has been previously reported that small changes in activin concentration induce the expression of different mesodermal genes in *Xenopus* animal caps (Green et al., 1992). As *Apod* was identified on account of its induction in animal caps following treatment with activin, we decided to further characterise *Apod*'s response to this mesoderm inducer. Increasing amounts of *activin* mRNA were injected into the animal pole region of 2-cell embryos. Animal caps were dissected at stage 9, cultured until stage 10.25 and then analysed by RNase protection for *Apod* mRNA expression. We also determined the expression of genes induced at high or low doses of activin, namely the dorsal gene *gsc* and the pan-mesodermal gene *Xbra* respectively (Green et al., 1992). *FGFr* mRNA expression was used as an internal loading control. As shown in Fig. 6, *Apod* mRNA expression increases with increasing activin concentrations. Interestingly, this is different to the *Xbra* mRNA induction profile where levels of mRNA peak and then decline as the amount of injected activin mRNA increases (see also Green et al., 1992; Gurdon et al., 1994). Thus, *Apod* responds to activin at low doses like *Xbra*, but with a profile that is more similar to that of *gsc* in that its expression is not down-regulated at high concentrations of activin.

Members of the TGF-β family of molecules with mesoderm-inducing capacity can be classified according to their abilities to dorsalise the mesoderm: *activin*, *bVg1* and *Xnr-2* can give rise to dorsal mesoderm tissues such as muscle and notochord, whereas *Bmp-4* differentiates the mesoderm into ventral derivatives such as blood (Asashima et al., 1990; Dale et al., 1992; Kessler and Melton, 1995; Jones et al., 1995). To determine which of these factors are able to influence *Apod* expression,

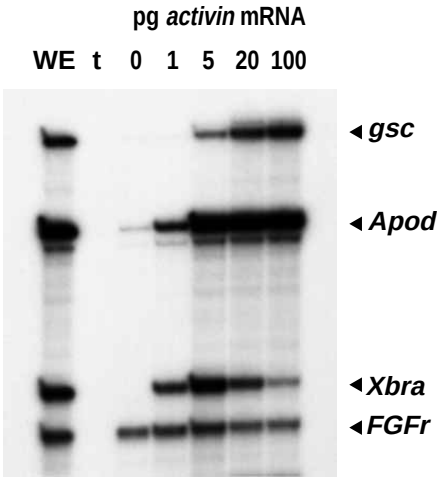

Fig. 6. *Apod* responds to activin in a dose responsive manner and with different kinetics to that of *Xbra*. *Activin* mRNA was injected into the animal pole region of 2-cell-stage embryos at the indicated pg doses. Animal caps were cut at stage 9 and cultured until sibling embryos were at stage 10.25. RNA was extracted from the equivalent of two embryos and analysed by RNase protection for expression of *Apod*, *Xbra* and *gsc*. Quantitation of gels following exposure to phosphorimager screens indicated that *Apod* mRNA expression increases with increasing *activin* doses, whereas *Xbra* mRNA is induced to a maximal level and then levels decline at higher *activin* concentrations. (t, tRNA control).

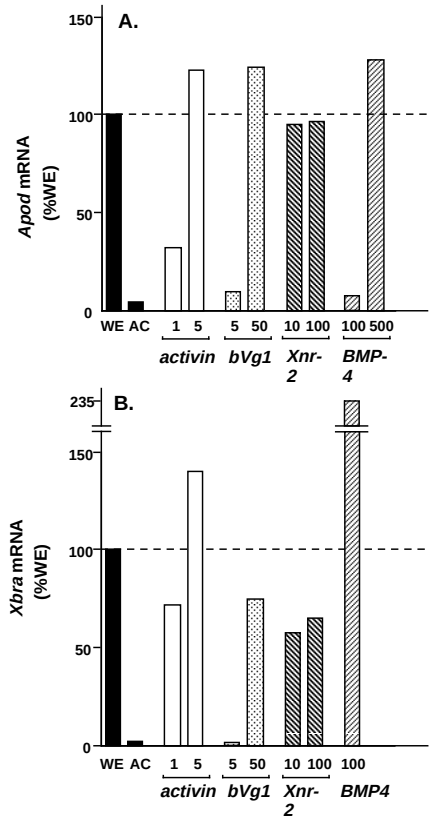

Fig. 7. *Apod* and *Xbra* show different responses to mesoderm-inducing molecules. (A,B) mRNAs for *activin*, *bVg1*, *Xnr-2* and *BMP-4* were individually injected at the indicated pg doses into the animal pole region of 2-cell-stage embryos. Animal caps were isolated at stage 9, cultured until sibling embryos had reached stage 10.25 and then RNA analysed by RNase protection assay for the expression of *Apod* (A) and *Xbra* (B) mRNA. Values for *Apod* mRNA levels in uninjected (AC) and mRNA-injected animal caps were obtained by exposing gels to phosporimager screens and were standardised with values for *FGFr* mRNA levels. Final values were expressed as a percentage of the standardised mRNA levels found in the whole embryo (WE). The dashed line at 100% represents the percentage of each gene found in the WE. These data demonstrate that *Apod* responds to all TGF-β molecules tested, regagrdless of their dorsalisation activity.

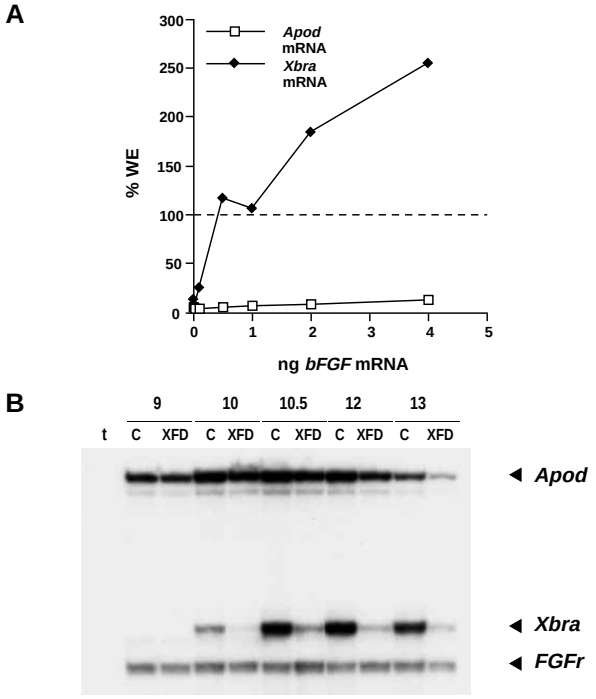

Fig. 8. Dependency of *Apod* and *Xbra* on bFGF. (A) *bFGF* mRNA was injected at the indicated ng doses into the animal pole region of 2-cell-stage embryos. Animal caps were isolated at stage 9, cultured until sibling embryos had reached stage 10.25 and then RNA analysed by RNase protection assay for the expression of *Apod* and *Xbra* mRNA. Values for *Apod* mRNA levels in uninjected (AC) and mRNA-injected animal caps were obtained by exposing gels to phosphorimager screens and were standardised with values for *FGFr* mRNA levels. The dashed line at 100% represents the percentage of each gene found in the WE. (B) 1 ng of the dominant negative FGFr construct (XFD) was injected radially in the equatorial zone of 2-cell-stage embryos. Embryos were allowed to develop until the indicated stage of development and the expression of *Apod* and *Xbra* mRNA was then determined in either XFD or uninjected control (C) embryos by RNase protection.

different doses of *activin*, *bVg1*, *Xnr-2* or *Bmp-4* mRNAs were overexpressed in animal caps and then *Apod* and *Xbra* mRNA levels determined (Fig. 7A, B respectively). Values were expressed as a percentage of mRNA levels found in the whole embryo. *Apod* mRNA was strongly induced by activin, bVg1, Xnr-2, and Bmp-4 to levels comparable to, or greater than, those found in the whole embryo. This suggests that the expression of *Apod* does not depend of the degree of dorsalisation promoted by these different TGF-β molecules. *Xbra* also responded to all mesoderm inducers tested. The most significant difference in the response of the two genes was that *Xbra* was considerably more responsive to *BMP-4* than was *Apod*. At a dose of 100 pg, *Apod* was barely induced whereas *Xbra* mRNA levels were over twice that observed in the whole embryo.

There is evidence to suggest that the FGF signalling pathway is required both for the formation of mesoderm with posteroventral characteristics and for the initiation and/or the maintenance of *Xbra* gene expression (Amaya et al., 1991; Isaacs et al., 1994; Schulte-Merker and Smith, 1995). In an attempt to determine the effect of FGF on *Apod* gene expression, we first overexpressed increasing doses of *bFGF* mRNA in animal caps. Surprisingly *Apod* was not induced by *bFGF*, even at high doses, whereas *Xbra* was induced by *bFGF* to levels several times greater than that found in the whole embryo (Fig. 8A).

To further examine the dependence of *Apod* on FGF signalling, we radially injected mRNA for the the dominant negative FGF receptor (XFD) into the equatorial region of 2-cell-stage embryos to abolish FGF signalling (Amaya et al., 1991). We subsequently assayed the level of *Apod* and *Xbra* mRNA by RNase protection assay at different stages of development to see how the expression of these genes was affected. As shown in Fig. 8B, *Apod* mRNA levels are relatively unaffected by XFD expression in the late blastula (stage 9) and early gastrula (stage 10) whereas *Xbra* mRNA levels are severely diminished at equivalent stages. Quantitation using a phoshorimager shows that the level of *Xbra* mRNA in *XFD*-injected, stage 10, early gastrula embryos is reduced to half that found in equivalent uninjected controls. In contrast, the level of *Apod* mRNA is still approximately 90% that observed in control embryos at the same time. From the mid gastrula stage onwards, *Apod*

mRNA levels are then reduced to approximately 60% of control values in *XFD*-injected embryos. In contrast, *Xbra* mRNA levels are markedly reduced to 20 to 30% that of controls. These results are also consistent with our observation that *Apod* mRNA induced in animal cap explants following injection with *activin* mRNA is only partly diminished by coinjection of XFD mRNA, whereas *Xbra* induction is severely blocked (data not shown).

The relative insensitivity of *Apod* to XFD expression compared to *Xbra* suggests that the requirement of these two T-box genes for FGF signalling is quite different. One explanation is that, whereas *Xbra* requires FGF signalling in an autoregulatory loop to maintain its later expression (Isaacs et al., 1994; Schulte-Merker and Smith, 1995), *Apod* does not.

Regulatory interactions exist among *Xenopus* T-box genes

The T-box gene, *Xbra*, is considered to be a marker that defines mesoderm (Smith et al., 1991). Now two more T-box genes, *Eomes* (Ryan et al., 1996) and *Apod*, have been cloned that are expressed pan-mesodermally and can specify a mesodermal cell fate. It is a likely possibility that these and potentially other T-box genes interact in overlapping pathways that bring about mesoderm differentiation and we have indeed already shown that *Apod* is able to induce the expression of *Eomes* and *Xbra* (Fig. 6D). We therefore examined the ability of *Eomes* and *Xbra* to regulate other *Xenopus* T-box genes by ectopically expressing each in the animal pole region of embryos and analysing gene expression by RNase protection in animal cap explants. The expression of *Xwnt-8* was used as a positive control since *Eomes* and *Xbra* have been shown to elevate its mRNA levels (Ryan et al., 1996).

We first examined what effect *Eomes* has on the expression of *Apod*. Fig. 9A shows that injection of 4 ng *Eomes* mRNA into the animal pole can strongly induce the expression of *Apod* mRNA in animal cap explants at stage 10.25. Thus, *Apod* and *Eomes* are able to induce each other's expression and may act in a cross-regulatory loop. *Eomes* is also able to activate *Xbra* (Ryan et al., 1996), suggesting that both *Apod* and *Eomes* are upstream components of the mesoderm induction pathway that is mediated by *Xbra*.

We then asked whether *Xbra* is able to induce *Apod* or *Eomes*. Injection of 4 ng *Xbra* mRNA is unable to increase the level of *Apod* mRNA at stage 10.25 above the low maternal level sometimes found in the large uninjected animal caps (Fig. 9A). Lower or higher concentrations of *Xbra* were still unable to induce *Apod* at this stage (data not shown). However, if caps are assayed at stage 11, some 2 hours later (see Fig. 9B), then induction of *Apod* in response to overexpression of *Xbra* becomes evident. One possible explanation for these observations is that, by stage 11, complex interactions between several genes have had time to occur and the induction of *Apod* mRNA by *Xbra* may now be indirect. Another possibility is that the early mechanisms regulating the initial zygotic expression of *Apod* are different to those regulating the later, maintained expression of *Apod*, and it is the latter on which *Xbra* is able to exert any influence.

These observations show that (1) *Apod* and *Eomes* can induce each others' expression and (2) *Apod* and *Eomes* can both induce *Xbra*. This suggests that once *Apod* and *Eomes* are induced, they may then participate in a regulatory loop where each maintains the others' expression. The mechanism of such 'cross-talk' for defining mesoderm identity will require further investigation.

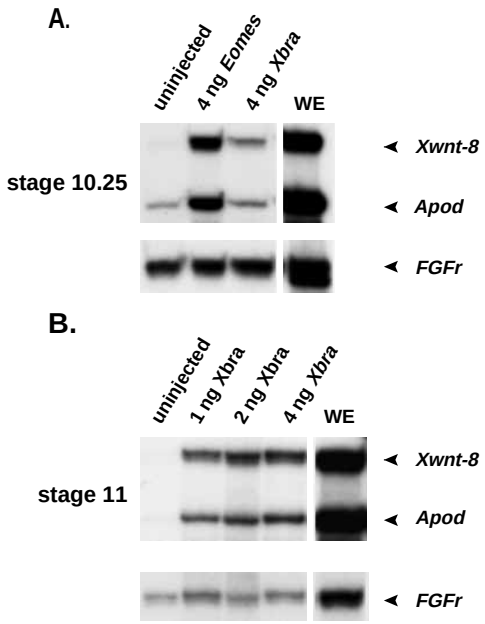

Fig. 9. *Xenopus* T-box genes are able to induce each others' expression. (A) 4 ng of *Xbra* or *Eomes* mRNA was injected into the animal pole region of 2-cell-stage embryos. Animal caps were cut at stage 9, cultured until sibling embryos were at stage 10.25 and then RNA analysed by RNase protection assay for expression of *Apod* and *Xbra* mRNA. All caps were also analysed for expression of *Xwnt-8*, which is not normally expressed in animal caps, but is inducible by *Eomes* and *Xbra*. The low level of *Apod* mRNA in the uninjected animal cap is sometimes observed in the early gastrula and is believed to be a small level of maternal *Apod* mRNA. *Eomes* induces *Apod*, but induction of *Apod* by *Xbra* is not apparent. (B) *Apod* and *Xwnt-8* mRNA levels in response to 1, 2 and 4 ng of *Xbra* mRNA were determined as before, but two hours later (at 23˚C) than in A when sibling embryos were at stage 11. *Xbra* mRNA injection now clearly induces *Apod* mRNA when assayed at this stage of development.

DISCUSSION

The new *Xenopus* T-box gene described here, *Antipodean* (*Apod*), encodes a putative transcription factor that is likely to be important in mesoderm formation for the following reasons: (1) a large maternal content of *Apod* mRNA is localised to the vegetal part of oocytes and early embryos, a region involved in mesoderm induction; (2) zygotic *Apod* transcription takes place in very early mesoderm cells, and can be induced by known mesoderm-inducing molecules of the TGF-β class; (3) ectopic expression of *Apod* in ectodermal (animal cap) cells changes their fate toward the mesoderm lineage.

Localised maternal mRNA of Apod

The study of chordate development (ascidians and *Xenopus*) has provided compelling evidence for the existence of localised maternal factors that play critical roles in the determination of cell fate during early embryogenesis. In ascidian embryos, cytoplasmic factors, probably mRNA molecules, are required for mesoderm, endoderm and epidermis cell differentiation (Nishida, 1996). In amphibians, mesoderm formation is thought to be largely initiated in the equatorial region of the embryo as the result of inductive interactions between vegetal and animal regions. Such processes can be reproduced in vitro

by incubating animal caps with diffusible molecules of the TGF-β and FGF families (reviewed by Slack, 1994) or can be artificially blocked by preventing cell-cell contact during the initial step of mesoderm formation in *Xenopus* embryos (Gurdon et al., 1984; Symes et al., 1988).

In contrast to this 'all-induction' model, Gurdon and colleagues (1985b) identified a subequatorial zone in fertilised eggs needed for the initiation of muscle differentiation in embryos as gastrulation proceeds. The possibility of an autonomous regulatory pathway in mesoderm formation has been further emphasised by the finding that the expression pattern of some genes involved in the definition of the dorso-ventral polarity, *goosecoid* and *Xwnt-8* respectively, does not depend on cell-cell interactions (Lemaire and Gurdon, 1994). Thus, it has been speculated that the accurate formation of mesoderm is dependent on both cell interaction and cell-autonomous pathways.

One way of relating the results that we report here to previous work is to envisage that Apod is an important molecule of both regulatory pathways and that vegetally localised maternal *Apod* mRNA may act as a maternal determinant for the mesoderm lineage in a subequatorial position. Two possibilities might be envisaged: (1) a subset of cells that inherit *Apod* mRNA are defined as potential mesodermal cells; (2) alternatively, maternal *Apod* mRNA may be primarily involved in establishing the specification of the endogenous mesoderm-inducing tissue, the endoderm. Further investigation will be necessary to distinguish between these possible roles of maternal *Apod* expression.

Apod appears to the first example of a trancription factor mRNA localised during oogenesis to the vegetal region. Other maternal mRNAs that are first localised to the vegetal pole at a similar time as *Apod* include *Xwnt-11* (Ku and Melton, 1993), *Xcat-2* (Mosquera et al., 1993; Forristall et al., 1995), *Xcat-3* (Elinson et al., 1993) and *Xlsirts* (Kloc et al., 1993). In contrast, *Vg1*, is localised to the cortex later than *Apod*, at stage 4 (Weeks and Melton, 1987). *Apod* is different from these other vegetally localised mRNAs in that it is predominantly expressed in the vegetal yolk mass of the mature oocyte, as opposed to the cortex. Furthermore, it is more concentrated in a subequatorial position, a novel location amongst characterised *Xenopus* mRNAs. It has been proposed that *Xcat-2* and *Xlsirts* move to the vegetal pole with the mitochondrial cloud as it moves from near the germinal vesicle to the vegetal cortex (Forristall et al., 1995; Kloc et al., 1993). The localisation of *Apod* to the vegetal pole at a similar time as these mRNAs suggests that it may also be dependent on this process.

Zygotic transcription of *Apod*

The early zygotic trancription of *Apod* is localised to the mesodermal region of gastrula embryos and, with *Eomes* (Ryan et al., 1996), it is the earliest mesodermally localised gene expression. We have shown here that *Apod* expression is induced by all molecules of the TGF-β class that we have tested, with an efficiency that is independent of their dorsalising activity. Surprisingly, and in contrast to *Xbra*, we have been unable to activate *Apod* by *bFGF*. Activin-inducible, mesodermal genes can be classified according to the extent to which they depend on FGF signalling. Expression of *Xbra*, *MyoD* and muscle actin require FGF signalling for their expression, whereas *Xlim-1*, *Xwnt-8*, *gsc* and *Mix.1* do not (Cornell and Kimelman, 1994; Cornell et al., 1995). The inability of *Apod* to respond to bFGF potentially places *Apod* in the latter class of genes. This may indicate that the TGF-β and FGF molecules induce/maintain mesoderm by activating different sets of T-box genes, which in turn control the expression of different subsets of mesodermal genes in *Xenopus* embryos.

All these activin inducible genes are expressed in mesoderm cells during gastrula stages in *Xenopus* development. However, only those that do not depend on FGF signalling, i.e. *Xlim-1*, *Xwnt-8*, *gsc*, *Mix.1*, are also expressed in endodermal cells at the same stages (Rosa, 1989; Cornell et al., 1995; Lemaire and Gurdon, 1994). Furthermore, their endodermal expression is dependent only on an intact activin or activin related signalling pathway (Hemmati-Brivanlou and Melton, 1992; Schulte-Merker et al., 1994; Cornell et al., 1995). In addition, TGF-β molecules seem to be important for patterning the expression of some endodermal genes along the dorsoventral axis (Henry et al., 1996). Taken together, these observations support the view that, despite the fundamental difference in behaviour, endoderm and mesoderm cells are subject to similar TGF-β signalling regulatory pathways and dorso-ventral patterning.

Our data show that *Apod* is dependent on TGF-β, but not FGF signalling. It is expressed maternally in the vegetal pole from which endodermal lineages arise, as well as zygotically in what may be both the mesoderm and parts of the endoderm. The possibility exists, therefore, that zygotic *Apod* may be involved with co-ordinating the initiation of both mesoderm and endoderm lineages in response to a TGF-β signal.

Ectopic expression of *Apod*

Developmentally important genes are generally able to activate the trancription of other very early genes, by over or ectopic expression. *Apod* has this ability, since the injection of *Apod* mRNA in animal caps induces most other early mesodermal genes, notably *Eomes* and *Xbra*. These genes are all expressed in an overlapping distribution throughout most of the mesoderm, *Xbra* following soon after *Eomes* and *Apod*. *Eomes* and *Apod* can induce each other, and both can induce *Xbra*. This may reflect a mechanism by which early T-box genes maintain and amplify mesoderm gene expression in the correct region of the embryo after its initiation.

By the late gastrula stages, *Apod* and *Xbra* expression is complementary; *Xbra* mRNA is expressed in the notochord, whereas *Apod* mRNA is associated with ventral structures and is excluded from notochord cells. The mechanisms responsible for this tissue specificity are likely to be quite complex, and may involve regulation by different signalling pathways.

In view of the localised maternal content of *Apod* mRNA, it is possible that its translation helps to initiate the mesodermally localised transcription of other T-box genes, thereby initiating a cascade of expression of this class of gene and leading to mesodermal differentiation.

We are very grateful to Dr Aaron Zorn for invaluable suggestions and discussions. We also thank Dr Ken Ryan for use of the *Eomesodermin* clone and unpublished information. This manuscript was improved upon critical reading by Drs Ken Ryan and Aaron Zorn. We thank Nigel Garrett, Andrew Mitchell and Dr Nobu Kikyo for excellent technical advice and Elizabeth Tweed for keeping the frog colony. This work was supported by the Cancer Research Campaign. F. S. is a Cancer Research Campaign Postdoctoral Fellow and G. C is a European Science Foundation and European Postdoctoral fellow.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell* **66**, 257-270.

Asashima, M., Nakano, H., Shimada, K., Kinoshita, K., Ishii, K., Shibai, H. and Ueno, N. (1990). Mesodermal induction in early amphibian embryos by activin A (erythroid differentiation factor) *Roux's Arch. Dev. Biol.* **198**, 330-335.

Bollag, R. J., Siegfried, A., Cebra-Thomas, J. A., Garvey, N., Davison, E. M. and Silver, L. M. (1994). An ancient family of embryonically expressed mouse genes sharing a conserved protein motif with the T-locus. *Nature Genet.* **7**, 383-389.

Campbell, C., Goodrich, K., Casey, G. and Beatty, B. (1995). Cloning and mapping of a human gene (*TBX2*) sharing a highly conserved protein motif with the *Drosophila omb* gene. *Genomics* **28**, 255-260.

Carnac, G., Kodjabachian, L., Gurdon, J. B. and Lemaire, P. (1996). The homeobox gene *Siamois* is a target of the wnt dorsalisation pathway and triggers organiser activity in the absence of mesoderm. *Development* (in press).

Christian, J. L., McMahon, J. A., McMahon, A. P. and Moon, R. T. (1991). *Xwnt-8*, a *Xenopus Wnt-1/int-1*-related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesodermal patterning during embryogenesis. *Development* **111**, 1045-1055.

Cornell, R. A. and Kimelman, D. (1994). Activin-mediated mesoderm induction requires FGF. *Development* **120**, 453-462.

Cornell, R. A., Musci, T. J. and Kimelman, D. (1995). FGF is a prospective competence factor for early activin-type signals in *Xenopus* mesoderm induction. *Development* **121**, 2429-2437.

Cunliffe, V. and Smith, J. C. (1992). Ectopic mesoderm formation in *Xenopus* embryos caused by widespread expression of a *Brachyury* homologue. *Nature* **358**, 427-430.

Dale, L., Howes, G., Price, B. M. and Smith, J. C. (1992). Bone morphogenetic protein 4: a ventralizing factor in early *Xenopus* development. *Development* **115**, 573-585.

Elinson, R. P., King, M. L. and Forristall, C. (1993). Isolated vegetal cortex from *Xenopus* oocytes selectively retains localized mRNAs. *Dev. Biol.* **160**,554-562.

Forristall, C., Pondel, M., Chen, L. and King, M. L. (1995). Patterns of localization and cytoskeletal association of two vegetally localized RNAs, *Vg1* and *Xcat-2*. *Development* **121**, 201-208.

Green, J. B. A., New, H. V. and Smith, J. C. (1992). Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell* **71**, 731-739.

Gurdon, J. B., Brennan, S., Fairman, S. and Mohun, T. J. (1984). Transcription of muscle-specific genes in early *Xenopus* development: nuclear transplantation and cell dissociation. *Cell* **38**, 691-700.

Gurdon, J. B., Fairman, S., Mohun, T. J. and Brennan, S. (1985a). The activation of muscle specific actin genes in *Xenopus* development by an induction between animal and vegetal cells of a blastula. *Cell* **41**, 913-922.

Gurdon, J. B, Mohun, T. J., Fairman, S. and Brennan, S. (1985b). All components required for the eventual activation of muscle specific genes are localised in the subequatorial region of an uncleaved egg. *Proc. Natl. Acad. Sci. USA* **82**, 139-143.

Gurdon, J. B. (1992). The generation of diversity and pattern in animal development. *Cell* **68**, 185-199.

Gurdon, J. B., Harger, P., Mitchell, A. and Lemaire, P. (1994). Activin signalling and response to a morphogen gradient. *Nature* **371**, 487-492.

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

Henry, G. L., Brivanlou, I. H., Kessler, D. S., Hemmati-Brivanlou, A. and Melton, D. A. (1996). TGF-β signals and a prepattern in *Xenopus laevis* endodermal development. *Development* **122**, 1007-1015.

Hemmati-Brivanlou, A. and Harland, R. A. (1989). Expression of an engrailed-related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development* **106**, 611-617.

Hemmati-Brivanlou, A., Frank, D., Bolce, M. E., Brown, R. D., Sive, H. L. and Harland, R. M. (1990). Localization of specific mRNAs in *Xenopus* embryos by whole mount in situ hybridization. *Development* **110**, 325-330.

Hemmati-Brivanlou, A. and Melton, D. A. (1992). A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. *Nature* **359**, 609-614.

Isaacs, H. V., Pownall, M. E. and Slack, J. M. W. (1994). eFGF regulates *Xbra* expression during *Xenopus* gastrulation. *EMBO J.* **13**, 4469-4481.

Jones, C. M., Kuehn, M. R., Hogan, B. L. M., Smith, J. C. and Wright, C. V. E. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121**, 3651-3662.

Jones, E. A. and Woodland, H. R. (1987). The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. *Development* **101**, 557-563.

Kessler, D. S. and Melton, D. A. (1995). Induction of dorsal mesoderm by soluble mature Vg1 protein. *Development* **121**, 2155-2164.

Kispert, A., Koschorz, B. and Herrmann, G. (1995). The T-protein encoded by *Brachyury* is a tissue specific transcription factor. *EMBO J.* **14**, 4763-4772.

Kloc, M., Spohr, G. and Etkin, L. D. (1993). Translocation of repetitive RNA sequences with the germ plasm in *Xenopus* oocytes. *Science* **262**, 1712-1714.

Ku, M. and Melton, D. A. (1993). *Xlwnt-11*: a maternally expressed *Xenopus* wnt gene. *Development* **119**, 1161-1173.

Lemaire, P. and Gurdon, J. B. (1994). A role for cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of the goosecoid and Xwnt-8 genes along the dorsoventral axis of early *Xenopus* embryos. *Development* **120,** 1191-1199.

Lemaire, P., Garrett, N. and Gurdon, J. B. (1995). Expression cloning of *Siamois*, a *Xenopus* homeobox gene expressed in dorsal-vegetal cells of blastulae and able to induce a complete secondary axis. *Cell* **81**, 85-94.

Liang, P. and Pardee, A. B. (1992). Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257**, 967-971.

Mosquera, L., Forristall, C., Zhou, Y. and King, M. L. (1993). A mRNA localized to the vegetal cortex of *Xenopus* oocytes encodes a protein with a nanos-like zinc finger domain. *Development* **117**, 377-386.

Nieuwkoop, P. D. and Faber, J. (1967). *Normal Table of Xenopus laevis (Daudin)*, 2nd ed. Amsterdam: North Holland.

Nishida, H. (1996). Vegetal cytoplasm promotes gastrulation and is responsible for specification of vegetal blastomeres in embryos of the ascidian *Halocynthia roretzi*. *Development* **122**, 1271-1279.

Pflugfelder, G. O., Roth, H., Poeck, B., Kerscher, S., Schwartz, H., Jonschker, B. and Heisenberg, M. (1992). The *lethal (1) optomotor-blind* gene of *Drosophila melanogaster* is a major organizer of optic lobe development: isolation and characterization of the gene. *Proc. Natl. Acad. Sci. USA* **89**, 1199-1203.

Rosa, F.M. (1989). *Mix.1*, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of *Xenopus* embryos. *Cell* **57,** 965-974.

Ryan, K., Garrett, N., Mitchell, A. and Gurdon, J. B. (1996). *Eomesodermin*, a key early gene in *Xenopus* mesoderm differentiation. *Cell*, in press.

Schulte-Merker, S., Smith, J. C. and Dale, L. (1994). Effects of truncated activin and FGF receptors and of follistatin on the inducing activities of BVg1 and activin: does activin play a role in mesoderm induction? *EMBO J.* **13,** 3533-3541.

Schulte-Merker, S. and Smith, J. C. (1995). Mesoderm formation in response to Brachyury requires FGF signalling. *Curr. Biol.* **5,** 62-67.

Slack, J. M. W. (1994). Inducing factors in *Xenopus* early embryos. *Curr. Biol.* **4**, 116-126.

Smith, J. C., Price, B. M., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a *Xenopus* homolog of *brachyury (T)* is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.

Symes, K., Yaqoob, M. and Smith, J. C. (1988). Mesoderm induction in *Xenopus* laevis: responding cells must be in contact for mesoderm formation but suppression of epidermal differentiation can occur in single cells. *Development*, **104**, 609-618.

Thompson, J. and Slack, J. M. (1992). Over-expression of fibroblast growth factors in *Xenopus* embryos. *Mech. Dev.* **38**, 175-182.

Thomsen, G., Woolf, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. and Melton, D. A. (1990). Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell* **63**,485-93.

Thomsen, G. H. and Melton, D. A. (1993). Processed Vg1 protein is an axial mesoderm inducer in *Xenopus*. *Cell* **74**, 433-441.

Weeks, D. L. and Melton, D. A. (1987). A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-β. *Cell* **51**, 861-867.

Wittbrodt, J. and Rosa, F. (1994). Disruption of mesoderm and axis formation in fish by ectopic expression of activin variants: the role of maternal activin. *Genes Dev.* **8**, 1448-1462.

(Accepted 28 October 1996)