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A novel homeobox gene *PV.1* mediates induction of ventral mesoderm in *Xenopus* embryos

(bone morphogenetic protein 4/mesodermal induction)

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ABSTRACT **The formation of ventral mesoderm has been traditionally viewed as a result of a lack of dorsal signaling and therefore assumed to be a default state of mesodermal development. The discovery that bone morphogenetic protein 4 (BMP4) can induce ventral mesoderm led to the suggestion that the induction of the ventral mesoderm requires a different signaling pathway than the induction of the dorsal mesoderm. However, the individual components of this pathway remained largely unknown. Here we report the identification of a novel *Xenopus* homeobox gene *PV.1* (posterior-ventral 1) that is capable of mediating induction of ventral mesoderm. This gene is activated in blastula stage *Xenopus* embryos, its expression peaks during gastrulation and declines rapidly after neurulation is complete. *PV.1* is expressed in the ventral marginal zone of blastulae and later in the posterior ventral area of gastrulae and neurulae. *PV.1* is inducible in uncommited ectoderm by the ventralizing growth factor BMP4 and counteracts the dorsalizing effects of the dominant negative BMP4 receptor. Overexpression of *PV.1* yields ventralized tadpoles and rescues embryos partially dorsalized by LiCl treatment. In animal caps, *PV.1* ventralizes induction by activin and inhibits expression of dorsal specific genes. All of these effects mimic those previously reported for BMP4. These observations suggest that *PV.1* is a critical component in the formation of ventral mesoderm and possibly mediates the effects of BMP4.**

The dorsal–ventral patterning of mesoderm is central to the specification of the vertebrate body axis. In *Xenopus*, cytoplasmically localized information and early embryonic inductions play important roles in this process. Dorsal–ventral polarity is established shortly after fertilization, when the cortex of the *Xenopus* egg rotates by about 30 degrees relative to the cytoplasm, thereby localizing important dorsal determinants (1, 2). As early as the 32-cell stage, the initial induction of mesoderm occurs with signals emanating from dorsal vegetal cells inducing dorsal mesoderm and ventral vegetal cells inducing ventral mesoderm (3, 4). Mesoderm is further patterned during gastrulation by dorsalizing signals arising from the Spemann organizer. Cells of the organizer induce the rest of the mesoderm in a graded fashion, regionalizing it into zones of somite, lateral plate, and blood islands (5).

A great deal of effort has focused on identifying the extracellular molecules that constitute these inducing signals. Several members of the transforming growth factor-β and fibroblast growth factor families are now clearly implicated as candidates for the putative dorsal and ventral mesoderm inducing signals, respectively (for review see ref. 6; refs. 7 and 8). Signaling proteins that modulate (rather than mediate) mesoderm induction, such as Xwnt 8 and ADMP-1, have also been identified (9, 10). Both of these factors have been proposed to be able to convert the fate of dorsal tissues to ventral. Recently, a potent dorsalizing factor (chordin), activated by organizer-specific genes, has been identified and shown to have a spatio-temporal expression pattern compatible with a role for this molecule in the regional subdivision of mesoderm occurring during gastrulation (11).

This classic view of *Xenopus* development, summarized in the three-signal model proposed by Dale and Slack (12), depicts the organizer as the sole source of a morphogen gradient responsible for the patterning of mesoderm. Ventral mesoderm is viewed as passive. Recent studies suggest that ventral mesoderm may play a more active role than originally thought in establishing dorsal–ventral pattern (13–17).

Bone morphogenetic protein 4 (BMP4), a transforming growth factor-β family member, has recently been identified as a very potent ventralizing factor in *Xenopus* embryos (13–17). Zygotic expression of BMP4 begins at or shortly after the midblastula transition and peaks during gastrulation. As shown by *in situ* hybridization, BMP4 transcription is present in the animal cap and in the ventral marginal zone of gastrulating embryos (18, 19). The effects of injected BMP4 mRNA or of added BMP4 protein of *Xenopus* or human origin have been studied in some detail (13–17). In animal caps, BMP4 is a weak inducer of ventral mesoderm. However, animal cap ectoderm treated with a combination of BMP4 and dorsalizing concentrations of activin results in the formation of ventral mesoderm. This suggests that BMP4 is a ventralizing factor that acts by modulating the dorsalizing signal provided by activin. In keeping with these observations, overexpression of this growth factor in intact embryos results in the suppression of dorso–anterior structures (13–17).

The importance of BMP4 in the ventralization of mesoderm has recently been confirmed by means of a truncated form of the BMP4 receptor, which specifically blocks endogenous BMP4 signaling (20, 21). Expression of this receptor converts ventral mesoderm to dorsal mesoderm resulting in the formation of a secondary axis. This suggests that an active BMP4 signal is required to produce ventral mesoderm. In the absence of the active ventralizing signal, dorsal tissues are formed (20, 21).

Mesoderm inducing growth factors are thought to provide positional information along the dorsal–ventral axis by regulating the expression of homeobox genes (22–24). In recent years, position-specific homeobox genes activated by dorsal mesoderm inducing signals have received much attention (25–30). These genes are thought to activate various subordinate genes that are necessary to further define dorsal meso-

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Abbreviations: BMP4, bone morphogenetic protein 4; RT, reverse transcription; DAI, dorsoanterior index; DNR, dominant negative receptor; C-actin, cardiac actin.

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derm and its derivatives. Observations that overexpression of these homeobox genes in uncommitted embryonic cells can give axis forming activity are consistent with this hypothesis (31, 32).

In this paper we describe a novel *Xenopus* homeobox gene that mediates the formation of ventral mesoderm. We show that this homeobox gene that we call posterior-ventral 1 (*PV.1*), functions in a ventral specific signaling pathway and is possibly an early nuclear target of BMP4 signaling.

MATERIALS AND METHODS

Isolation of *PV.1* cDNA. Using degenerate primers for the conserved homeobox sequences PRTAFT and KTWFQN, we screened a subtracted, gastrula-specific cDNA library (33). The reaction cycles and conditions were performed as described (29). The PCR fragment encoding the *PV.1* homeobox was used to screen a stage 13 *Xenopus* cDNA library (34).

PCR Reactions. Amplification of cDNA was performed in 10-μl reactions containing 1× PCR buffer (Perkin–Elmer), 200 μM of dNTPs, 1 μM of each primer, and 1 unit of *Taq* polymerase (Perkin–Elmer). Cycling parameters were 94°C, 0 s; 55°C, 0 s; 72°C, 40 s for each cycle. An air thermocycler (Idaho Technology) was used for all experiments. Input cDNA for each sample was normalized to histone H4. The primers used for amplification of Brachyury, Goosecoid, cardiac actin, histone H4, and Xhox3 are as described in Niehrs *et al.* (35). PCR products were electrophoresed on a 2% agarose gel that was stained with SYBR Green I Nucleic Acid Gel Stain (Molecular Probes) and imaged using a Molecular Dynamics Fluorescent Scanner.

Embryos. Eggs and embryos were obtained and cultured as described (36). Developmental stages of embryos were determined according to Nieuwkoop and Faber (37).

Preparation of RNA and Reverse Transcription (RT). Total RNA for PCR reactions was isolated from animal caps using TRIzol (Life Technologies, Gaithersburg, MD) and treated with DNase (amplification grade, Life Technologies). RT reactions were done with Superscript II (Life Technologies) as described by the manufacturer with 2 μg of total RNA per reaction. Total RNA for the developmental Northern blot was prepared using the procedure of Sargent *et al.* (36). Five micrograms of RNA was loaded on 1.2% agarose gels containing 5 mM methylmercury hydroxide (38). Gels were blotted to Nytran filters using vacuum transfer. DNA probes were made using the BRL random primer labeling system with [α-^{32}P]dCTP. Hybridization was performed as described by Church and Gilbert (39).

Induction Experiments. Animal caps were excised from stage 8 embryos and treated with 100 pM of activin A or control solution.

Whole-Mount *in Situ* Hybridization. *In situ* hybridization was performed following the method of Harland (40).

Microinjection of RNA. Capped RNA was made from linearized plasmid DNA using the mMessage mMachine kit from Ambion (Austin, TX). Embryos kept in 1 × MMR and 4% Ficoll 400 were microinjected at the 4-cell stage with BMP4 or *PV.1* RNA. The amount of injected RNA and the site of the injection are indicated in the figure legends.

LiCl Treatment. Embryos were treated with 0.3 M LiCl solution at the 32-cell stage for 15 min and than transferred into 0.1 × MMR containing gentamycin (41).

RESULTS

Isolation, Characterization, and Spatio-Temporal Expression of *PV.1*. *PV.1* was isolated from a gastrula-specific cDNA library using degenerate primers and PCR-based technology (42). The homeobox sequence of *PV.1* (Fig. 1*A*) is most similar (63% identical) to that of the mouse homeobox gene Emx1 (43). Because of this low similarity, we believe that *PV.1* homologues in other species have not yet been isolated.

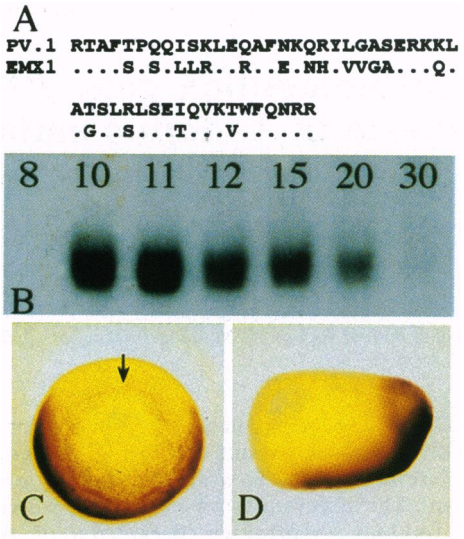

FIG. 1. Identification of a ventral-specific homeobox gene. (*A*) *PV.1* homeodomain sequence compared with the murine Emx1. Dots indicate identities. (*B*) Temporal expression pattern of *Xenopus PV.1*. Northern blot analysis of *Xenopus* total RNA isolated from embryos at indicated stages. Equal amounts of RNA were loaded in each lane. The blot was probed with a fragment of *PV.1* cDNA; a single 1.5-kb transcript was detected. *PV.1* expression is first detected at stage 10, peaks at stage 11, slowly decreases and is not detectable by stage 30. (*C* and *D*) Spatial expression pattern of *PV.1*. Transcripts were detected by whole mount *in situ* hybridization of albino embryos using digoxigenin-labeled *PV.1* antisense probe. (*C*) Vegetal view of stage 11 embryos showing the ventral marginal zone expression of *PV.1*. Arrow marks the position of the organizer. (*D*) Lateral view of stage 14 embryos (anterior is to the left and dorsal on top). *PV.1* expression is evident throughout the ventral and posterior regions, whereas the dorso–anterior region is negative.

Northern blot analysis of *Xenopus* total RNA reveals that zygotic expression of *PV.1* begins at or shortly after midblastula transition, peaks at stage 11 (early gastrula), and is much reduced by stage 30 (Fig. 1*B*). *PV.1* mRNA is not detectable in the *Xenopus* oocyte (data not shown). *In situ* hybridization experiments using digoxigenin-labeled antisense probe were preformed to assess the spatial localization of *PV.1* transcript during development. *PV.1* expression is first detectable in the ventral and lateral marginal zone regions of the late blastula, whereas the dorsal marginal zone is negative. This dorsal–ventral asymmetry of *PV.1* expression persists throughout gastrulation in the invaginating mesoderm (Fig. 1*C*). At stage 10, *PV.1* expression begins to spread on the ventral side toward the animal cap until at stage 11.5 *PV.1* expression forms a gradient across the cap with higher levels detected ventrally than dorsally (data not shown). At the end of gastrulation, *PV.1* transcripts are predominately localized to the ventral and lateral regions of the closing slit blastopore (Fig. 1*D*). Using RT PCR on RNA from microdissected endoderm we were able to detect a low level of *PV.1* expression (not shown). Fig. 1*C* shows that this RNA is predominantly localized in the ventral endodermal region.

Induction of *PV.1* Expression by BMP4. That *PV.1* transcription peaks during the early gastrula stages and is localized to ventral and lateral regions of the marginal zone suggests a potential role for this gene in the formation of ventral mesoderm. Interestingly, the spatio-temporal expression pattern of *PV.1* closely resembles that of the potent ventralizing agent BMP4 (13–17). We therefore investigated whether *PV.1* is inducible by BMP4 (Fig. 2). Four-cell stage embryos were injected with BMP4 mRNA and allowed to develop to stages

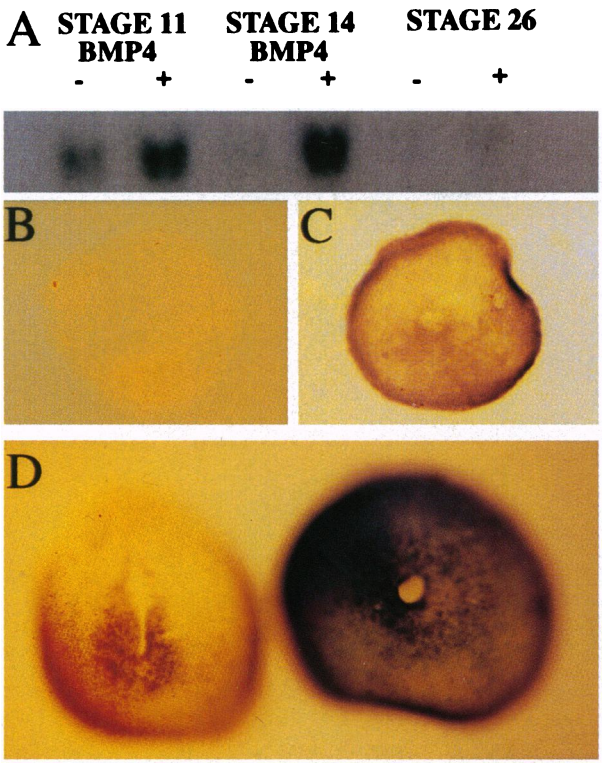

FIG. 2. Induction of *PV.1* by BMP4. (*A*) Northern blot analysis of total RNA isolated from embryos injected with 1.0 ng of BMP4 mRNA and from uninjected control embryos. Total RNA was isolated from stages 11, 14, and 26 embryos. Five micrograms of total RNA was loaded on each lane. Hybridization of the blot with *PV.1* probe revealed that *PV.1* is strongly induced by overexpression of BMP4. (*B* and *C*) *In situ* hybridization of *PV.1* to animal caps from uninjected embryos (*B*) or to animal caps from embryos overexpressing BMP4 (*C*). Animal caps were dissected at stage 9 and cultured until stage 12. (*D*) *In situ* hybridization of *PV.1* to a stage 14 embryo injected with BMP4 (right) and an uninjected stage 14 embryo (left).

11 and 14. Northern blot analyses of total RNA isolated from these embryos and from uninjected controls revealed that *PV.1* transcript levels increase significantly in embryos overexpressing BMP4 (Fig. 2*A*). This observation was confirmed by whole mount *in situ* hybridization using *PV.1* antisense RNA probe. In this experiment we observed a dramatic increase in *PV.1* transcription throughout the entire embryo (Fig. 2*D*). Furthermore, *PV.1* is strongly induced in uncommited ectoderm excised from embryos overexpressing BMP4 (Fig. 2 *B* and *C*).

Ventralization of Embryos Injected by *PV.1* RNA. The above observations raise the possibility that *PV.1* may function downstream of BMP4 in the ventralization of mesoderm. To assess the role of *PV.1*, synthetic mRNA was injected into the two dorsal or two ventral blastomeres of 4-cell stage *Xenopus* embryos. Embryos were cultured until stage 36, and graded according to the dorsoanterior index (DAI) of Kao and Elinson (41). Embryos dorsally injected with 1 ng of *PV.1* mRNA develop normally up to the early gastrula stage. At this stage, however, development slows significantly and the blastopore does not completely close. By stage 36 all embryos injected dorsally appear extremely ventroposteriorized with DAI scores ranging from 0 to 3 (n = 61) (Fig. 3*A*). Dorsal injections of mRNA encoding β-galactosidase do not perturb development. Embryos injected ventrally with *PV.1* transcript likewise develop normally (not shown).

The ventralized phenotype produced by dorsal injection of *PV.1* is dose-dependent. We found that a 10-fold dilution of injected mRNA increases the mean DAI of the resulting embryos from 1.2 to 4.5 (data not shown). Embryos receiving the lower doses of *PV.1* mRNA appear normal through

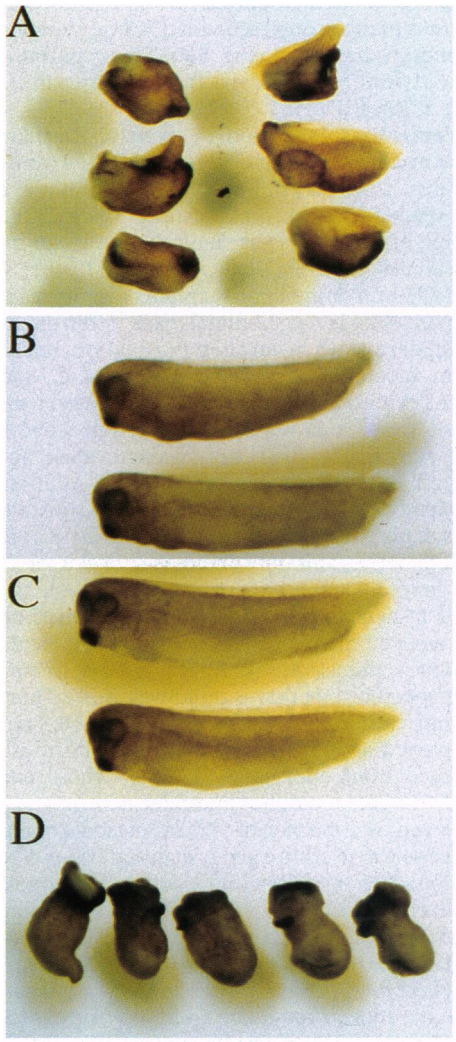

FIG. 3. Misexpression of *PV.1* in dorsal mesoderm ventroposteriorizes intact embryos and rescues embryos partially dorsalized by LiCl treatment. (*A*) Lateral view of stage 36 embryos dorsally injected with 1 ng of *PV.1* mRNA. *PV.1* injected embryos appear extremely ventroposteriorized. (*B*) Control embryos dorsally injected with 1 ng of β-galactosidase mRNA. (*C*) Lateral view of stage 36 embryos ventrally injected at the 4-cell stage with 1.5 ng of *PV.1* mRNA and treated at the 32-cell stage with 0.3 M LiCl. (*D*) Control embryos treated with 0.3 M LiCl alone. Ventral injection of *PV.1* mRNA rescues embryos partially dorsalized by LiCl.

gastrulation with complete closing of the blastopore. By stage 36, however, these embryos appear microcephalic to acephalic and have enlarged posterior ends (data not shown). Thus misexpression of *PV.1* in the dorsal marginal zone has a potent ventroposteriorizing effect on the developing *Xenopus* embryo.

***PV.1* Prevents Dorsalization of Embryos Treated with LiCl.** In keeping with these observations, we found that ventral injection of *PV.1* mRNA rescues dorsalization by LiCl treatment. Full LiCl treatment yields embryos that completely lack trunk structure and have radially symmetric heads, often with a radial band of cement gland and/or eye pigmentation (44). Partial LiCl treatment results in the formation of embryos that lack trunk and tail structures but are not entirely radially symmetric (18, 19). We found that embryos treated with LiCl show much reduced expression of *PV.1* RNA (data not shown). To test whether *PV.1* can prevent dorsalization of embryos by LiCl, we injected *PV.1* mRNA into the ventral marginal zone of 4-cell stage embryos and followed with a partial LiCl treatment at the 32-cell stage (Fig. 3). Twenty-seven of 31

injected embryos formed entirely normal posterior axes (Fig. 3*C*), whereas in the group receiving LiCl treatment alone, only 1 of 30 embryos had a tail (Fig. 3*D*). Thus, ventral injection of *PV.1* mRNA restores trunk and tail structures in LiCl-treated embryos, as does BMP4 (13–17).

***PV.1* Ventralizes Induction by Activin.** Results of animal pole explant experiments suggest that BMP4 ventralizes *Xenopus* embryos by overriding endogenous dorsal mesoderm inducing signals (13–17). To determine whether the apparent ventralizing effect of *PV.1* is similarly due to an interference with dorsal mesoderm inducing signals we further studied the effects of *PV.1* on animal pole explants (animal caps) induced with activin A (Fig. 4). Animal caps differentiate only as atypical epidermis when cultured in isolation, but form dorsal mesoderm including notochord and muscle, and undergo convergent extension movements when treated with activin A (45, 46). Animal caps were removed from stage 8 embryos injected with either *PV.1* or β-galactosidase mRNA and treated with 100 pM activin A or a control solution. Whereas control caps treated with activin elongated substantially (Fig. 4*A*), caps expressing *PV.1* elongated very little in response to activin (Fig. 4*C*). This morphological effect of *PV.1* could reflect a general suppression of mesoderm induction by *PV.1*, or instead, a specific ventralization of dorsal mesoderm induction. We assayed the expression of several molecular markers of mesoderm induction by RT-PCR. *Goosecoid (gsc)*, an organizer specific gene that is rapidly expressed in response to activin, and cardiac actin (C-actin), a muscle specific gene, were employed as markers of dorsal mesoderm (25, 31, 47). The *Xenopus* homeobox gene, *Xhox 3*, whose expression is restricted to ventroposterior mesoderm in normal embryos, was employed as a marker of ventral mesoderm (22, 23), and the *Xbra* gene was used as a general mesoderm marker (48, 49).

Induction of *gsc* and C-actin by activin was greatly reduced in animal caps injected with *PV.1* mRNA, whereas *Xhox3* expression was significantly enhanced. Activin induced *Xbra* transcription in these caps appeared roughly equivalent to controls. Likewise, *Xhox3* expression was enhanced and *gsc* RNA levels reduced in intact embryos overexpressing *PV.1* (data not shown). These results suggest that *PV.1* selectively down-regulates the formation of dorsal mesoderm and up-regulates ventral mesoderm formation without affecting general mesoderm induction by activin. Suprisingly, we also found that animal caps derived from embryos receiving injections of *PV.1* mRNA, but not exposed to exogenous growth factor, express very low levels of *Xbra*. This observation suggests that *PV.1* itself might be capable of inducing ventral-type mesoderm in animal caps.

***PV.1* Counteracts the Dorsalizing Effects of the Dominant Negative BMP4 Receptor.** All of the above observations mimic those previously reported for BMP4 (13–17). To further address whether *PV.1* participates in the BMP4 signaling pathway, we attempted to rescue embryos ectopically expressing the BMP4 dominant negative receptor (DNR) by coexpressing *PV.1*. The BMP4 DNR lacks the entire intracellular kinase domain. Its ectopic expression specifically blocks BMP4 signaling and converts ventral to dorsal mesoderm resulting in the formation of a secondary axis (20, 21). Embryos were injected ventrally at the 4-cell stage with a mixture of mRNAs encoding the BMP4 DNR and *PV.1* or with mRNA encoding the DNR alone. DNR mRNA was injected at a concentration that completely neutralizes the ventralizing activity of coinjected BMP4 mRNA (data not shown). Embryos were cultured until stage 36 and were scored according to the DAI. As shown in Fig. 5, *PV.1* completely counteracted the dorsalizing effects of the dominant negative BMP4 receptor and resulted in the development of embryos with normal ventroposterior structure. Thus, *PV.1* is a likely effector of the BMP4 signaling pathway activating a ventral-specific program of gene expression.

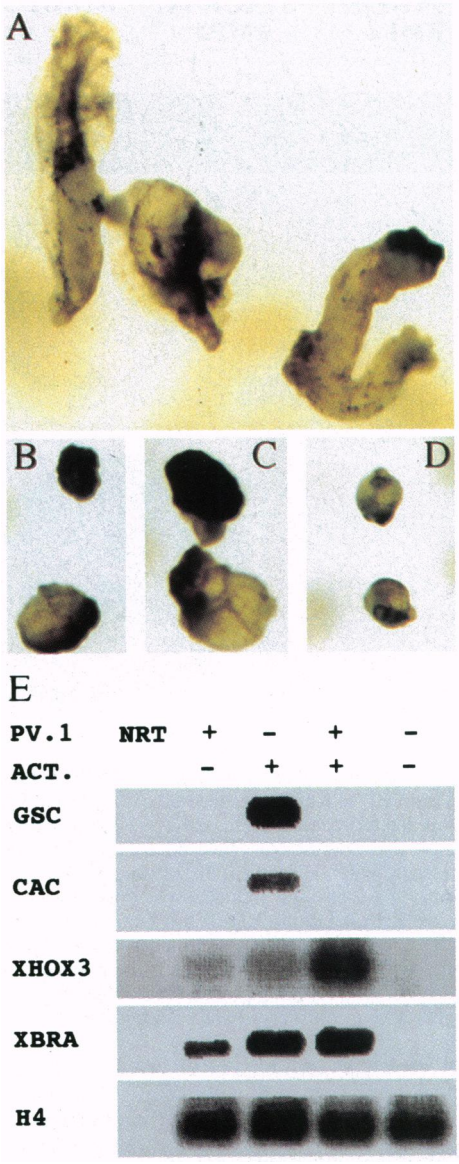

FIG. 4. (*A–D*) *PV.1* inhibits elongation of animal caps in response to activin. Two-cell stage embryos were injected in the animal pole with 1.5 ng of mRNA encoding *PV.1* or β-galctosidase. Animal caps were excised from stage 8 injected embryos and were treated with 100 pM activin A or a control solution and cultured for two days. Caps excised from β-galactosidase-injected embryos and treated with activin A (*A*) show significant elongation relative to untreated controls (*B*). Elongation in response to activin is inhibited in caps overexpressing *PV.1* (*C*). No elongation is observed in caps overexpressing *PV.1* and not treated with activin (*D*). (*E*) Overexpression of *PV.1* in animal caps inhibits induction of *gsc* and C-actin and enhances *Xhox3* expression in response to activin. Animal caps overexpressing *PV.1* and control caps were treated with activin A or a control solution. Caps were cultured until siblings reached stage 11. Total RNA was isolated and assayed for expression of *gsc*, C-actin, and *Xhox3* by RT-PCR. Expression levels of *Xbra* were measured as an indicator of generic mesoderm induction by activin. Histone H4 was used to normalize between samples. Template from which RT was omitted (indicated as NRT in the figure) was used to confirm absence of contaminating genomic DNA sequences. Relative to activin-induced control caps, caps overexpressing *PV.1* show significant reduction in *gsc* and C-actin expression levels and enhancement of *Xhox3* in response to activin. Caps overexpressing *PV.1* but not induced with activin A express low levels of *Xbra* and *Xhox3* relative to uninduced control caps.

DISCUSSION

***PV.1* Is Expressed in the Ventral Marginal Zone of *Xenopus* Embryos.** We have isolated a novel *Xenopus* homeobox gene,

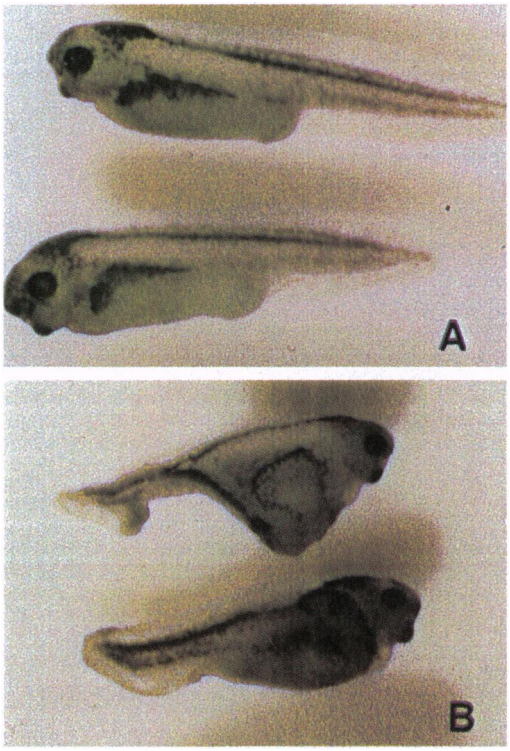

FIG. 5. *PV.1* counteracts the dorsalizing effects of the dominant negative BMP4 receptor. Embryos were ventrally injected at the 4-cell stage with a mixture of mRNAs encoding the DNR (2 ng) and *PV.1* (1.5 ng) (*A*) or with mRNA encoding the DNR alone (2 ng) (*B*). Embryos were cultured until stage 42 and were scored according to the DAI. Embryos receiving injections of mRNA encoding the DNR alone display DAIs ranging from 6–8 (n = 30), whereas embryos injected with mRNA encoding the DNR plus PV.1 develop normally (DAI = 5; n = 28).

PV.1, which is expressed in the ventral marginal zone of blastula and gastrula stage embryos. In the late gastrula and early neurula, *PV.1* transcripts are localized to the ventral half of the slit blastopore, a region fated to form ventral-most mesodermal derivatives (27). This expression pattern within the prospective mesoderm correlates with that observed for the potent ventralizing agent BMP4 suggesting a relationship between the two molecules (22, 23). Like BMP4, *PV.1* transcripts are also detected in the animal pole ectoderm of *Xenopus* embryos and overexpression of BMP4 in animal caps significantly enhances *PV.1* RNA synthesis.

Overexpression of *PV.1* Mimics the Ventralizing Effects of BMP4 and Counteracts the Dorsalizing Effects of the Dominant Negative BMP4 Receptor. The observations that *PV.1* and BMP4 share a similar expression pattern and that *PV.1* is strongly induced by BMP4 raise the possibility that this gene may function as an effector in the ventralization of mesoderm by BMP4. We have investigated this possibility by assessing the functional properties of *PV.1* and comparing them with those of BMP4. Like BMP4 we found that misexpression of *PV.1* in dorsal mesoderm is sufficient to ventralize intact embryos and to rescue embryos dorsalized by LiCl treatment. Furthermore, by both morphological and molecular criteria, *PV.1* ventralizes induction by activin. This ability has been previously described for BMP4 as well. Finally, *PV.1* counteracts the dorsalizing effects of the dominant negative BMP4 receptor. This is the first transcriptional factor described to have this ability. All of these experiments suggest that *PV.1* is a ventral-specific transcription modulator that is an early (possibly the earliest) nuclear target of BMP4 signaling.

It is unlikely that the effects of *PV.1* are exclusively due to a feedback loop in which *PV.1* activates BMP4 transcription. In the rescue experiments using the BMP4 DNR, receptor mRNA was injected at a concentration that completely blocked the ventralizing activity of coinjected BMP4 mRNA. The ability of *PV.1* to nonetheless counteract the dorsalizing effects of the BMP4 DNR excludes the possibility that *PV.1* is acting solely through such a feedback loop. Furthermore, in other experiments designed to determine whether *PV.1* can activate BMP4 transcription, we were unable to show BMP4 activation by *PV.1*.

For many years the generally held view has been that ventral is a default state that occurs in the absence of an active dorsalizing signal (12). Observations of the effects of the dominant negative BMP4 receptor provided the first indication that an active signal is required to produce ventral mesoderm (20, 21). However, this work left open the possibility that this ventralizing signal simply inhibits dorsal signals and that ventral gene expression is a default state that occurs in the absence of these signals. The early expression pattern of *PV.1* and its ability to mimic BMP4 function provides convincing evidence that there is an active ventral-specific program of gene expression. The formation of ventral mesoderm is thus the result of an active process in which *PV.1* activates genes necessary for the formation of ventral mesoderm.

Two other genes, *Xbra* and *Xhox3*, have also been implicated in the formation of posterior ventral tissues. While they are all likely to be involved in the formation of posterior structures and none of the three genes has been unequivocably shown to be the direct target of BMP4 signaling, we feel that *PV.1* is the best candidate to be a mediator of ventroposterior mesoderm formation by BMP4. *PV.1* has at all times a ventroposterior expression pattern most similar to that of BMP4, whereas, *Xbra* shows a significant expression in the dorsal mesoderm including the notochord (18, 19). Furthermore, both BMP4 and *PV.1* suppress muscle-specific markers, whereas *Xbra*-injected embryos show elevated levels of muscle-specific actin (49). Therefore, we consider *Xbra* to be a marker of dorsal as well as ventral posterior mesoderm.

Similarly, the spatial expression pattern of *Xhox3* indicates an involvement of this gene in the specification of dorsal as well as ventral posterior structures and is significantly different from that of *PV.1* and BMP4. *Xhox 3* is expressed in a graded fashion along the anteroposterior axis. However, in later stage embryos, *Xhox3* seems to be expressed predominantly in the dorsal posterior mesoderm (22, 23). At the same stage *PV.1* and BMP4 are ventroposteriorly expressed.

Neither of these genes is able to induce a high amount of ventral mesoderm in isolated animal caps suggesting that a combination of these or additional transcription factors will be needed to form large quantities of ventral mesoderm.

Recently a *Xenopus* homeobox gene, called *Xvent-1* has been identified and shown to have an expression pattern and a ventralizing activity similar to that of *PV.1* (50). *Xvent1* belongs to the same subfamily of homeobox genes and differs only in two amino acids from *PV.1* in its homeodomain. Our preliminary experiments indicate that other members of this novel homeobox subfamily exist and that they too may play a role in the patterning of ventral mesoderm.

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