The EMBO Journal Vol.18 No.21 pp.6062–6072, 1999

The zinc finger gene *Xblimp1* controls anterior endomesodermal cell fate in Spemann's organizer

Flávio S.J.de Souza, Volker Gawantka, Aitana Perea Gómez[1], Hajo Delius[2], Siew-Lan Ang[1] and Christof Niehrs[3]

Division of Molecular Embryology and [2]Division of Applied Tumour Virology, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany and [1]Institut de Génétique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/Université Louis Pasteur/Collège de France, BP163, 67404 Illkirch cedex, CU de Strasbourg, France

[3]Corresponding author

The anterior endomesoderm of the early *Xenopus* gastrula is a part of Spemann's organizer and is important for head induction. Here we describe *Xblimp1*, which encodes a zinc finger transcriptional repressor expressed in the anterior endomesoderm. *Xblimp1* represses trunk mesoderm and induces anterior endomesoderm in a cooperative manner with the pan-endodermal gene *Mix.1*. Furthermore, *Xblimp1* can cooperate with the BMP inhibitor *chordin* to induce ectopic heads, while a dominant-negative *Xblimp1* inhibits head formation. The head inducer *cerberus* is positively regulated by *Xblimp1* and is able to rescue microcephalic embryos caused by dominant-negative *Xblimp1*. Our results indicate that *Xblimp1* is required for anterior endomesodermal cell fate and head induction.

Keywords: Blimp-1/cerberus/endoderm/head formation/PRDI-BF1

Introduction

The study of early endoderm development has been a relatively neglected topic until very recently, when the availability of early endodermal markers allowed the identification of genes important for endoderm formation in *Xenopus* embryos. Transforming growth factor-β (TGF-β) signalling by Vg1 and nodal-related proteins is strongly implicated in early endoderm formation (Henry *et al.*, 1996; Joseph and Melton, 1998; Zorn *et al.*, 1999). Transcription factors involved in endoderm specification include members of the HMG-box (Hudson *et al.*, 1997), T-box (Zhang *et al.*, 1998) and paired-homeobox (Ecochard *et al.*, 1998; Henry and Melton, 1998; Lemaire *et al.*, 1998) families. These genes are expressed throughout the whole embryonic endoderm and are implicated in pan-endodermal development.

The expression of many organizer genes reveals that the anterior endoderm is already regionalized in the early gastrula. At this stage, the anterior endodermal cells are mixed with mesoderm precursors and are therefore referred to as anterior endomesoderm. These cells are the first to migrate under the blastocoel roof during gastrulation, and give rise to liver, foregut and prechordal endomesoderm (Pasteels, 1949; Nieuwkoop and Florschütz, 1950; Keller, 1991; Bouwmeester *et al.*, 1996). The activity of one gene expressed in the anterior endomesoderm, *cerberus*, has given strong molecular support to the idea that this region is crucial in the process of head induction (reviewed in Slack and Tannahill, 1992; Gilbert and Saxen, 1993; Bouwmeester and Leyns, 1997; Niehrs, 1999). Cerberus is a secreted factor able to induce ectopic heads including forebrain, eye, cement gland and heart in *Xenopus* (Bouwmeester *et al.*, 1996). Independent evidence for the importance of endoderm in forebrain induction comes from studies in mouse, where ablation of anterior visceral endodermal cells (Thomas and Beddington, 1996) as well as inactivation of genes such as *nodal* (Varlet *et al.*, 1997) and *otx2* (Rhinn *et al.*, 1998) in visceral endoderm cause rostral truncations of the neural tube (for a review, see Beddington and Robertson, 1999). The anterior portion of the visceral endoderm (AVE) lies beneath the future forebrain at the beginning of gastrulation and expresses markers similar to those found in the anterior endomesoderm of *Xenopus*, including *cerberus-like* (Belo *et al.*, 1997; Biben *et al.*, 1998; Shawlot *et al.*, 1998), the homeobox gene *Hex* (Newman *et al.*, 1997; Thomas *et al.*, 1998) and the murine homologue of *dkk1* (Glinka *et al.*, 1998; Pearce *et al.*, 1999), suggesting that the AVE and the anterior endomesoderm of *Xenopus* are homologous structures.

In addition to *cerberus*, *frzb* (Leyns *et al.*, 1997; Wang *et al.*, 1997) and *dkk1* (Glinka *et al.*, 1998) are expressed in *Xenopus* anterior endomesoderm. These genes encode secreted proteins that inhibit signalling by Wnt family members, and when co-expressed with BMP inhibitors such as Chordin (Sasai *et al.*, 1994) and Noggin (Smith and Harland, 1992) can induce a head (Glinka *et al.*, 1997). According to the two-inhibitor model (Glinka *et al.*, 1997), the head organizer, consisting of anterior endomesoderm, secretes BMP and Wnt inhibitors, while the trunk organizer, corresponding to the more posterior chordamesoderm, secretes predominantly BMP inhibitors. It was found recently that the head inducer *cerberus* can bind to and inactivate BMP, Wnt and nodal-related molecules, suggesting that the inhibition of nodal signalling is also important for head induction (Piccolo *et al.*, 1999).

While this presents a framework for the signals involved in Spemann organizer function, little is known about transcription factors specifying the anterior endomesoderm. Here, we present *Xblimp1*, a gene expressed in the anterior endomesoderm of the *Xenopus* organizer. *Xblimp1* encodes a zinc finger transcriptional repressor which is the *Xenopus* homologue of human PRDI-BF1 (Keller and Maniatis, 1991) and mouse Blimp-1 (Turner *et al.*, 1994). In mammals, PRDI-BF1 represses transcription from the

β-interferon promoter after viral infection (Keller and Maniatis, 1991) and Blimp-1 promotes survival and maturation of B-cell precursors in the immune system (Turner *et al.*, 1994; Messika *et al.*, 1998) by a mechanism that involves the direct repression of the c-*myc* promoter (Lin *et al.*, 1997).

Here we show that *Xblimp1 is* expressed in the anterior endomesoderm and its overexpression represses trunk mesoderm and induces anterior endomesoderm. Studies with a dominant-negative *Xblimp1* suggest that the gene is required for *cerberus* expression and head formation. In addition, in co-injections with the BMP inhibitor *chordin*, *Xblimp1* can induce ectopic heads. The results suggest an important role for *Xblimp1* in the anterior endomesoderm, controlling *cerberus* expression and head formation.

Results

Cloning of Xblimp1

We are carrying out a large-scale *in situ* hybridization screen to identify genes differentially expressed during early *Xenopus* development. During this screen, a 1 kb cDNA, *23E9.1*, was found to be expressed in the anterior endomesodermal cells (Gawantka *et al.*, 1998) and was chosen for further characterization. A 3.4 kb cDNA clone was recovered subsequently, which contained a complete open reading frame (ORF) coding for a putative protein of 780 amino acids. The deduced amino acid sequence is most similar to the zinc finger protein known as PRDI-BF1 in humans (Keller and Maniatis, 1991) and Blimp-1 in the mouse (Turner *et al.*, 1994), and we named the gene *Xblimp1*. As Figure 1 shows, Xblimp1 and mouse Blimp-1 proteins have nearly the same size and share an overall identity of 71%. The identity is distributed along the whole sequence, but is particularly high (93%) in the five zinc finger motifs found near the C-terminus of the proteins. Along with the mammalian sequences, an ORF in *Caenorhabditis elegans* (Wilson *et al.*, 1994) encodes a putative protein containing five zinc finger motifs which has 35% overall identity and 42% similarity to Xblimp1, indicating that the PRDI-BF1/Blimp-1 factor is of ancient origin.

We conclude that *Xblimp1 is* a *Xenopus* homologue of the mammalian zinc finger gene *PRDI-BF1*/*Blimp-1*.

Xblimp1 is expressed in anterior endomesoderm and prechordal plate

By RT–PCR analysis, *Xblimp1* transcription starts after mid-blastula transition (stage 8.5), and transcripts can still be found at least until tadpole stages (not shown). Whole-mount *in situ* hybridization of sagittal cuts of early gastrulae show that *Xblimp1* transcripts are present in anterior endomesodermal cells, the region primarily fated to become liver and prechordal plate (Pasteels, 1949; Nieuwkoop and Florschütz, 1950; Keller, 1991), in a pattern similar to that of *cerberus* (stage 10.5, Figure 2A and B) (Bouwmeester *et al.*, 1996), but in a narrower arc. In addition to deep cells, *Xblimp1* is also weakly expressed in the ectoderm. By the end of gastrulation (stage 13, Figure 2D), *Xblimp1* is no longer expressed in the anterior-most endodermal cells, but maintained in the prechordal plate, in both the mesodermal and endodermal layers. Transcripts are also seen in both neural plate and future epidermis in three longitudinal stripes of expression (Figure 2C, asterisks). Expression is also found in 2–4 stripes near the dorsal midline of the embryo (Figure 2C), and transversal cuts indicate that the expression is in paraxial mesoderm (not shown). By tadpole stages (stage 30, Figure 2E), *Xblimp1* is expressed in a variety of tissues: the presumptive pituitary gland, the eyes, the dorsal part of the otic vesicles, the pharyngeal arches (especially the third and fourth), the fin and two stripes in the forming somites near the tip of the tail, which correspond to future somites and can be regarded as a continuation of the expression in the paraxial mesoderm seen at stage 13.

Since the expression of the mammalian homologues of *Xblimp1* has not been studied during development, we performed whole-mount *in situ* hybridizations on mouse embryos. Transcripts can first be detected soon after gastrulation starts. As shown in Figure 2F, mouse

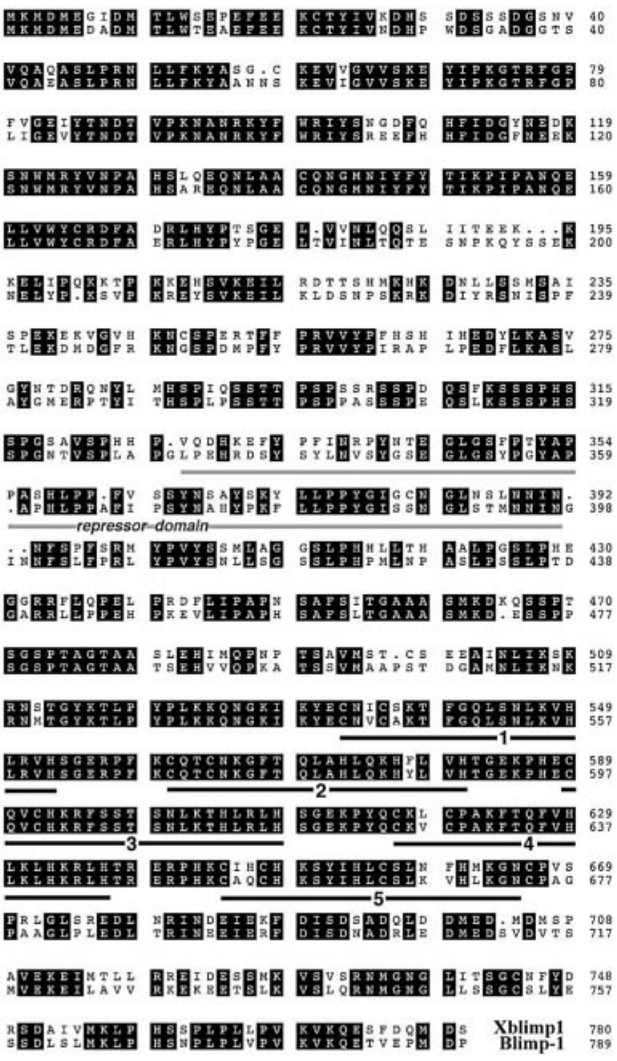

Fig. 1. Alignment of the deduced amino acid sequences of Xblimp1 (top) and mouse Blimp-1 (bottom; Turner *et al.*, 1994). Boxes show identical residues. The five zinc finger motifs near the C-termini are underlined in black. Underlined in grey is the domain mediating transcriptional repression by co-repressors of the Groucho family (repressor domain; Ren *et al.*, 1999). The identity between both proteins is 71% overall and 93% in the zinc finger motifs.

Blimp-1 is weakly expressed at 6.5 days post-coitum (d.p.c.) in the anterior and posterior visceral endoderm and in presumptive definitive endoderm cells. At 7.0 d.p.c. (Figure 2G and H), transcripts are found in the anterior region of the embryo in the prechordal plate as well as in the anterior definitive endoderm. At head fold stage, *Blimp-1* expression is seen in the foregut and adjacent mesoderm (Figure 2I and J). At the 2–3 somite stage, expression is detectable in the anterior neural ridge. Later in development (8.5 d.p.c. onwards), expression is found in the otic placode, ventral telencephalon, branchial arches and somites (not shown). A full description of early *Blimp-1* expression will be published elsewhere (A.P.Gómez and S.-L.Ang, in preparation).

In conclusion, *Xblimp1* and *Blimp-1* are expressed in equivalent regions in the early gastrula, namely anterior endomesoderm and prechordal plate in *Xenopus*, and AVE and prechordal plate in the mouse.

Xblimp1 inhibits mesoderm development

The expression of *Xblimp1* during early gastrulation in the anterior endomesoderm suggested that the gene could play a role in the dorso-anterior region of the embryo, particularly in the endodermal layer. To gain insight into the role of *Xblimp1* during gastrula stages, we carried out a series of overexpression experiments by microinjecting synthetic *Xblimp1* RNA into early *Xenopus* embryos. Increasing doses of *Xblimp1* RNA cause progressive reduction of the antero-posterior axis of the embryos, with retardation of gastrulation and spina bifida defects appearing with higher doses of injected RNA (Figure 3B). These effects suggest that the formation of the main axial structures of the embryo, such as somites and notochord, is impaired by *Xblimp1* overexpression.

To find out which changes in gene expression can be caused by *Xblimp1* during gastrulation, dorsal and ventral marginal zone explants (DMZ and VMZ) injected with *Xblimp1* RNA were analysed by RT–PCR (Figure 3C). The trunk mesoderm markers *Xbra* (pan-mesodermal, Smith *et al.*, 1991), *chordin* (prospective notochord, Sasai *et al.*, 1994) and *myf5* (muscle, Hopwood *et al.*, 1991; Dosch *et al.*, 1997) are repressed by *Xblimp1*. The dorsal homeobox gene *Xlim1* (Taira *et al*., 1994) is also repressed by *Xblimp1*. In the VMZ, the dorso-anterior markers *goosecoid* (Cho *et al.*, 1991), *otx2* (Blitz and Cho, 1995;

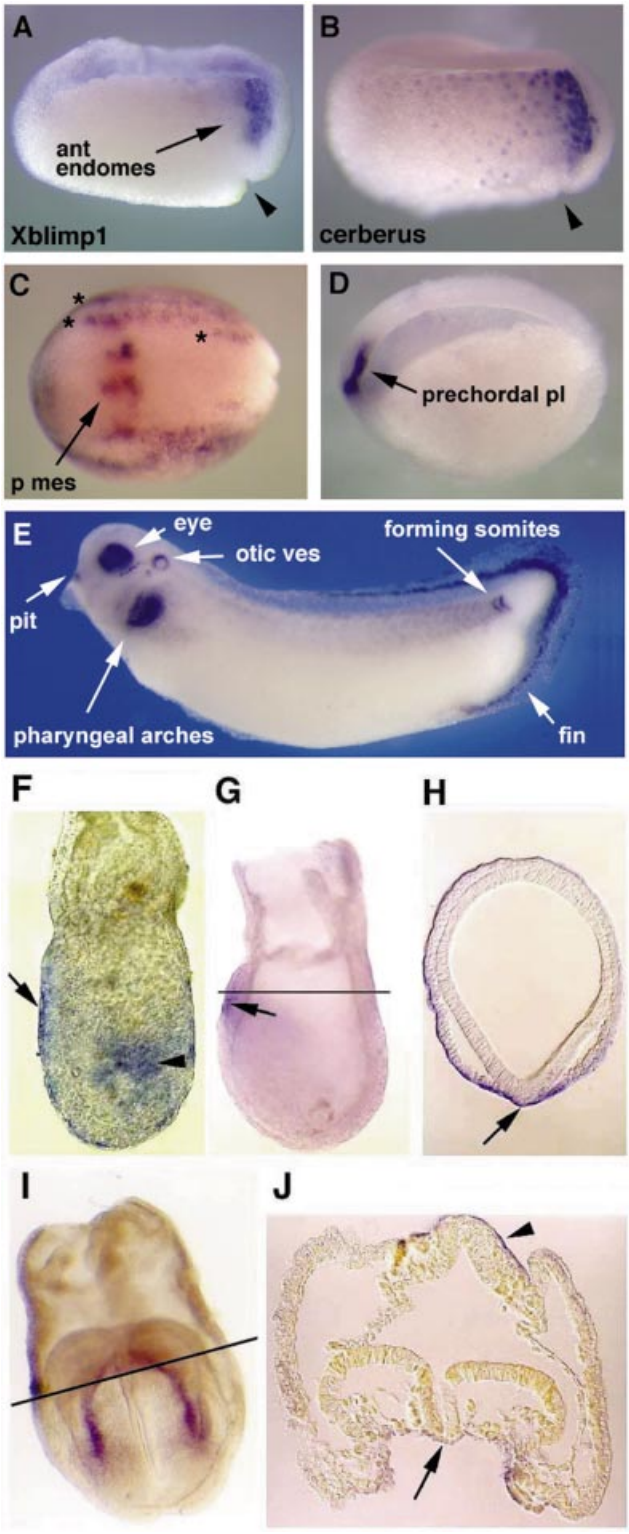

Fig. 2. Expression of *Blimp1* in *Xenopus* and mouse. Whole-mount *in situ* hybridizations are shown in all panels. (**A** and **B**) Sagittal cuts of *Xenopus* gastrulae (stage 10.5) showing the expression of *Xblimp1* and *cerberus*. The dorsal lip is indicated by an arrowhead. Note that both genes are expressed similarly in deep anterior endomesodermal cells (ant endomes). (**C**) Dorsal view of a *Xenopus* neurula (stage 13) showing *Xblimp1* expression in stripes in the neural plate and epidermis (asterisks) and blocks of paraxial mesoderm (p mes). Anterior is to the left. (**D**) Expression of *Xblimp1* in the prechordal plate seen in a sagittally cut neurula. Anterior is to the left. (**E**) Expression of *Xblimp1* in a whole-mount tailbud embryo (stage 30). Transcripts are detected in the future pituitary gland (pit), eye, otic vesicle, pharyngeal arches, fin and forming somites. (**F**) Lateral view of a 6.5 d.p.c. mouse embryo; anterior is to the left. *Blimp-1* is weakly expressed in the anterior (arrow) and posterior visceral endoderm. Expression is also seen in presumptive definitive endoderm (arrowhead). (**G**) Lateral view of a 7.0 d.p.c. mouse embryo; anterior is to the left. *Blimp-1* expression becomes more restricted to the anterior side of the embryo (arrow). The black line shows the plane of the section shown in (H). (**H**) Transversal cut of the embryo shown in (G); anterior is to the bottom. *Blimp-1* is expressed in the prechordal plate and in more lateral anterior definitive endoderm. (**I**) Head-fold-stage embryo in anterior view. *Blimp-1* transcripts are found in the foregut and midgut. The black line indicates the plane of the section shown in (J). (**J**) Transversal cut of the embryo in (I), showing *Blimp-1* expression in the foregut (arrow) and in the endoderm overlying the posterior-most part of the primitive streak (arrowhead).

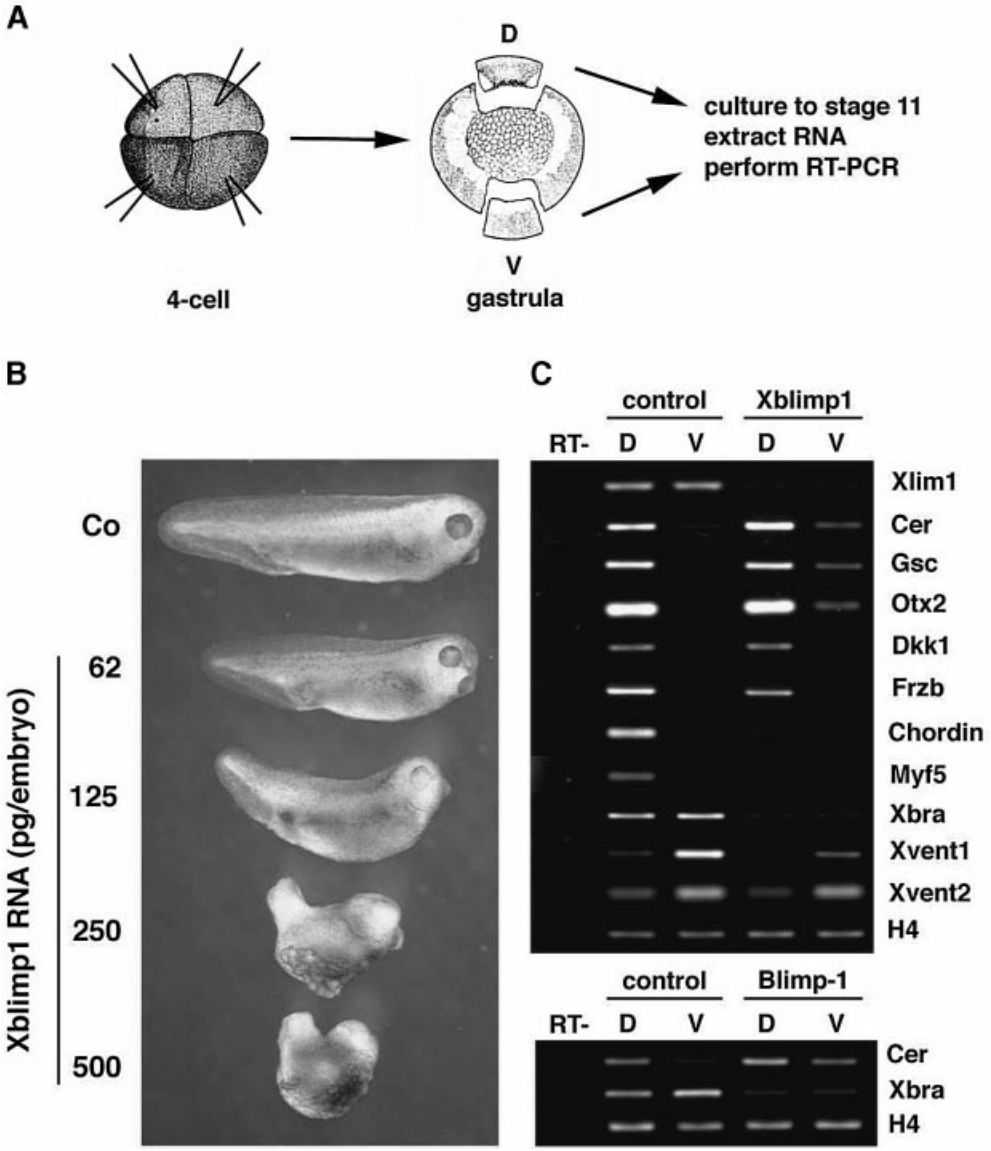

Fig. 3. *Xblimp1* inhibits mesoderm. (**A**) Scheme of the marginal zone experiment. Embryos are injected with RNA into all blastomeres at the 4-cell stage, and dorsal (D) and ventral (V) marginal zone explants are cut at the gastrula stage. Explants are cultured until stage 11, total RNA is extracted and analysed by RT–PCR. (**B**) The indicated amounts of *Xblimp1* RNA were injected into all blastomeres of 4-cell stage embryos. Note that the axis is reduced with increasing amounts of RNA injected. (**C**) Embryos were either uninjected (control) or injected with *Xblimp1* RNA (100 pg/blastomere), and dorsal (D) and ventral (V) marginal zone explants were analysed at stage 11 by RT–PCR for the expression of the indicated genes. Note that the trunk mesoderm markers *Xbra*, *chordin* and *myf5* are inhibited by *Xblimp1*, while the anterior markers *cerberus* (Cer), *goosecoid* (Gsc) and *otx2* are induced. The lower panel shows the same experiment performed with mouse *Blimp-1* (100 pg/blastomere) at stage 13. H4, histone 4; RT–, uninjected whole embryo control sample without reverse transcription.

Pannese *et al.*, 1995) and *cerberus* (Bouwmeester *et al.*, 1996), which are co-expressed with *Xblimp1* in the anterior endomesoderm, are weakly induced. Like the *Xenopus* gene, mouse *Blimp-1* injection represses axial structures (not shown), induces *cerberus* and represses *Xbra* in marginal zone explants (Figure 3C, lower panel), suggesting that both genes have similar properties. Since *Xblimp1* represses mesodermal markers, it is likely that the reduction of the axis seen in injected embryos is caused by a deficiency in the development of axial mesodermal structures such as notochord and somitic mesoderm. We conclude that *Blimp* genes are able to repress mesoderm.

Xblimp1 induces endoderm and cooperates with Mix.1 in inducing cerberus

Since mesoderm inhibitory effects similar to that of *Xblimp1* are typically observed for endoderm-promoting genes, such as *Mix.1* and *Milk* (Rosa, 1989; Ecochard *et al.*, 1998; Lemaire *et al.*, 1998), we tested whether *Xblimp1* could induce endoderm in animal cap explants. RT–PCR analyses (Figure 4B) show that *Xblimp1* can induce pan-endodermal markers such as *endodermin* (Sasai *et al.*, 1996), *Xsox17*α (Hudson *et al.*, 1997) and the new pan-endodermal marker *26D10.1* (Gawantka *et al.*, 1998) in ectoderm. *Xblimp1* could also cause some neuralization, as observed by neural cell adhesion

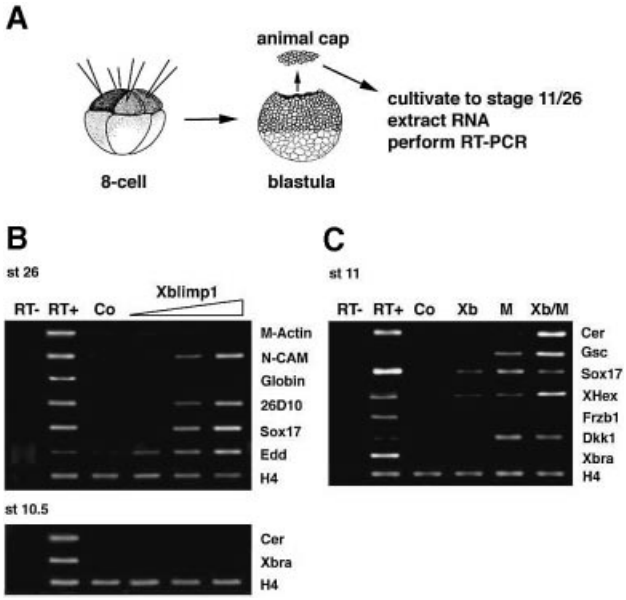

Fig. 4. *Xblimp1* induces anterior endomesoderm. (**A**) Diagram of animal cap experiments. Embryos were microinjected with RNA into four animal blastomeres at the 8-cell stage, animal caps were cut at late blastula stages and explants were cultivated up to stages 26 and 10.5 (B) or 11 (C) and total RNA was extracted and analysed by RT–PCR. (**B**) Animal caps were either uninjected (Co) or injected with increasing concentrations of *Xblimp1* RNA (50, 100 and 200 pg/blastomere). At stage 26, explants injected with *Xblimp1* express the endodermal markers *Xsox17α*, *endodermin* (Edd) and *26D10.1*, as well as the neural marker *NCAM*. At stage 10.5, neither *Xbra* nor *cerberus* is induced by *Xblimp1*. (**C**) *Xblimp1* (Xbl, 100 pg/blastomere) and *Mix.1* (M, 12.5 pg/blastomere) RNAs were either injected alone or in combination (Xbl/M). Note the induction of *cerberus* expression (Cer) by the combination. M-Actin, muscle actin; H4, histone 4; RT+, RT–, uninjected whole embryo control sample with or without reverse transcription, respectively.

molecule (NCAM) induction (Kintner and Melton, 1987), which occurred in the absence of mesoderm, since no *Xbra* or muscle actin (Mohun *et al.*, 1984) was induced (Figure 4B). In some experiments, the blood marker α-globin (Banville and Williams, 1985) was induced (not shown). Finally, *Xblimp1* did not induce *cerberus* in animal caps, unlike in VMZs (see Figure 3C).

The fact that *Xblimp1* induces *cerberus* in VMZs, but not in animal caps (Figure 4B), suggested that a competence factor is missing in ectoderm. Recently, it was found that *Mix.1* serves as a competence factor to *siamois* in the induction of *cerberus* (Lemaire *et al.*, 1995, 1998). Figure 4C shows that *Mix.1* also serves as a competence factor to *Xblimp1*, with both genes cooperating to induce high levels of *cerberus*, while neither *Xblimp1* nor *Mix.1* can induce considerable *cerberus* expression when injected alone. Among other genes expressed in the anterior endomesoderm, *goosecoid* and *XHex* (Newman *et al.*, 1997) are also induced by the combination, while the head inducers *dkk1* and *frzb* (Leyns *et al.*, 1997; Wang *et al.*, 1997; Glinka *et al.*, 1998) are not induced in a cooperative manner. The competence effect was specific to *Mix.1*, because the co-expression of *Xblimp1* and another endoderm-promoting transcription factor, *Xsox17α* (Hudson *et al.*, 1997), was not able to induce anterior endomesodermal markers cooperatively (not shown).

In conclusion, *Xblimp1* is an endodermal inducer in ectodermal explants, and it can cooperate specifically with the pan-endodermal gene *Mix.1* in inducing anterior endomesoderm.

Xblimp1 is required for cerberus expression and head formation

The mammalian zinc finger PRDI-BF1/Blimp-1 works as a transcriptional repressor of the promoters of the β-interferon and c-*myc* genes (Keller and Maniatis, 1991; Lin *et al.*, 1997), and it was shown recently that this repression is mediated by members of the Groucho family of transcriptional co-repressors, which can bind to a repressor domain in the middle of human PRDI-BF1 protein (Figure 1, Ren *et al.*, 1999). In view of that, we constructed a dominant-negative version of *Xblimp1* (*XbVP*, Figure 6A) by fusing its zinc finger domain with the transcriptional activator domain of the viral protein VP16 (Friedman *et al.*, 1988), an approach employed successfully for other transcriptional repressors (Ferreiro *et al.*, 1998; Onichtchouk *et al.*, 1998; Latinkic and Smith, 1999). As a control, we fused the zinc finger domain of Xblimp1 with the transcriptional repressor domain of *Drosophila* Even-skipped (Han and Manley, 1993). This construct (*Xbeve*, Figure 5A) should repress transcription of its target genes, like wild-type Xblimp1.

The injection of RNA for the repressor construct *Xbeve* causes a phenotype similar to that of wild-type *Xblimp1*, with reduction of the dorso-anterior axis and gastrulation defects (Figure 5B). *Xbeve* also induced *cerberus* and repressed *Xbra* and *chordin* in marginal zones, as did wild-type *Xblimp1* (Figure 5C). Importantly, *Xbeve* was able to cooperate with *Mix.1* in inducing *cerberus*-like wild-type *Xblimp1*, while *XbVP* was not (Figure 5D). We conclude that, like its mammalian homologues, *Xblimp1* acts as a transcriptional repressor during early *Xenopus* development.

RNA injection of the activator construct *XbVP* into the dorsal side of embryos led to a microcephalic phenotype, with embryos lacking cement gland and eyes or having cyclopic eyes, indicating disturbances in the induction and/or patterning of the head (Figure 6B). The phenotype is specific, since it can be rescued by the co-injection of *XbVP* with wild-type *Xblimp1* RNA (Figure 6B; Table I, I-1), but not by the co-injection with RNA for another zinc finger gene, *GATA2* (Table I, I-3; Zon *et al.*, 1991).

The microcephaly caused by *XbVP* may be due to a direct interference with the prospective neuroectoderm, since *Xblimp1* is also weakly expressed in the ectoderm, or it could be due to indirect interference with head-inducing anterior endomesoderm. To distinguish between these possibilities, *XbVP* RNA was injected into dorso-vegetal blastomeres of 8-cell-stage embryos, so that the prospective neuroectoderm was not targeted by the injection. Embryos injected in this manner also have head defects and can again be rescued by co-injections with wild-type *Xblimp1* RNA (Table I, I-2), suggesting that the phenotype induced by *XbVP* is caused by an interference with *Xblimp1* function in the anterior endomesoderm.

The anterior endomesoderm secretes the head inducers Cerberus, Frzb and Dkk1, and the microcephaly caused by *XbVP* could indicate that it inhibits the expression of one or more of these factors. To test this, RT–PCR analyses were carried out with gastrula dorsal explants injected with either *XbVP* alone or combinations of *XbVP* and

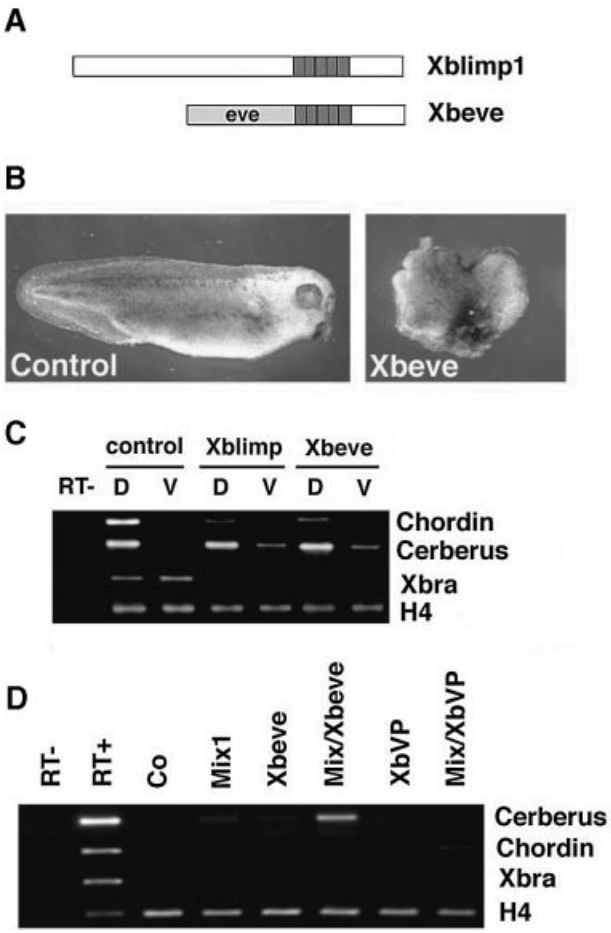

Fig. 5. *Xblimp1* acts as a transcriptional repressor during early development. (**A**) Scheme of wild-type Xblimp1 protein and the Xbeve construct, in which the N-terminal domain of Xblimp1 is replaced by the repressor domain of Even-skipped (eve). Zinc fingers are shown in dark grey. (**B**) Embryo injected into all blastomeres at the 4-cell stage with *Xbeve* RNA (250 pg/blastomere) has axial defects. The phenotype is similar to that of *Xblimp1* RNA injection (Figure 3B). (**C**) RT–PCR of dorsal (D) and ventral (V) marginal zones at stage 10.5 injected with *Xblimp1* (125 pg/blastomere) and *Xbeve* (250 pg/blastomere) RNAs. Note that both *Xblimp1* and *Xbeve* can induce *cerberus* and repress *Xbra* and *chordin*. (**D**) RT–PCR of animal cap explants at stage 11 injected with *Mix.1* (12.5 pg/blastomere), *Xbeve* (beve, 60 pg/blastomere) or *XbVP* (bVP, 30 pg/blastomere) RNAs alone or in combination. Note the *cerberus* induction by the co-injection of *Mix.1* and *Xbeve* (M/beve), but not by *Mix.1* and *XbVP* (M/bVP). Co, uninjected control; H4, histone 4; RT+, RT–, uninjected whole embryo control sample with or without reverse transcription, respectively.

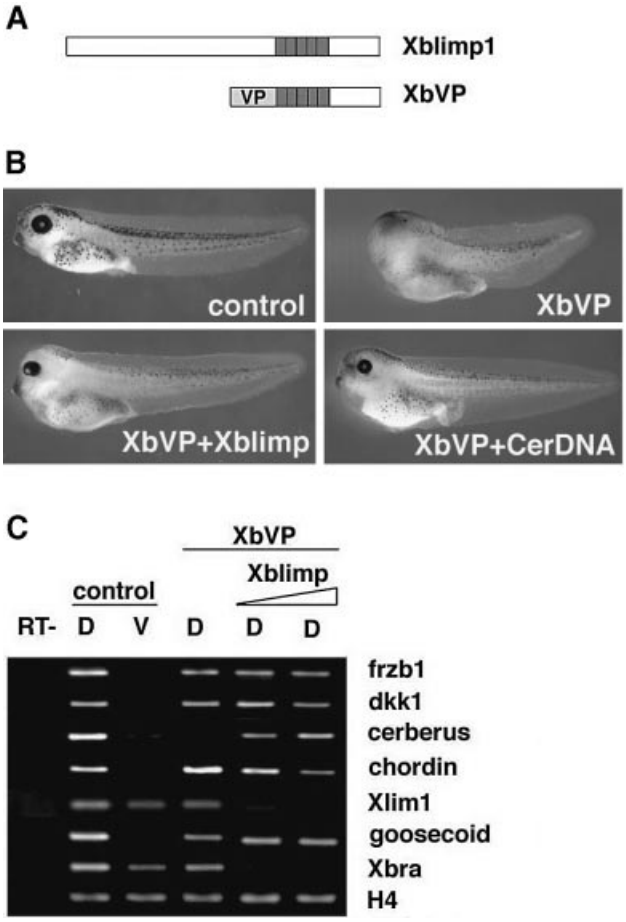

Fig. 6. Requirement for *Xblimp1* in head formation. (**A**) Scheme of wild-type Xblimp1 protein and the dominant-negative construct XbVP, in which the N-terminus of Xblimp1 was substituted by the activator domain of VP16 (VP). The five zinc fingers are in dark grey. (**B**) Embryos were either uninjected (control) or injected into two dorsal blastomeres at the 4-cell stage with 125 pg of *XbVP*, or co-injected with 125 pg of *XbVP* and either 50 pg of *Xblimp1* RNA or 50 pg of *cerberus* DNA. Note that both wild-type *Xblimp1* and *cerberus* can rescue the microcephalic phenotype caused by *XbVP*. (**C**) RT–PCR analysis of stage 10.5 dorsal (D) or ventral (V) marginal zones explanted from embryos that were either uninjected (control) or injected with the indicated RNAs into two dorsal blastomeres at the 4-cell stage. *XbVP* injection (125 pg/embryo) inhibits *cerberus* expression, and this effect can be rescued by co-injection with *Xblimp1* (100–200 pg/embryo). H4, histone 4; RT–, uninjected whole embryo control sample without reverse transcription.

Xblimp1 RNAs. *XbVP* injection reduces the expression of *cerberus*, but not that of the head inducers *frzb* and *dkk1* (Figure 6C). Importantly, the co-injection of wild-type *Xblimp1* RNA rescues *cerberus* expression repressed by *XbVP* (Figure 6C). These results suggest a genetic interaction between *Xblimp1* and *cerberus* in the anterior endomesoderm.

Since *cerberus* was the only gene among the known head inducers whose expression was affected by *XbVP*, we tried to rescue the microcephaly by co-injecting *XbVP* RNA with *cerberus*. Cerberus protein is a nodal inhibitor, and RNA injections interfere precociously with nodal signalling, inhibiting endomesoderm and axis formation (Bouwmeester *et al.*, 1996; Piccolo *et al.*, 1999). To circumvent this, we overexpressed *cerberus* from plasmid DNA after mid-blastula transition. The co-injection of *XbVP* RNA and *cerberus* DNA could rescue head formation, increasing the number of embryos with normal heads up to 60% (Figure 6B; Table I, I-4).

The results indicate that *Xblimp1* works as a transcriptional repressor during early *Xenopus* development, and suggest that it is essential for *cerberus* expression and head induction.

Xblimp1 can induce ectopic heads together with the BMP inhibitor chordin

Even though the results of overexpressing wild-type and dominant-negative *Xblimp1* indicated that the gene plays a role in head formation, we were unable to induce ectopic heads by overexpressing *Xblimp1* alone (not shown). We showed previously that the combined action of BMP and

Table I. *XbVP* causes a microcephalic phenotype that can be rescued by *Xblimp1* RNA and *cerberus* DNA

RNA or DNA injected (pg/embryo)		Two eyes (%)	Cyclopia (%)	No eyes (%)	No. of embryos
I-1	*XbVP* (100–130)	5.2	34.8	60	90
	XbVP + *Xblimp1* (25)	33.1	60.6	6.4	37
	XbVP + *Xblimp1* (50)	65.7	17.1	17.4	75
	XbVP + *Xblimp1* (100)	59.1	10.9	30	27
I-2	*XbVP* (100–200)	18	59.1	22.5	48
	XbVP + *Xblimp* (50–100)	76.2	18.8	5	60
I-3	*XbVP* (100)	13.1	47.2	29	39
	XbVP + *GATA2* (62)	13.6	56.6	29.8	37
	XbVP + *GATA2* (125)	23.4	55.6	21.1	38
	XbVP + *GATA2* (250)	13.5	32.5	54.1	37
I-4	*XbVP* (120)	17.2	33.5	49.4	58
	XbVP + DNA Cer (25)	31.9	33.7	34.5	42
	XbVP + DNA Cer (50)	57.9	12.8	29.2	65
	XbVP + DNA Cer (100)	38.4	45	29.1	43

Four-cell (I-1, I-3 and I-4) or 8-cell (I-2) stage embryos were microinjected into two dorsal or dorso-vegetal blastomeres with *XbVP* RNA alone or in combination with *Xblimp1* (I-1 and I-2) or *GATA2* (I-3) RNAs or with plasmid DNA for *cerberus* (I-4). Only embryos without gastrulation defects (spina bifida) were scored.

Wnt inhibitors can induce secondary axes that contain a well-formed head, while overexpression of any inhibitor alone is unable to do so (Glinka *et al.*, 1997, 1998). To test whether *Xblimp1* could cooperate with a BMP inhibitor in inducing a head, *Xblimp1* RNA was co-injected with RNA encoding the BMP antagonist Chordin into two ventral blastomeres at the 4-cell stage. We found that co-expression of *Xblimp1* and *chordin* can induce ectopic heads in up to 35% of injected embryos, with one cement gland and one or two eyes (Figure 7A; Table II). Neither different doses of *chordin* alone nor co-injections with other BMP inhibitors lead to significant head induction (Glinka *et al.*, 1997; data not shown).

To test whether the expression of any head inducer was superinduced by the combination of *Xblimp1* and *chordin*, we carried out RT–PCRs in injected ventral marginal zones. As shown in Figure 7B, no cooperation could be found in the expression of any of the known head inducers *cerberus*, *frzb* or *dkk1*. Furthermore, the ectopic heads are not induced by the activation of the early axis-inducing Wnt pathway (Moon and Kimelman, 1998), since *siamois* expression is not stimulated by the combination (Figure 7B).

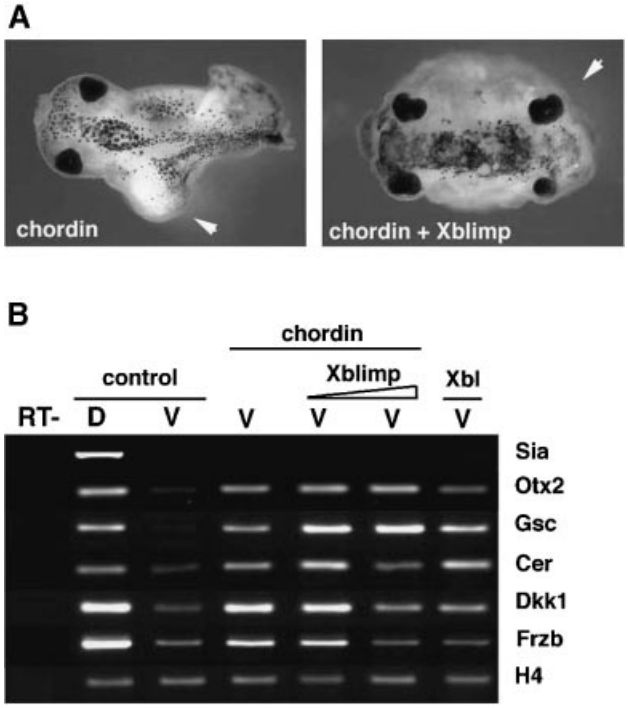

Fig. 7. *Xblimp1* induces an ectopic head together with *chordin*. (**A**) Left panel: 400 pg of *chordin* RNA injected into two ventral blastomeres at the 4-cell stage induce an incomplete secondary axis (arrowhead). Right panel: ventral injection of 400 pg of *chordin* plus 200 pg of *Xblimp1* RNA induces a secondary head (arrowhead). (**B**) RT–PCR analysis of stage 10.5 dorsal (D) or ventral (V) marginal zones explanted from embryos that were either uninjected (control) or injected with the indicated RNAs into two ventral blastomeres at the 4-cell stage. The co-injection of *chordin* (400 pg/embryo) and *Xblimp1* (100–200 pg/embryo) RNAs does not significantly affect the expression of the head inducers *cerberus* (Cer), *frzb* and *dkk1 in* ventral explants in relation to *chordin* or *Xblimp1* (Xbl, 200 pg/embryo) RNAs alone. Sia, *siamois*; Gsc, *goosecoid*; H4, histone 4; RT–, uninjected whole embryo control sample without reverse transcription.

Discussion

In this study, we describe *Xblimp1*, a gene expressed in the anterior endomesoderm of the *Xenopus* organizer, and we present evidence that *Xblimp1* plays a role in specifying this group of cells and in the process of forebrain, or head, induction since: (i) it is necessary and sufficient to induce anterior endomesodermal markers; (ii) it is necessary and sufficient together with BMP inhibitors to confer the head-inducing activity characteristic for this tissue; and (iii) it is expressed at the right time in the anterior endomesoderm to perform this role *in vivo*.

Xblimp1 is expressed in the anterior endomesoderm of Spemann's organizer

Xblimp1 is a *Xenopus* homologue of the mammalian zinc finger transcriptional regulator *PRDI-BF1*/*Blimp-1*. In fibroblasts, *PRDI-BF1* mediates the repression of β-interferon after viral infection (Keller and Maniatis, 1991). In B cells, ectopic expression of *Blimp-1* can partially drive their terminal differentiation into immunoglobulin-secreting plasma cells (Turner *et al.*, 1994). In addition to these roles in adults, PRDI-BF1/Blimp1 and its *Xenopus* homologue are likely to play multiple roles during embry-

Table II. *Xblimp1* induces secondary heads together with the BMP inhibitor *chordin*

RNA (pg/embryo)	Incomplete 2nd axis (%)	Ectopic cement gland (%)	One ectopic eye (%)	Two ectopic eyes (%)	No. of embryos
chordin (400)	90	0	0.6	0	67
chordin + *Xblimp1* (50)	18.5	31.5	21	8	51
chordin + *Xblimp1* (100)	28	43	22	7.5	69
chordin + *Xblimp1* (200)	22.5	34	32.5	5	84

Four-cell stage embryos were injected into two ventral blastomeres with 400 pg of *chordin* RNA alone or in combination with the indicated amounts of *Xblimp1* RNA. Embryos were scored for the formation of ectopic cement glands and one or two eyes.

Table III. Primers used in RT–PCR

Gene	Forward primer 5′–3′	Reverse primer 5′–3′
α-Globin	AGGCTGCAGTTGTTGCTC	TGGTGAGCTGCCCTTGCTGA
*Xsox17*α	CAGAGCAGATCACATCCAACCG	GGAAAGGACAGAAGAAATGGGC
26D10.1	GGGGCAGACGATCAGCAAACGC	AATTGGCATCGGATTCTCTGGC
XHex	TTCACCCTGCCTTCACCCACCC	TTCTGCTCGGCGCTCAAACACC
Xblimp1	AAGATTATGCAGAAAGGGAGGG	GAAAGGAGAAATACAGAGAAGGGG

onic development, since these genes start being expressed in early embryogenesis in both frogs and mice in many different regions.

In *Xenopus*, *Xblimp1* is expressed in the anterior endomesoderm, while *Blimp-1* is expressed in the AVE of the mouse gastrula. Even though the visceral endoderm is an extra-embryonic tissue and does not contribute to the fetus, there is strong evidence indicating that mouse AVE is the equivalent to the anterior endomesoderm of *Xenopus*. Many genes expressed in the anterior endomesoderm from *Xenopus* are also expressed in the mouse AVE (Beddington and Robertson, 1999). Other similarities in the early expression of *Xblimp1* and *Blimp-1* include the expression in the prechodal plate and foregut, and later the expression in the forebrain, otic vesicle, branchial arches and somites.

Like the mammalian homologues, Xblimp1 also functions as a transcriptional repressor during early *Xenopus* development, since injections of the repressor construct *Xbeve* and wild-type *Xblimp1* have similar effects. Consistent with this, the effects of overexpression of *Xenopus* and mouse *Blimp-1* are indistinguishable.

Xblimp1 regulates the fate of anterior endomesoderm

The *Xblimp1*-expressing anterior endomesoderm of the gastrula is fated to become liver, foregut and prechordal mesoderm (Pasteels, 1949; Nieuwkoop and Florschütz, 1950; Keller, 1991; Bouwmeester *et al.*, 1996). *Xblimp1* can repress mesoderm and cause reductions in the anteroposterior axis, a characteristic that it shares with other endoderm regulators such as *Mix.1* and *Milk*. Both genes are expressed in the whole endoderm and act as potent mesodermal repressors, and it was proposed that their role is to restrict mesoderm differentiation to the marginal zone (Ecochard *et al.*, 1998; Lemaire *et al.*, 1998). Apart from repressing mesoderm, *Xblimp1* can induce anterior endomesodermal markers, such as *cerberus* and *goosecoid*, suggesting that it has a role in regulating the fate of anterior endomesoderm. However, *Xblimp1* seems to regulate a subroutine of the anterior endomesodermal differentiation programme, since *Xlim1*, *frzb* and *dkk1* are regulated independently.

Interestingly, even though *Xblimp1 can* induce endodermal markers in animal caps, the induction of the anterior gene *cerberus* can only be achieved when *Xblimp1* is co-expressed with the pan-endodermal gene *Mix.1*. This suggests that in the anterior endomesoderm of the gastrula, where *Mix.1* and *Xblimp1* are co-expressed, both genes may act cooperatively to induce and/or maintain the expression of the head inducer *cerberus*. It was shown previously by Lemaire *et al.* (1998) that the homeobox gene *siamois* can induce *cerberus* in animal caps only when co-expressed with *Mix.1*. Thus, *Mix.1* seems to work as an endodermal competence modifier for both *siamois* and *Xblimp1* in the induction of *cerberus*.

We found that co-injections of *Xblimp1* and the BMP inhibitor *chordin* induce secondary heads. Since BMP inhibitors induce ectopic heads when co-expressed with Wnt inhibitors (Glinka *et al.*, 1997), this suggested that *chordin* and *Xblimp1* might cooperate to induce high levels of Wnt inhibitor expression. However, *cerberus*, *frzb* and *dkk1* are not induced cooperatively. It may be that *Xblimp1* and *chordin* induce an unknown Wnt inhibitor, or that *Xblimp1* inhibits Wnt signalling intracellularly in the endomesoderm.

To corroborate these gain-of-function results, we investigated the requirement for *Xblimp1* using the dominant-negative *XbVP*. A concern working with an artificial transcriptional activator is the specificity of its effects, due to the possibility of unphysiological activation of target genes normally not controlled by the wild type. In this context, the major criterion used for assessing the specificity is the ability of the wild type to rescue the dominant-negative effects. We show that wild-type *Xblimp1* fully rescues the *XbVP* phenoytpe and its effects on marker gene expression. However, even the rescue cannot formally exclude the possibility of artificial activation of some target genes.

XbVP causes microcephaly and inhibits *cerberus* expression. Since the expression of the head-inducing genes *frzb* and *dkk1* was not repressed by *XbVP*, it is possible that the microcephaly is a direct consequence of inhibiting *cerberus* expression in anterior endomesoderm. Consistent with this, we found that injection of *cerberus* can rescue

head formation in *XbVP*-injected embryos. A dominant-negative *Mix.1* (*enRMix.1*) has also been shown to be essential for *cerberus* expression and for head formation (Lemaire *et al.*, 1998; Latinkic and Smith, 1999). Taken together with the cooperative effects between *Xblimp1* and *Mix.1*, this suggests that these genes act in parallel to promote *cerberus* expression and head induction in anterior endomesoderm.

Recent work by Schneider and Mercola (1999) casts doubt over the role of *cerberus* as a head inducer. The authors removed *cerberus*-expressing anterior endoderm from dorsal explants and embryos at stage 10 gastrulae, and observed that head formation was unaffected. The removal of the anterior endoderm plus the prechordal plate, however, inhibited head formation, and the authors concluded that *cerberus*-expressing anterior endoderm is not necessary for head induction. These results may suggest that the head deficiencies observed following *XbVP* overexpression are not caused by the down-regulation of *cerberus*. However, the anterior endoderm could not be removed by Schneider and Mercola prior to early gastrula stage. Since *cerberus* is expressed before gastrulation starts, a role for *cerberus* in head formation prior to this stage could not be ruled out. In addition, the authors did not analyse whether the operated explants had recovered *cerberus* expression by the time forebrain induction takes place. The study, nevertheless, highlights the fact that the prechordal plate is important in head induction and patterning. *Xblimp1* is expressed in both anterior endoderm and prechordal plate, and may play a role in head induction in one or both of these regions. Other factors may exist that are important for head induction and are regulated by *Xblimp1* in the prechordal plate.

cerberus induces ectopic hearts (Bouwmeester *et al.*, 1996), and Schneider and Mercola (1999) showed that *cerberus*-expressing anterior endoderm plays an essential role in heart induction. Since *Xblimp1* acts as a *cerberus* regulator, it may also have a role in the regulation of heart formation. Future work should address this issue.

Along with the similarities in marker gene expression discussed, there is strong evidence that the *Xenopus* anterior endomesoderm and mouse AVE are responsible for forebrain induction in both organisms (Bouwmeester and Leyns, 1997; Beddington and Robertson, 1999). In particular, specific inactivation of *Otx2* and *nodal* in the visceral endoderm causes loss of AVE-specific gene expression and forebrain truncations (Varlet *et al.*, 1997; Rhinn *et al.*, 1998). *Blimp-1* null-mutant embryos die before 9.0 d.p.c. (M.M.Davis, personal communication), confirming that the gene is essential during early vertebrate development. In view of our results with the *Xenopus* homologue, it would be interesting to test whether the specific inactivation of mouse *Blimp-1* in the visceral endodermal layer would cause loss of *cerberus-like* expression and forebrain deficiencies.

Materials and methods

Embryos and explants

In vitro fertilization, embryo culture, staging, microinjection and culture of marginal zone and animal cap explants were carried out as described previously (Gawantka *et al.*, 1995). Marginal zone explants were cultured in 0.3× MBS medium and animal cap explants in 0.5× MBS medium.

Constructs

23E9.1 cDNA (Gawantka *et al.*, 1998) was used to isolate a full-length *Xblimp1* cDNA clone (pBSXblimp1) from a stage 10 cDNA library in λZap II by filter hybridization. The recovered clone (3.4 kb) contains the whole coding region but lacks part of the 3′-untranslated region. The coding region was subcloned into pCS2+ (Rupp *et al.*, 1994) (pCSXblimp1). The DDBJ/EMBL/GenBank accession No. of *Xblimp1* is AF182280.

To construct pCSXbVP, a 261 bp fragment containing the *VP16* activation domain (Friedman *et al.*, 1988) was PCR-amplified from pCMV-GLVP2(H) (Wang *et al.*, 1994) using primers f: GGG**GAATTC**C-TG**ATG**GACTCCCAGCAGCC (*Eco*RI site and starting ATG in bold) and r: GGG**GTCGAC**CTCGTCAATTCCAAGGGC (*Sal*I site in bold). The region coding for amino acids 500–780 of Xblimp1 was PCR-amplified using primers f: GGG**GTCGAC**GAAGAAGCCATCAAT (*Sal*I site in bold) and r: GGG**TCTAGA**TTTCTG**TTA**AGAGTCCAT (*Xba*I site and stop codon in bold). The *Xblimp1* and *VP16* fragments were joined by *Sal*I and cloned into pCS2+ (*Eco*RI and *Xba*I).

To construct pCSXbeve, a fragment containing the *even-skipped* repressor domain (Han and Manley, 1993) was PCR-amplified from eve-BSK (*eve*, kind gift of G.Ryffel) using primers f: GGG**GAATTCATG**A-GCACGATCAAGGTGTGG (*Eco*RI site and starting ATG in bold) and r: GGG**GTCGAC**CGCCTCAGTCTTGTAGGG (*Sal*I site in bold). The region coding for amino acids 500–780 of Xblimp1 was PCR-amplified (same primers as for pCSXbVP cloning), joined to the *even-skipped* fragments and cloned into the pCS2+ vector. PCR amplifications were carried out using the Expand™ High Fidelity kit (Roche).

In situ hybridization

Whole-mount *in situ* hybridizations were carried out as described for mouse (Conlon and Herrmann, 1993) and *Xenopus* (Harland, 1991) with modifications (Gawantka *et al.*, 1995). For wax sections (7 μm), embryos were fixed overnight in Bouin's fixative, dehydrated and embedded in wax. For cryostat sections (14 μm), embryos were post-fixed in 4% paraformaldehyde, equilibrated in 20% sucrose and embedded in OCT (Tissue-Tek, Miles).

Microinjection experiments

The plasmids pCSXblimp1, pCSXbeve and pCSXbVP were linearized with *Not*I, psP64T-GATA2a (Walmsley *et al.*, 1994) was linearized with *Xba*I, and all templates were transcribed with SP6 RNA polymerase using the Megascript kit (Ambion) and a cap:GTP ratio of 5:1. pCS2Cerberus-flag was used in plasmid microinjection experiments (Piccolo *et al.*, 1999).

RT–PCR

RT–PCR assays were carried out as described previously (Gawantka *et al.*, 1995). Gene-specific primers were as follows: H4, *Xvent1*, muscle actin, *goosecoid* (Gawantka *et al.*, 1995), *Xbra* and *chordin* (Glinka *et al.*, 1996), *otx2*, *siamois* and *Xlim1* (Glinka *et al.*, 1997), *NCAM* (Hemmati-Brivanlou *et al.*, 1994), *Xvent2* (Onichtchouk *et al.*, 1996), *dkk1* (Glinka *et al.*, 1998), *cerberus* (Bouwmeester *et al.*, 1996), *endodermin* (Sasai *et al.*, 1996) and *Frzb* (Wang *et al.*, 1997). Other primers are shown in Table III.

Acknowledgements

We thank T.Bouwmeester, M.M.Davis and R.Patient for reagents, and W.Wu and C.Kiecker for comments on the manuscript. F.S.J.S. is supported by the Deutscher Akademischer Austauschdienst. This work was supported by the Deutsche Forschungsgemeinschaft.

References

Banville,D. and Williams,J.G. (1985) The pattern of expression of the *Xenopus laevis* tadpole α-globin genes and the amino acid sequence of the three major tadpole α-globin polypeptides. *Nucleic Acids Res.*, **13**, 5407–5421.

Beddington,R.S.P. and Robertson,E.J. (1999) Axis development and early asymmetry in mammals. *Cell*, **96**, 195–209.

Belo,J.A., Bouwmeester,T., Leyns,L., Kertesz,N., Gallo,M., Follettie,M. and De Robertis,E.M. (1997) Cerberus-like is a secreted factor with neuralizing activity expressed in the anterior primitive endoderm of the mouse gastrula. *Mech. Dev.*, **68**, 45–57.

Biben,C. *et al*. (1998) Murine cerberus homologue mCer-1: a candidate anterior patterning molecule. *Dev. Biol.*, **194**, 135–151.

Blitz,I.L. and Cho,K.W.Y. (1995) Anterior neuroectoderm is progressively induced during gastrulation: the role of the *Xenopus* homeobox gene *orthodenticle*. *Development*, **121**, 993–1004.

Bouwmeester,T. and Leyns,L. (1997) Vertebrate head induction by anterior primitive endoderm. *BioEssays*, **19**, 855–863.

Bouwmeester,T., Kim,S.-H., Sasai,Y., Lu,B. and De Robertis,E.M. (1996) Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature*, **382**, 595–601.

Cho,K.W., Blumberg,B., Steinbeisser,H. and De Robertis,E.M. (1991) Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid*. *Cell*, **67**, 1111–1120.

Conlon,R.A. and Herrmann,B.G. (1993) Detection of messenger RNA by *in situ* hybridization to postimplantation embryo whole mounts. *Methods Enzymol.*, **225**, 373–383.

Dosch,R., Gawantka,V., Delius,H., Blumenstock,C. and Niehrs,C. (1997) Bmp-4 acts as a morphogen in dorsoventral mesoderm patterning in *Xenopus*. *Development*, **124**, 2325–2334.

Ecochard,V., Cayrol,C., Rey,S., Foulquier,F., Caillol,D., Lemaire,P. and Duprat,A.P. (1998) A novel *Xenopus Mix*-like gene *milk* involved in the control of the endomesodermal fates. *Development*, **125**, 2577–2585.

Ferreiro,B., Artinger,M., Cho,K.W.Y. and Niehrs,C. (1998) Antimorphic goosecoids. *Development*, **125**, 1347–1359.

Friedman,A.D., Triezenberg,S.J. and McKnight,S.L. (1988) Expression of a truncated viral trans-activator selectively impedes lytic infection by its cognate virus. *Nature*, **335**, 452–454.

Gawantka,V., Delius,H., Hirschfeld,K., Blumenstock,C. and Niehrs,C. (1995) Antagonizing the Spemann organizer: role of the homeobox gene *Xvent-1*. *EMBO J.*, **14**, 6268–6279.

Gawantka,V., Pollet,N., Delius,H., Pfister,R., Vingron,M., Nitsch,R., Blumenstock,C. and Niehrs,C. (1998) Gene expression screening in *Xenopus* identifies molecular pathways, predicts gene function and provides a global view of embryonic patterning. *Mech. Dev.*, **77**, 95–141.

Gilbert,S.F. and Saxen,L. (1993) Spemann's organizer: models and molecules. *Mech. Dev.*, **41**, 73–89.

Glinka,A., Delius,H., Blumenstock,C. and Niehrs,C. (1996) Combinatorial signalling by *Xwnt-11* and *Xnr3 in* the organizer epithelium. *Mech. Dev.*, **60**, 221–231.

Glinka,A., Wu,W., Onichtchouk,D., Blumenstock,C. and Niehrs,C. (1997) Head induction by simultaneous repression of Bmp and wnt signalling in *Xenopus*. *Nature*, **389**, 517–519.

Glinka,A., Wu,W., Delius,H., Monaghan,P.A., Blumenstock,C. and Niehrs,C. (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature*, **391**, 357–362.

Han,K. and Manley,J.L. (1993) Transcriptional repression by the *Drosophila* even-skipped protein: definition of a minimal repression domain. *Genes Dev.*, **7**, 491–503.

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

Hemmati-Brivanlou,A., Kelly,O.G. and Melton,D.A. (1994) *Follistatin*, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell*, **77**, 283–295.

Henry,G.L. and Melton,D.A. (1998) *Mixer*, a homeobox gene required for endoderm development. *Science*, **281**, 91–96.

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

Hopwood,N.D., Pluck,A. and Gurdon,J.B. (1991) *Xenopus Myf-5* marks early muscle cells and can activate muscle genes ectopically in early embryos. *Development*, **111**, 551–560.

Hudson,C., Clements,D., Friday,R.V., Stott,D. and Woodland,H.R. (1997) *Xsox17*α and -β mediate endoderm formation in *Xenopus*. *Cell*, **91**, 397–405.

Joseph,E.M. and Melton,D.A. (1998) Mutant Vg1 ligands disrupt endoderm and mesoderm formation in *Xenopus* embryos. *Development*, **125**, 2677–2685.

Keller,A.D. and Maniatis,T. (1991) Identification and characterization of a novel repressor of β-interferon gene expression. *Genes Dev.*, **5**, 868–879.

Keller,R. (1991) Early embryonic development of *Xenopus laevis*. *Methods Cell Biol.*, **36**, 61–113.

Kintner,C.R. and Melton,D.A. (1987) Expression of *Xenopus N-CAM* RNA in ectoderm is an early response to neural induction. *Development*, **99**, 311–325.

Latinkic,B.V. and Smith,J.C. (1999) Goosecoid and Mix.1 repress brachyury expression and are required for head formation in *Xenopus*. *Development*, **126**, 1769–1779.

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.

Lemaire,P., Darras,S., Caillol,D. and Kodjabachian,L. (1998) A role for the vegetally expressed *Xenopus* gene *Mix1 in* endoderm formation and in the restriction of mesoderm to the marginal zone. *Development*, **125**, 2371–2380.

Leyns,L., Bouwmeester,T., Kim,S.-H., Piccolo,S. and De Robertis,E.M. (1997) *Frzb-1 is* a secreted antagonist of wnt-signals expressed in the Spemann organizer. *Cell*, **88**, 747–756.

Lin,Y., Wong,K. and Calame,K. (1997) Repression of c-*myc* transcription by Blimp-1, an inducer of terminal B cell differentiation. *Science*, **276**, 596–599.

Messika,E.J., Lu,P.S., Sung,Y.J., Yao,T., Chi,J.T., Chien,Y.H. and Davis,M.M. (1998) Differential effect of B lymphocyte-induced maturation protein (Blimp-1) expression on cell fate during B cell development. *J. Exp. Med.*, **188**, 515–525.

Mohun,T.J., Brennan,S. and Gurdon,J.B. (1984) Region-specific regulation of the actin multi-gene family in early amphibian embryos. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **307**, 337–342.

Moon,R.T. and Kimelman,D. (1998) From cortical rotation to organizer gene expression: toward a molecular explanation of axis specification in *Xenopus*. *BioEssays*, **20**, 536–545.

Newman,C.S., Chia,F. and Krieg,P.A. (1997) The *XHex* homeobox gene is expressed during development of the vascular endothelium: overexpression leads to an increase in vascular endothelial cell number. *Mech. Dev.*, **66**, 83–93.

Niehrs,C. (1999) Head in the Wnt—the molecular nature of Spemann's head organizer. *Trends Genet.*, **15**, 314–319.

Nieuwkoop,P.D. and Florschütz,P.A. (1950) Quelques caracteres speciaux de la gastrulation et de la neurulation de l'oeuf de *Xenopus laevis*, Daud. et de quelques autres Anoures. I. *Arch. Biol.*, **61**, 113–150.

Onichtchouk,D., Gawantka,V., Dosch,R., Delius,H., Hirschfeld,K., Blumenstock,C. and Niehrs,C. (1996) The *Xvent-2* homeobox gene is part of the BMP-4 signaling pathway controlling dorsoventral patterning of *Xenopus* mesoderm. *Development*, **122**, 3045–3053.

Onichtchouk,D., Glinka,A. and Niehrs,C. (1998) Requirement for *Xvent-1* and *Xvent-2* gene function in dorsoventral patterning of *Xenopus* mesoderm. *Development*, **125**, 1447–1456.

Pannese,M., Polo,C., Andreazzoli,M., Vignali,R., Kablar,B., Barsacchi,G. and Boncinelli,E. (1995) The *Xenopus* homologue of *Otx2 is* a maternal homeobox gene that demarcates and specifies anterior body regions. *Development*, **121**, 707–720.

Pasteels,J. (1949) Observations sur la localisation de la plaque prechordale et de l'entoblaste presomtifs au cours de la gastrulation chez *Xenopus laevis*. *Arch. Biol.*, **60**, 235–250.

Pearce,J.J.H., Penny,G. and Rossant,J. (1999) A mouse *cerberus/dan*-related gene family. *Dev. Biol.*, **209**, 98–100.

Piccolo,S., Agius,E., Leyns,L., Bhattacharyya,S., Grunz,H., Bouwmeester,T. and De Robertis,E.M. (1999) The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature*, **397**, 707–710.

Ren,B., Chee,K.J., Kim,T.H. and Maniatis,T. (1999) PRDI-BF1/Blimp-1 repression is mediated by corepressors of the Groucho family of proteins. *Genes Dev.*, **13**, 125–137.

Rhinn,M., Dierich,A., Shawlot,W., Behringer,R.R., Le Meur,M. and Ang,S.L. (1998) Sequential roles for *Otx2 in* visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification. *Development*, **125**, 845–856.

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.

Rupp,R.A., Snider,L. and Weintraub,H. (1994) *Xenopus* embryos regulate the nuclear localization of *XMyoD. Genes Dev.*, **8**, 1311–1323.

Sasai,Y., Lu,B., Steinbeisser,H., Geissert,D., Gont,L.K. and De Robertis,E.M. (1994) *Xenopus* chordin: a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell*, **79**, 779–790.

Sasai,Y., Lu,B., Piccolo,S. and De Robertis,E.M. (1996) Endoderm induction by the organizer-secreted factors chordin and noggin in *Xenopus* animal caps. *EMBO J.*, **15**, 4547–4555.

Schneider,V.A. and Mercola,M. (1999) Spatially distinct head and heart inducers within the *Xenopus* organizer region. *Curr. Biol.*, **9**, 800–809.

Shawlot,W., Deng,J.M. and Behringer,R.R. (1998) Expression of the mouse cerberus-related gene, *Cerr1*, suggests a role in anterior neural induction and somitogenesis. *Proc. Natl Acad. Sci. USA*, **95**, 6198–6203.

Slack,J.M. and Tannahill,D. (1992) Mechanism of anteroposterior axis specification in vertebrates. Lessons from the amphibians. *Development*, **114**, 285–302.

Smith,J.C., Price,B.M., Green,J.B., 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.

Smith,W.C. and Harland,R.M. (1992) Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell*, **70**, 829–840.

Taira,M., Otani,H., Jamrich,M. and Dawid,I.B. (1994) Expression of the LIM class homeobox gene *Xlim-1 in* pronephros and CNS cell lineages of *Xenopus* embryos is affected by retinoic acid and exogastrulation. *Development*, **120**, 1525–1536.

Thomas,P. and Beddington,R. (1996) Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr. Biol.*, **6**, 1487–1496.

Thomas,P.Q., Brown,A. and Beddington,R.S. (1998) *Hex*: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. *Development*, **125**, 85–94.

Turner,C.J., Mack,D.H. and Davis,M.M. (1994) Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. *Cell*, **77**, 297–306.

Varlet,I., Collignon,J. and Robertson,E.J. (1997) *nodal* expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. *Development*, **124**, 1033–1044.

Walmsley,M.E., Guille,M.J., Bertwistle,D., Smith,J.C., Pizzey,J.A. and Patient,R.K. (1994) Negative control of *Xenopus GATA-2* by activin and noggin with eventual expression in precursors of the ventral blood islands. *Development*, **120**, 2519–2529.

Wang,S., Krinks,M., Lin,K., Luyten,F.P. and Moos,M. (1997) Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell*, **88**, 757–766.

Wang,Y., O'Malley,B.W.,Jr, Tsai,S.Y. and O'Malley,B.W. (1994) A regulatory system for use in gene transfer. *Proc. Natl Acad. Sci. USA*, **91**, 8180–8184.

Wilson,R. *et al*. (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C.elegans*. *Nature*, **368**, 32–38.

Zhang,J., Houston,D.W., King,M.L., Payne,C., Wylie,C. and Heasman,J. (1998) The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. *Cell*, **94**, 515–524.

Zon,L.I., Mather,C., Burgess,S., Bolce,M.E., Harland,R.M. and Orkin,S.H. (1991) Expression of GATA-binding proteins during embryonic development in *Xenopus laevis*. *Proc. Natl Acad. Sci. USA*, **88**, 10642–10646.

Zorn,A.R., Butler,K. and Gurdon,J.B. (1999) Anterior endomesoderm specification in *Xenopus* by Wnt/β-catenin and TGF-β signalling pathways. *Dev. Biol.*, **209**, 282–297.

Received July 30, 1999; revised September 10, 1999; accepted September 13, 1999