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Localized BMP-4 Mediates Dorsal/Ventral Patterning in the Early *Xenopus* Embryo

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Mesoderm is initially induced in the amphibian embryo by events that occur in the early cleavage stages prior to the midblastula transition (MBT) and morphogenesis. These inductive interactions establish the mesoderm at the equator and create a distinction between the dorsal and the ventral regions. After the MBT, zygotic factors pattern the mesoderm and induce the neuroectoderm on the dorsal side of the embryo. Most previous studies have focused on the effects of signals originating in the dorsal mesoderm. We show that BMP-4 transcripts are localized to the ventral side of the gastrula embryo and provide evidence that localized expression of BMP-4 is important for regulating the expression of mesodermal and neural genes. We show that ectopic expression of BMP-4 inhibits the formation of dorsal and lateral mesoderm and reduces the size of the neural plate. Elimination of BMP-4 signaling with a dominant-negative BMP receptor expands the lateral mesoderm and neural plate without expanding the expression of genes along the dorsal midline. These results suggest that BMP-4 may act to oppose the action of dorsalizing signals and neural-inducing signals that originate in the dorsal organizer region. We suggest that BMP-4 may have an analogous role to the *Drosophila* gene, *dpp*, in dorsal/ventral pattern formation.

INTRODUCTION

Dorsal/ventral polarity is established upon fertilization of the *Xenopus* egg when the rotation of the cortex relative to the cytoplasm activates a dorsal mesoderm-inducing signal on one side of the egg (Gerhart *et al.*, 1989). The identity of the dorsal mesoderm inducer is unknown, but it has been proposed to be a maternal Wnt, noggin, or an activated Vg1 (Smith and Harland, 1991, 1992; Sokol *et al.*, 1991; Thomsen and Melton, 1993). Possibly as early as the 32-cell stage, a combination of signaling molecules, including the dorsal mesoderm inducer, an activin-like factor, and an FGF, establish the presumptive mesodermal region at the equator of the embryo and specify a dorsal/ventral difference within the presumptive mesoderm (reviewed in Kimelman *et al.*, 1992; Sive, 1993; R. Cornell, T. Musci, and D. Kimelman, submitted for publication). Thus, by the late blastula stage, the marginal zone is fated to form mesoderm and has acquired either a dorsal or ventral character, with the dorsal marginal zone specified to become the region known as Spemann's organizer. We refer to these early signaling events as "primary mesoderm induction." In the classical view, the mesoderm is further patterned during gastrulation by dorsalizing signals arising from the organizer region (Smith and Slack, 1983; Slack *et al.*, 1984; Dale *et al.*, 1985; Smith *et al.*, 1985). Neuroectoderm is also induced and patterned during gastrulation by signals from the organizer region (reviewed in Slack and Tannahill, 1992).

This "dorsal-centric" view of development depicts the ventral side as passive, with dorsalizing signals from the organizer converting lateral marginal zone cells from a default ventral fate to a more dorsal fate (Smith and Slack, 1983; Slack *et al.*, 1984; Dale *et al.*, 1985; Smith *et al.*, 1985). Recent results have suggested that the ventral side of the embryo may play an active role in establishing the dorsal/ventral pattern (Dale *et al.*, 1992; Jones *et al.*, 1992; Moon and Christian, 1992; Sive, 1993). One factor that might regulate ventral development is BMP-4, a TGF-β family member closely related to Vg1, mouse Vgr-1, BMP-2, BMP-3, and the *Drosophila decapentaplegic* (*dpp*) gene (Padgett *et al.*, 1987; Weeks and Melton, 1987; Wozney *et al.*, 1988; Lyons *et al.*, 1989; Koster *et al.*, 1991; Dale *et al.*, 1992). *Xenopus* BMP-4 is initially expressed at the midblastula transition (MBT), with peak levels reached during gastrulation (Dale *et al.*, 1992). Injection of RNA encoding BMP-4 into the animal pole of early cleavage-stage embryos produces "ventralized" embryos. Ectopic expression of BMP-4 induces ventral mesoderm in animal cap explants (Koster *et al.*, 1991; Dale *et al.*, 1992; Jones *et al.*, 1992) and overrides the dorsal mesoderm-inducing capacity of activin in these explants (Dale *et al.*, 1992;

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Jones *et al.*, 1992). These experiments support a role for BMP-4 in the patterning of ventral mesoderm. However, localization of BMP-4 transcripts by microdissection and RNase protection analysis demonstrated that BMP-4 RNA was uniformly distributed throughout the embryo (Dale *et al.*, 1992). These reported findings have made it difficult to conceive of BMP-4 as a ventral morphogen.

We have extended these studies on the role of BMP-4 in mesoderm induction and pattern formation in gastrula and neurula embryos by examining the effects of ectopic BMP-4 expression within the whole embryo using specific markers of mesoderm and neuroectoderm. We have compared these results to the effects on whole embryos caused by expression of a dominant-negative BMP receptor. We show that ectopic expression of BMP-4 eliminates or decreases the expression of dorsal and lateral mesodermal genes and leads to the formation of a diminished neural plate. Conversely, elimination of BMP-4 signaling causes an expansion of dorsal paraxial mesoderm at the expense of ventral mesoderm. In addition, elimination of BMP-4 signaling greatly expands the size of the neural plate, as revealed by the presence of neural gene expression on the ventral side of the embryo. Finally, we show that BMP-4 transcripts are excluded from the dorsal side of the embryo during gastrulation and thereafter. These results lead us to conclude that the localized expression of BMP-4 acts to regulate mesodermal and neural patterning along the dorsal/ventral axis by opposing the dorsalizing signal(s) originating in the organizer. In addition, we suggest that BMP-4 may have an analogous role to that of the closely related molecule, *dpp*, which is involved in regulating the extent of neuroectoderm formed in the early *Drosophila* embryo (Irish and Gelbart, 1987; Ferguson and Anderson, 1992a,b).

MATERIALS AND METHODS

Embryos and Animal Caps

Fertilized *Xenopus* embryos were prepared as described previously (Newport and Kirschner, 1982). After the jelly coat was removed with 2% cysteine (pH 7.8), the eggs were washed in 0.1× MMR (1× MMR is 0.1 *M* NaCl, 2 m*M* KCl, 1.0 m*M* $MgSO_4$, 2.0 m*M* $CaCl_2$, 5.0 m*M* Hepes, and 0.1 m*M* EDTA). The upper portion of the animal hemisphere, corresponding to roughly one-fourth of the embryo, was manually separated at stage 8–9 with care taken to remove adhering vegetal cells and cultured in 1× MMR. Where applicable, Activin A was added to the buffer. Activin A (a kind gift of Genentech) was used at 10 ng/ml in the presence of 100 μg/ml of bovine serum albumin.

RNA Synthesis and Microinjection

Injection RNAs were synthesized using the mMessage mMachine kit (Ambion) according to the protocol provided. The RNAs were purified by one extraction with phenol:chloroform (1:1) followed by two rounds of concentration and separation in Microcon 100 microconcentrators (Amicon) to separate the RNA from unincorporated nucleotides. RNA was microinjected as previously described (Moon and Christian, 1989). Five to ten nanoliters of RNA was injected per blastomere. The dorsal side of four-cell embryos was identified by pigment and cell size differences between dorsal and ventral blastomeres at this stage (Nieuwkoop and Faber, 1967). Embryos were fixed in 1× MEMFA (Harland, 1991).

In Situ Hybridization and Probe Synthesis

Whole-mount *in situ* hybridization was performed using digoxigenin-labeled RNA probes (Harland, 1991), with the modifications that levamisol was omitted from the alkaline phosphatase buffer and that RNase was not used following hybridization of the probe. Antisense probes were synthesized from cDNAs encoding BMP-4, *Xnot*, *gsc*, *MyoD*, *Xwnt-8*, *Xbra*, *Xpo*, and *Hairy II*, using plasmids linearized with *Sal*I, *Hin*dIII, *Eco*RI, *Hin*dIII, *Bam*HI, *Eco*RV, *Hin*dIII, and *Bam*HI, respectively. SP6 RNA polymerase was used for the BMP-4 and *gsc* probes and T7 was used for the *Xnot*, *Xbra*, *Xpo*, *Hairy II*, *MyoD*, and *Xwnt-8* probes. Whole-mount embryos were photographed using Kodak Ektachrome 160T film or Kodak Tmax 100 film.

RNA Isolation and RNase Protection

RNA was prepared by homogenization in an SDS-Proteinase K buffer (Cornell and Kimelman, 1994) and analyzed by the RNase protection assay (Melton *et al.*, 1984). Probes for *Xbra*, muscle actin, *MyoD*, and EF-1α were prepared as previously described (Cornell and Kimelman, 1994). A *Xhox3* probe was synthesized from a 370-bp subclone of pcXhox3 (Ruiz i Altaba and Melton, 1989) inserted into the *Bam*HI and *Pst*I sites of BluescriptII (SK^+) (Stratagene). This plasmid was linearized with *Bam*HI and transcribed with T7 polymerase. Probes were hybridized with RNA samples overnight at 45°C and then treated for 1 hr at room temperature with 10 μg/ml of RNase A (Sigma) and 0.5 μg/ml of RNase T1 (Sigma) for all probes except *Xhox3*, which was digested with RNase T1 alone. Protected fragments were separated on 8% acrylamide–urea gels and exposed to film.

RESULTS

Overexpression of BMP-4 Ventralizes Embryos, Eliminating Dorsal and Anterior Structures at Increasing Doses

Previous studies demonstrated that overexpression of BMP-4 in the animal pole of *Xenopus* embryos resulted in the formation of completely ventralized embryos

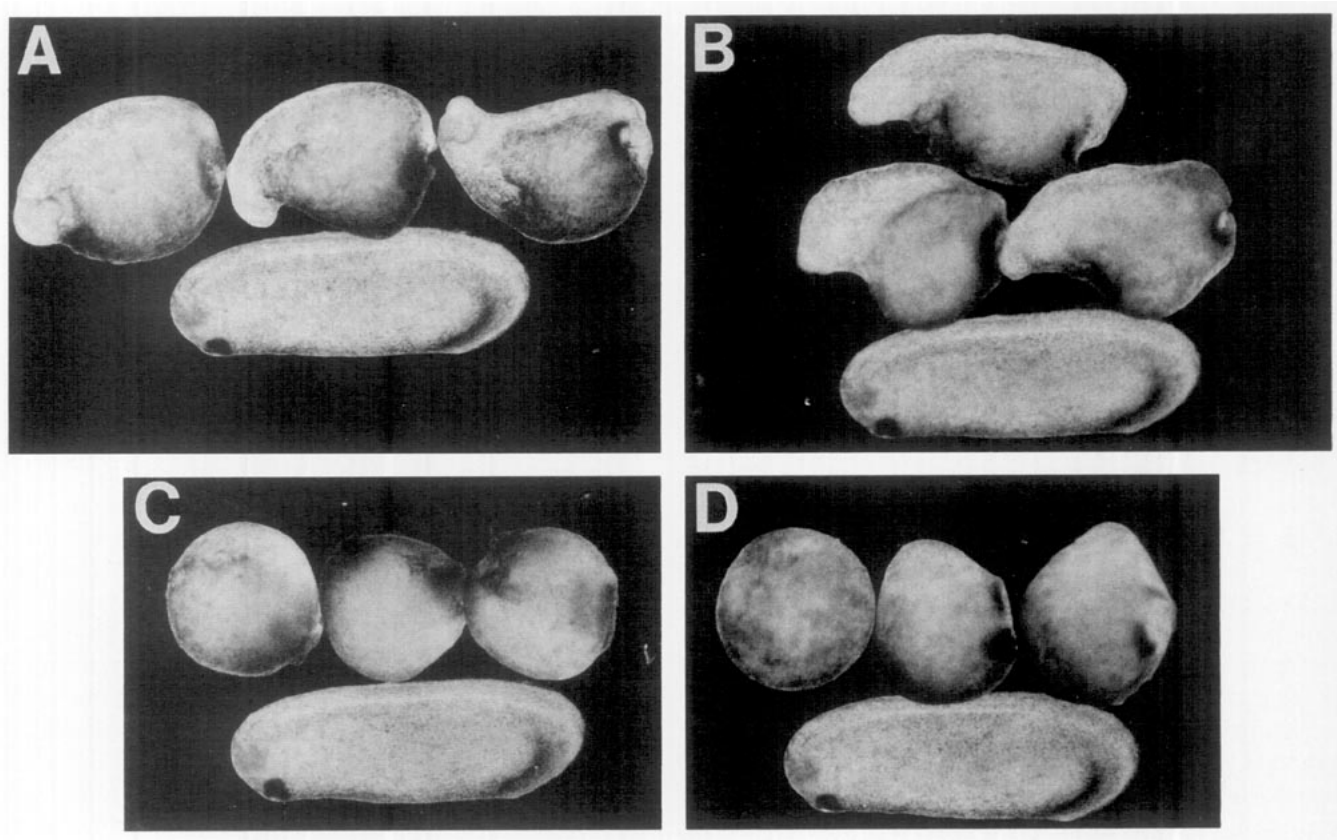


FIG. 1. Phenotypic comparison of dorsal and ventral injections of BMP-4 RNA. Four-cell embryos were injected into the two dorsal (A and C) or the two ventral (B and D) blastomeres with 2 ng (A and B) or 4 ng (C and D) of BMP-4 RNA. In each panel, the top three embryos were injected with BMP-4 RNA, and the bottom embryo is a control, uninjected embryo at the same stage (Stage 26). Complete ventralization can be achieved with different doses of BMP-4 RNA in embryos from different females; therefore, the embryos presented here are from the same experiment. Anterior is to the left in the control embryos and in embryos injected with 2 ng BMP-4 RNA. The closed blastopore is to the right in embryos injected with 4 ng BMP-4 RNA.

(Dale *et al.*, 1992; Jones *et al.*, 1992). When embryos were injected with BMP-4 RNA into one dorsal or one ventral cell at the four-cell stage, dorsal-injected embryos were completely ventralized and ventral-injected embryos were only partially ventralized (Dale *et al.*, 1992). We have repeated these experiments with some modifications. To compare the phenotype of dorsal injections with ventral injections, we injected embryos either dorsally or ventrally into two of four cells, rather than into one of four cells or the animal pole as was done previously. This assured that the RNA was evenly distributed throughout the dorsal or ventral half of the embryo. Different batches of embryos showed different sensitivities to a given dose of RNA; complete ventralization was routinely obtained using 2 to 4 ng of RNA. A representative example of the phenotypic effects of two different doses of RNA injected dorsally or ventrally is shown in Fig. 1. When these embryos were injected either dorsally or ventrally with 2 ng of BMP-4 RNA and allowed to develop until tailbud or tadpole stages, there was a noticeable increase in size of the ventral/posterior region of the embryo and a corresponding decrease in the size of dorsal/anterior structures (Figs. 1A and 1B). Ventral injections produced the same phenotypic effect, although the embryos developed slightly longer axes than those injected dorsally. When 4 ng of BMP-4 RNA was injected dorsally, embryos lost all axial structures and appeared completely ventralized (Fig. 1C). The same dose of BMP-4 RNA injected ventrally produced a predominance of completely ventralized embryos, although a slight elongation was occasionally observed (Fig. 1D). In summary, although the dorsal side of the embryo is more sensitive to a particular dose of BMP-4 RNA as reported earlier (Dale *et al.*, 1992), these results demonstrate that, under the conditions used here, both dorsal and ventral injections result in the same series of dose-dependent phenotypes. The discrepancy between our results and those of Dale *et al.* (1992) likely can be accounted for by the even distribution of the RNA over the dorsal or ventral half of the embryo in our experiments compared to RNA distribution over only a portion of the dorsal or ventral side in the previous work.

Ventralization of BMP-4-Injected Embryos Alters Pattern Formation and Gene Expression in the Gastrula-Stage Embryo

During gastrulation, the expression pattern of many genes becomes increasingly restricted to specific regions within the embryo. *Xnot*, for example, is expressed throughout the blastula embryo but becomes restricted to the presumptive notochord during gastrulation (von Dassow *et al.*, 1993). Many of the genes expressed in specific patterns during gastrulation are transcription factors that are thought to define the fate of embryonic cells. Therefore, changes in the expression patterns of these genes are likely to presage phenotypic changes seen during later embryonic stages.

Previous studies demonstrated that the transcript levels of *Xhox3* (Dale *et al.*, 1992; Jones *et al.*, 1992) and *Xbra* (Jones *et al.*, 1992) were altered as a result of BMP-4 RNA injections. However, these studies did not show whether changes in the levels of gene expression were accompanied by alterations in the spatial distribution of these transcripts. With the development of whole-mount *in situ* hybridization (Harland, 1991) and the isolation of several new genes which demarcate additional territories within the gastrula and neurula embryos, we can more effectively study the effects of ectopic BMP-4 expression on patterned gene expression in whole embryos during the stages in which BMP-4 is likely to be functioning.

Among the identified genes that are expressed solely within the organizer region of the gastrula embryo are *goosecoid* (*gsc*), which is expressed in the presumptive head mesoderm (Cho *et al.*, 1991), and *Xnot*, which is expressed in the future notochord (von Dassow *et al.*, 1993). We have previously shown that the expression of *Xnot* is eliminated when BMP-4 RNA is injected into the dorsal side of cleavage-stage embryos (von Dassow *et al.*, 1993), consistent with the elimination of dorsal axial development in embryos injected with BMP-4 RNA. Likewise, injection of BMP-4 RNA into the two dorsal cells of a four-cell embryo eliminated *gsc* expression in early gastrula embryos (Figs. 2A and 2B; Table 1).

The results obtained with genes expressed on the ventral side of the embryo were unexpected. *MyoD* is normally expressed in the ventral and lateral marginal zone during gastrulation and is excluded from the dorsal marginal zone in the region of the future notochord (Fig. 2C; Frank and Harland, 1991). In embryos injected dorsally with BMP-4 RNA, *MyoD* was no longer cleared from the dorsal midline and, instead, there was continuous expression of *MyoD* on the dorsal side (Fig. 2D; Table 1). Surprisingly, *MyoD* expression was greatly reduced or eliminated from the ventral and lateral marginal zone (Fig. 2D). *Xwnt-8* is also expressed in the lateral and ventral marginal zone, but during normal development is cleared from a broader region on the dorsal side than is *MyoD* (Fig. 2E; Christian *et al.*, 1991; Smith and Harland, 1991). *Xwnt-8* was completely eliminated from the ventral side of embryos injected dorsally with BMP-4 RNA (Fig. 2F; Table 1). In addition, the dorsal clearing of *Xwnt-8* expression either was eliminated (data not shown) or was much smaller in size than in control embryos (Figs. 2E and 2F). Similar results were observed with both *MyoD* and *Xwnt-8* when BMP-4 RNA was injected ventrally.

Given the unexpected results that injections of BMP-4 RNA eliminated the expression of both *MyoD* and *Xwnt-8* on the ventral side, we examined the effects of ventral BMP-4 RNA injections on the *Xenopus Brachyury* homolog, *Xbra* (Smith *et al.*, 1991), to determine if all mesodermal gene expression was eliminated on the ventral side by ectopic BMP-4 RNA injections. *Xbra* is expressed in what is thought to be the entire mesodermal region of the early gastrula embryo (Fig. 2G; Smith *et al.*, 1991). The expression of *Xbra* on the ventral side of the embryo was not eliminated by the ventral injection of BMP-4 RNA, but instead, its expression was expanded into the animal hemisphere (Fig. 2H; Table 1). The expansion of *Xbra* expression into the animal hemisphere is likely to be due to the mesoderm inducing properties of BMP-4 (Koster *et al.*, 1991; Dale *et al.*, 1992; Jones *et al.*, 1992; Fig. 5A). To assure that the results obtained with markers of ventral mesoderm were not due to variations in sensitivity between batches of embryos, in one experiment, fixed embryos were divided between *Xwnt-8* and *Xbra* probes. *Xwnt-8* expression was eliminated from the ventral side, whereas the expression of *Xbra* remained and was expanded. The domain of expression of *Xpo*, a marker of posterior mesoderm and ectoderm (Sato and Sargent, 1991), was also expanded animally on the ventral side of embryos injected ventrally with BMP-4 RNA (data not shown).

We expanded our study to examine the regulation of neural patterning by BMP-4, using the recently described neural plate marker, *Hairy II* (Turner and Weintraub, 1994). At stage 13, *Hairy II* is expressed in a stripe that outlines the open neural plate and in a region along the dorsal midline (Fig. 2I). In embryos injected with BMP-4 RNA, *Hairy II* expression was either eliminated or diffuse and faint (data not shown), or the region outlining the neural plate was greatly diminished in size (Fig. 2J; Table 1).

These results demonstrate that the diminishment of dorsal and anterior structures observed in later stage embryos injected with BMP-4 RNA is due to perturbations in early gene expression. Surprisingly, the expression of two genes that are localized to the lateral and ventral marginal zone of uninjected gastrula embryos,

MyoD and *Xwnt-8*, was shifted to the dorsal side of the embryo and ventral expression was eliminated. This observation suggests that the increase in ventral/posterior tissue in BMP-4 RNA-injected embryos is not simply due to enhancement of all genes expressed within the ventral marginal zone as might be expected from the ventralized phenotype.

BMP-4 Transcripts Are Absent from the Dorsal Side of the Gastrula Embryo

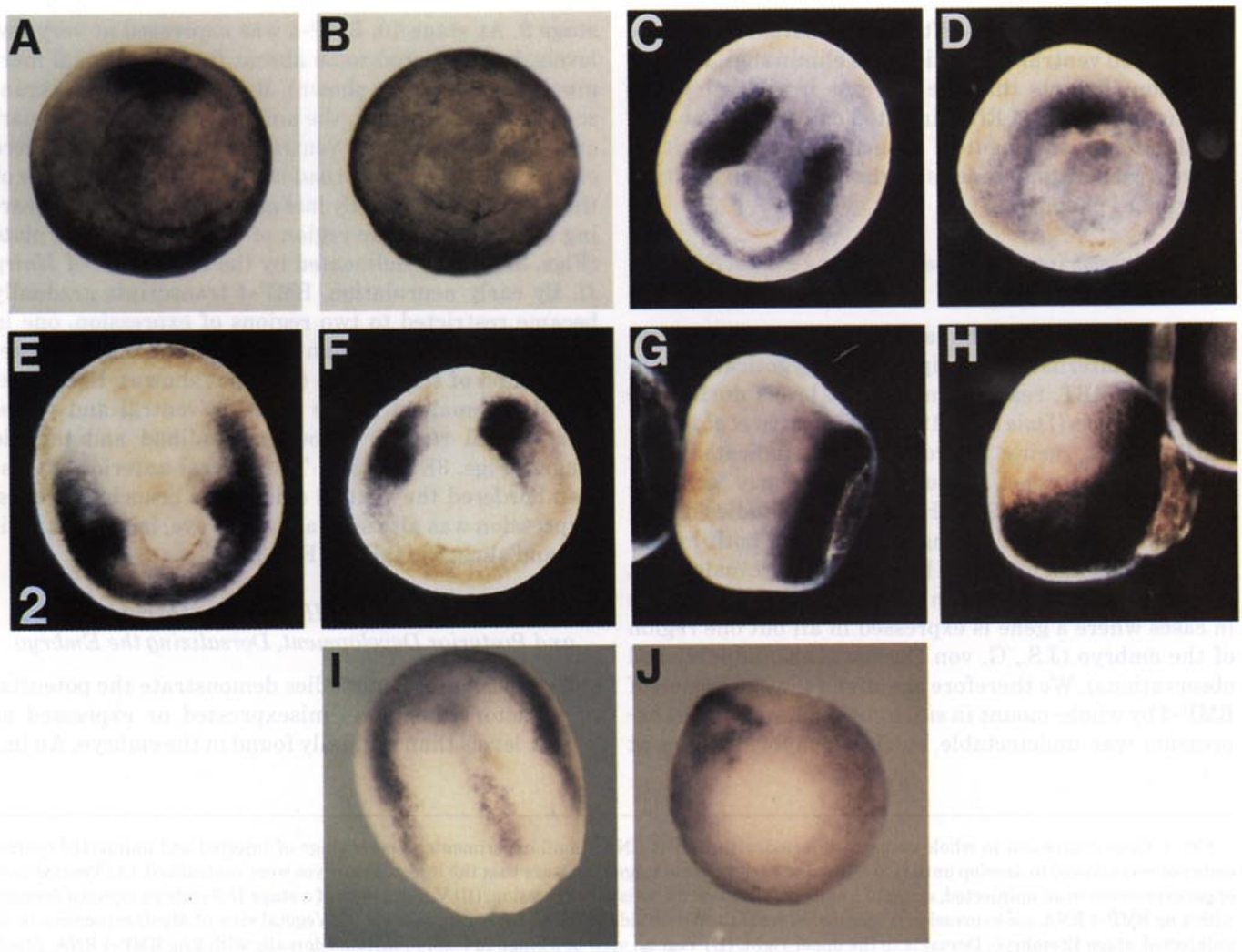
BMP-4 is present in the early embryo at very low levels as a maternal transcript and is zygotically transcribed at MBT, reaching maximum levels during the gastrula stages (Dale *et al.*, 1992; Nishimatsu *et al.*, 1992). Earlier work, using microdissection, indicated that BMP-4 is expressed throughout the early gastrula embryo (Dale *et al.*, 1992). However, our studies on another gene expressed during gastrulation, both by microdissection and by *in situ* hybridization, revealed that microdissection studies can provide misleading results in cases where a gene is expressed in all but one region of the embryo (J.S., G. von Dassow, D.K., unpublished observations). We therefore examined the expression of BMP-4 by whole-mount *in situ* hybridization. BMP-4 expression was undetectable by *in situ* hybridization at stage 9. At stage 10, BMP-4 was expressed at very low levels, but appeared to be absent from the dorsal marginal zone (data not shown). By stage 11, BMP-4 transcripts were present in the animal cap and at an apparently lower level in the ventral marginal zone, but were clearly absent from a broad region on the dorsal side of the embryo (Fig. 3A). By late gastrula stages, this clearing approximated the region of the entire neural plate (Figs. 3B–3D) as delineated by the expression of *Hairy II*. By early neurulation, BMP-4 transcripts gradually became restricted to two regions of expression, one in the ventral anterior region and one in the ventral posterior region of the embryo (data not shown). BMP-4 expression remained in the anterior/ventral and posterior/ventral regions throughout tailbud and tadpole stages (Figs. 3E and 3F). The ventral anterior expression bordered the ventral side of the branchial arches. Expression was also seen above the eye, in the otic vesicle, and along the tail fin (Fig. 3F).

A Dominant-Negative BMP Receptor Disrupts Ventral and Posterior Development, Dorsalizing the Embryo

Ectopic expression studies demonstrate the potential of a factor when it is misexpressed or expressed at higher levels than normally found in the embryo. An im-

FIG. 2. Gene expression in whole embryos injected with BMP-4 RNA. In all experiments, a percentage of injected and uninjected control embryos was allowed to develop until late tailbud or early tadpole stages to assure that the injected embryos were ventralized. (A) Vegetal view of *gsc* expression in an uninjected, stage 10.5 embryo. Dorsal is up, as is *gsc* expression. (B) Vegetal view of a stage 10.5 embryo injected dorsally with 4 ng BMP-4 RNA. *gsc* expression is eliminated from the dorsal side of the embryo. Dorsal is up. (C) Vegetal view of *MyoD* expression in an uninjected, stage 12 embryo. Dorsal is to the upper right. (D) Vegetal view of a stage 12 embryo injected dorsally with 2 ng BMP-4 RNA. *MyoD* expression is no longer cleared from the dorsal midline. The ventral expression is absent and the lateral expression is diminished. Dorsal is up. (E) Vegetal view of *Xwnt-8* expression in an uninjected, stage 12 embryo. Dorsal is up. (F) Vegetal view of a stage 12 embryo injected dorsally with 2 ng BMP-4 RNA. *Xwnt-8* is no longer expressed on the ventral side of the embryo. Two lateral patches of expression remain and the dorsal clearing has narrowed. Dorsal is up. Note that these embryos are from the same batch of embryos as those stained for *MyoD*. (G) Lateral view of *Xbra* expression in an uninjected, stage 11 embryo. *Xbra* is expressed throughout the marginal zone in the presumptive mesoderm. Dorsal is up. (H) Lateral view of a stage 11 embryo injected ventrally with 2 ng BMP-4 RNA. *Xbra* expression has expanded from the ventral marginal zone into the animal pole. Dorsal is up. (I) Dorsal view of *Hairy II* expression in an uninjected, stage 12.5 albino embryo. Anterior is up. *Hairy II* is expressed in a stripe that outlines the neural plate as well as within the dorsal midline. (J) Dorsal view of a stage 12.5 albino embryo injected with 2 ng BMP-4 RNA. Note that the embryo shown here was injected without dorsal or ventral distinction since pigmentation could not be used to differentiate dorsal from ventral. The neural plate as outlined by *Hairy II* has been greatly reduced in size. The results were identical to the results seen with dorsal and ventral injections of BMP-4 RNA into pigmented embryos (data not shown). The blastopore is often larger in embryos injected with BMP-4 RNA than in control embryos; however, most injected embryos that are allowed to develop until tailbud or tadpole stages completed gastrulation. In B, D, and F, embryos injected dorsally with BMP-4 RNA are shown. Since the results did not differ significantly when embryos were injected ventrally, only dorsal-injected embryos are shown.

FIG. 3. BMP-4 is cleared from the dorsal side of gastrula-stage embryos. Albino embryos at various stages were hybridized with an antisense BMP-4 RNA probe. (A) Lateral view of a stage 11 embryo, dorsal is up. The blastopore is to the upper right. BMP-4 expression is absent from the dorsal side of the embryo. Expression is strongest in the animal pole and appears to be at a lower level in the ventral marginal zone. An arrowhead indicates the ventral marginal zone. (B) Lateral/vegetal view of a stage 12 embryo. Staining is visible adjacent to the blastopore on the ventral side of the embryo. (C) Lateral view of the same embryo shown in B, anterior is to the left, dorsal is up. BMP-4 expression is absent from the dorsal side of the embryo in a region that approximates the future neural plate. (D) Future anterior view of the same embryo shown in B, the blastopore (arrowhead) and dorsal are to the right. A sharp boundary of expression is visible in this region. (E) Lateral view of a stage 22 embryo, anterior is to the left, dorsal is up. BMP-4 expression has resolved to two ventral regions, one anterior and one posterior. Additional expression can be seen above the eyes and along the future dorsal tail fin. (F) Lateral view of a stage 28 embryo, anterior is to the left, dorsal is up. The ventral posterior expression is reduced to a small narrow region; the ventral anterior expression is below the branchial arches. The expression above the eye is still present as well as expression along the tail fin. BMP-4 expression is now also visible in the otic vesicle. No specific staining was detected with the sense probe (not shown).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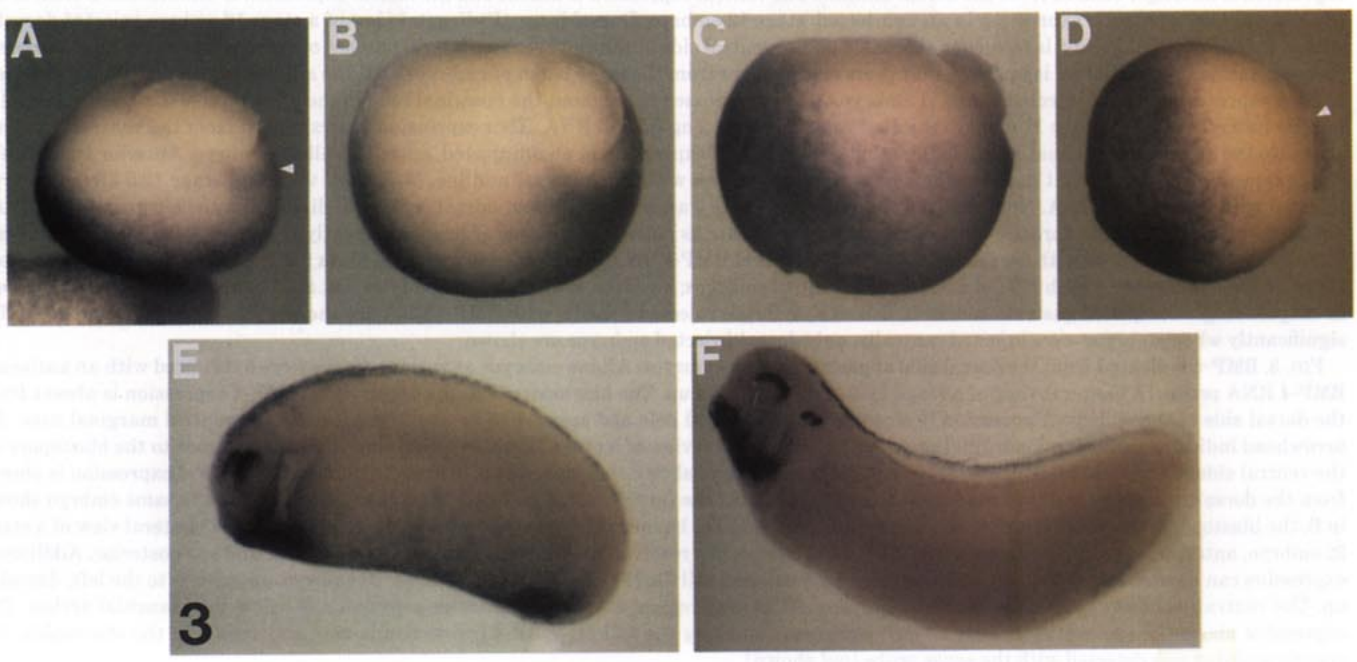
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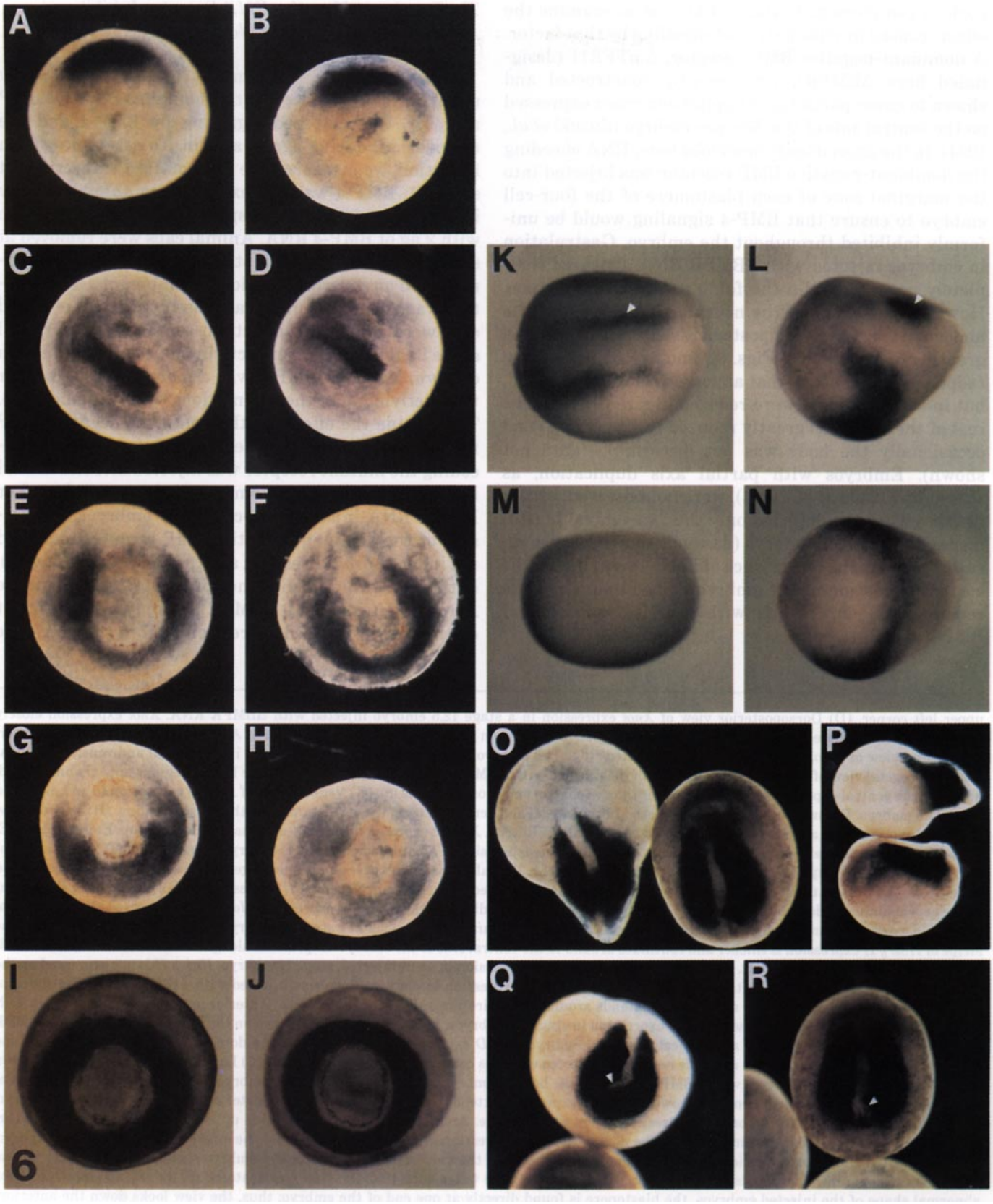

FIG. 6. Gene expression in gastrula and neurula embryos injected with dominant-negative BMP receptor RNA. (A) Vegetal view of *gsc* expression in an uninjected stage 10 embryo. Dorsal is up. (B) Vegetal view of *gsc* expression in a stage 10 embryo injected with ΔBMPR RNA. *gsc* expression is unchanged. Dorsal is up. (C) Dorsoposterior view of *Xnot* expression in an uninjected, stage 12.5 embryo. Anterior is at the

portant complement to these studies is to examine the effects caused by elimination of signaling by that factor. A dominant-negative BMP receptor, ΔmTFR11 (designated here ΔBMPR), was recently constructed and shown to cause partial axis duplication when expressed on the ventral side of the *Xenopus* embryo (Suzuki *et al.*, 1994). In the experiments described here, RNA encoding the dominant-negative BMP receptor was injected into the marginal zone of each blastomere of the four-cell embryo to ensure that BMP-4 signaling would be uniformly inhibited throughout the embryo. Gastrulation in embryos injected with ΔBMPR RNA appeared completely normal, as did the folding of the neural tube. However, the shape of the neurula-stage embryos was abnormal in that the posterior/ventral region was greatly reduced in size (Figs. 6O and 6P). The resulting tadpoles formed heads that appeared relatively normal, but in some embryos were reduced in size (Fig. 4). The rest of the body was greatly reduced in size (Fig. 4) and occasionally the body was not discernible (data not shown). Embryos with partial axis duplication, as shown by Suzuki *et al.* (1994), were not observed, except in cases where we injected only the two ventral blastomeres of a four-cell embryo (data not shown). These results establish the importance of BMP-4 signaling in the correct formation of the embryonic body plan and are reciprocal to our results with ectopic expression of BMP-4.

The Dominant-Negative BMP Receptor Inhibits Signaling by BMP-4 but Not by Activin

To test whether signaling by other TGF-β family members is also blocked by the dominant-negative BMP receptor, we compared signaling by BMP-4 and the mesoderm inducing agent, activin. To assay mesoderm induction, we measured the level of *Xbra* transcripts at stage 11. BMP-4 was overexpressed in animal caps by injecting the animal hemisphere of a one-cell embryo with 2 ng of BMP-4 RNA. Animal caps were removed at stage 9 and cultured until stage 11. To measure activin-mediated mesoderm induction, recombinant activin protein was added to animal caps dissected at stage 9, and caps were maintained in activin-containing buffer until stage 11. Both BMP-4 and activin induced the expression of *Xbra*, although BMP-4 was a more effective inducer than activin under these conditions (Fig. 5A, lanes 1–3). To examine the effects of the dominant-negative BMP receptor on signaling by these growth factors, RNA encoding the mutant receptor was injected into the animal hemisphere of both blastomeres of either uninjected or BMP-4 RNA-injected two-cell-stage embryos. Expression of the dominant-negative BMP receptor alone did not induce *Xbra* expression, as expected (Fig. 5A, lane 5), but it greatly diminished the ability of BMP-4 to induce *Xbra* (Fig. 5A, lane 4). ΔBMPR had only a minor effect on the activin-induced expression of *Xbra* (Fig. 5A, lane

upper left corner. (D) Dorsoposterior view of *Xnot* expression in a stage 12.5 embryo injected with ΔBMPR RNA. *Xnot* expression closely resembles that in uninjected embryos, although the region of expression may not extend as far anteriorly. Anterior is at the upper left corner. (E) Vegetal view of *MyoD* expression in an uninjected stage 11.5 embyro. Dorsal is up. *MyoD* is expressed in the lateral and ventral marginal zone. (F) Vegetal view of *MyoD* expression in embryos injected with ΔBMPR RNA. *MyoD* expression in the lateral regions appears unchanged; however, the ventral expression appears stronger than in control embryos. Dorsal is up. (G) Vegetal view of *Xwnt-8* expression in an uninjected stage 11.5 embryo. Dorsal is up. *Xwnt-8* is expressed in the lateral and ventral marginal zone distant from the dorsal midline. (H) Vegetal view of *Xwnt-8* expression in a stage 11.5 embryo injected with ΔBMPR RNA. *Xwnt-8* expression is nearly eliminated. Dorsal is up. Embryos in D, E, F, and G are from the same experiment. (I) Vegetal view of *Xbra* expression in an uninjected, stage 11 embryo. Dorsal is up. (J) Vegetal view of *Xbra* expression in a stage 11 embryo injected with ΔBMPR RNA. Dorsal is up. *Xbra* expression is unchanged in injected embryos as compared to controls. (K) Dorsolateral view of *Hairy II* expression in an uninjected, stage 13 embryo. *Hairy II* expression is in a stripe along the border of the neural plate and does not extend to the blastopore (at right). In addition to this stripe of expression, *Hairy II* is expressed along the dorsal midline (arrowhead). Anterior is to the left. (L) Dorsolateral view of *Hairy II* expression in a stage 13 embryo injected with ΔBMPR RNA. The stripe of *Hairy II* expression is thicker and continues around to the ventral side of the embryo. Expression along the dorsal midline (arrowhead) is shortened. Anterior is to the left. (M) Ventral view of *Hairy II* expression in an uninjected, stage 13 embryo. *Hairy II* is not expressed on the ventral side. Anterior is to the left. (N) Ventral view of *Hairy II* expression in a stage 13 embryo injected with ΔBMPR RNA. The stripe of *Hairy II* expression bordering the neural plate extends around the entire ventral side of the embryo. Anterior is to the left. Embryos in K–N are albino embryos. (O) Dorsal view of *MyoD* expression in stage 15 embryos, anterior is up. The embryo on the left was injected with ΔBMPR RNA, the embryo on the right is an uninjected control embryo. *MyoD* expression is cleared from the dorsal midline in both injected and uninjected embryos. The posterior region is reduced in injected embryos compared to control embryos. (P) Lateral view of the embryos shown in O. The top embryo was injected with ΔBMPR RNA and the bottom embryo is an uninjected control embryo. No *MyoD* expression is present on the ventral side of the control embryo; however, *MyoD* expression extends around the entire ventroposterior region of the injected embryo. Morphological changes in the embryo are already evident at this stage. Anterior is to the left, dorsal is up. (Q) View of the blastopore (arrowhead) in the same embryo as in O and P. The dorsal paraxial expression of *MyoD* extends around the entire ventral side of the embryo. Dorsal is up. (R) Dorsoposterior view of the blastopore (arrowhead) of the uninjected, stage 15 control embryo shown in O and P. No ventral expression is apparent. Anterior is up. Photographs for Q and R were taken such that the view was centered on the blastopore. Due to the abnormal shape of the injected embryos, the blastopore is found directly at one end of the embryo; thus, the view looks down the anterior/posterior axis. The blastopore of uninjected embryos at stage 15 is on the dorsoposterior surface of the embryo; therefore, the view is dorsoposterior.

TABLE 1

	BMP-4 RNA injections			ΔBMPR RNA injections		
	n	% normal expression	% altered expression	*n*	% normal expression	% altered expression
gsc	19	0	100[a]	13	100	0[f]
Xnot	—	—	—	15	100	0[g]
MyoD	94	3	69[b]	17	0	100[h]
Xwnt-8	56	0	96[c]	16	0	100[i]
Xbra	68	15	71[d]	16	100	0[j]
Hairy II	84	1	99[e]	42	0	100[k]

Note. Percentages do not add up to 100 in all cases since some embryos have altered phenotypes that are not represented by the descriptions given below which represent the majority phenotype. In all experiments, a portion of the embryos were left to develop to tailbud or tadpole stages to ensure that the injection produced the expected phenotype.

[a] *gsc* expression is absent (4 ng, dorsal injection, one experiment).

[b] No dorsal clearing; ventral *MyoD* expression is diminished or absent (2–4 ng, dorsal and ventral injections, seven experiments), 13% retained dorsal clearing but had diminished or absent ventral *MyoD* expression; 7% had radial expression.

[c] Dorsal clearing is reduced or absent; ventral *Xwnt-8* expression is absent (2–4 ng, dorsal and ventral injections, six experiments).

[d] Ventral *Xbra* expression expanded into animal region (2 ng, ventral injection, five experiments).

[e] *Hairy II* expression is diminished or absent (2–4 ng, dorsal and ventral injections, four experiments).

[f] *gsc* expression in all embryos appeared normal (4 ng, one experiment).

[g] *Xnot* expression in all embryos appeared normal; however, in some stage 12.5 embryos, expression appeared shortened along the anterior/posterior axis (4 ng, two experiments).

[h] *MyoD* expression was enhanced on the ventral side in stage 11.5 embryos and expanded to the ventral side in stage 15 embryos (4 ng, two experiments).

[i] *Xwnt-8* expression was eliminated or greatly reduced (4 ng, two experiments).

[j] *Xbra* expression in all embryos appeared normal; however, in some stage 12.5 embryos, notochordal expression appeared shortened along the anterior/posterior axis (4 ng, two experiments).

[k] *Hairy II* expression bordering the neural plate was expanded to the ventral side (4 ng, four experiments).

6). Therefore, the effects of the dominant-negative BMP receptor are not due to interference with activin signaling.

The effects of BMP-4 and the dominant-negative BMP receptor were also compared by assaying the expression of the muscle actin gene (Mohun *et al.*, 1984) in undissected embryos from the above experiment that were allowed to develop to stage 31 (Fig. 5B). Injection of BMP-4 RNA eliminated the expression of muscle actin (Fig. 5B, lanes 1 and 2), consistent with the ability of BMP-4 to ventralize embryos. Injection of ΔBMPR RNA did not, by itself, alter the levels of muscle actin (Fig. 5B, lane 3). However, when expressed in embryos injected with BMP-4 RNA, the dominant-negative BMP receptor inhibited the BMP-4-mediated ventralization of the embryo and rescued the wild-type expression of muscle actin (Fig. 5B, lane 4), and it partially rescued the ventralized phenotype of the embryo (data not shown). Similarly, injection of both BMP-4 and ΔBMPR RNAs into the dorsal marginal zone significantly rescued the ventralized phenotype (Suzuki *et al.*, 1994). Therefore, the dominant-negative BMP receptor can inhibit the BMP-4-mediated ventralization of the embryo without inhibiting mesoderm induction.

Ectopic Expression of the Dominant-Negative BMP Receptor Alters Gene Expression in Gastrula- and Neurula-Stage Embryos

To determine the effects of eliminating BMP-4 signaling on pattern formation and gene expression in gastrula and neurula embryos, RNA encoding the dominant-negative BMP receptor was injected into the marginal zone of all four blastomeres of the four-cell embryo in order to disrupt BMP-4 signaling uniformly throughout the embryo. The embryos were harvested, along with controls, for whole-mount *in situ* hybridization. Since embryos injected with ΔBMPR RNA have heads that appear normal or slightly reduced in size (Fig. 4), we examined the expression of the presumptive head mesoderm marker, *gsc*. As shown in Figs. 6A and 6B, *gsc* expression was unaffected by the truncated BMP receptor (Table 1). *Xnot*, which is also expressed in the dorsal marginal zone, was only slightly shortened along the an-

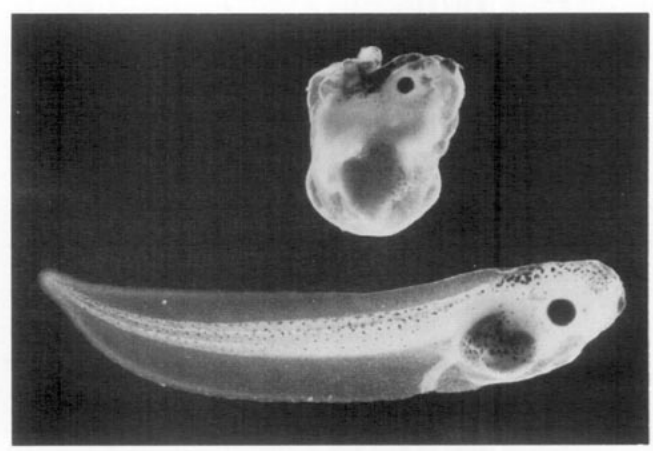

FIG. 4. Phenotypic effects of eliminating BMP-4 signaling in the early embryo. The top embryo was injected into the marginal zone of each cell at the four-cell stage with a total of 4 ng of the dominant-negative BMP receptor RNA. The head of this injected embryo is reduced in size, but appears relatively normal in structure compared to the control embryo below. The body of the injected embryo has not elongated and is extremely reduced in size. Embryos are at stage 47, anterior is to the right, dorsal is up.

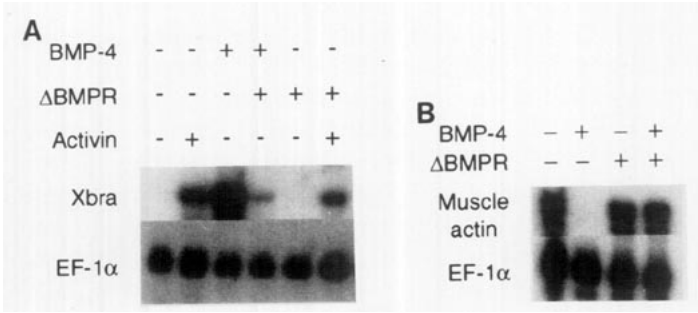

FIG. 5. The dominant-negative BMP receptor blocks BMP-4 signaling, but not activin signaling. (A) Animal caps were isolated at stage 9 from uninjected, BMP-4 RNA- or ΔBMPR RNA-injected embryos or from embryos injected with both BMP-4 and ΔBMPR RNAs. In some cases, activin was added to the animal caps at stage 9. In all cases, RNA was harvested at stage 11. The levels of *Xbra* and the ubiquitously expressed gene, EF-1α, were determined by RNase protection analysis. Lane 1, control caps. Lane 2, activin-treated caps. Lane 3, animal caps from embryos injected with 2 ng BMP-4 RNA. Lane 4, animal caps from embryos injected with 2 ng BMP-4 RNA and 2 ng ΔBMPR RNA. Lane 5, animal caps from embryos injected with 2 ng ΔBMPR RNA. Lane 6, animal caps from embryos injected with 2 ng ΔBMPR RNA and treated with activin. (B) Embryos injected with BMP-4 RNA and/or ΔBMPR RNA from the same experiment shown in A were allowed to develop until stage 31. RNA was isolated and analyzed for muscle actin expression by RNase protection analysis. Lane 1, uninjected embryos. Lane 2, embyros injected with 2 ng BMP-4 RNA. Lane 3, embryos injected with 2 ng ΔBMPR RNA. Lane 4, embryos injected with 2 ng BMP-4 RNA and 2 ng ΔBMPR RNA. EF-1α was used as a control for loading.

terior/posterior axis by the truncated BMP receptor (Figs. 6C and 6D; Table 1). In contrast, the expression of genes with transcripts localized to the lateral and ventral regions of the mesoderm were altered by injection of ΔBMPR RNA. *MyoD* is expressed within the ventral and lateral marginal zone at stage 11.5, although staining is more intense within the lateral regions comprising the future dorsal somitic mesoderm (Fig. 6E). Injection of the dominant-negative BMP receptor RNA did not change the overall spatial distribution of *MyoD* at this stage, although the ventral expression became more intense, more closely resembling the level of staining in the lateral regions (Fig. 6F; Table 1). This effect was more pronounced at later stages of development. By neurulation, normal *MyoD* expression was localized to the dorsal side of the embryo flanking the notochord, and no ventral *MyoD* expression was observed (Figs. 6O, 6P, and 6R). In embryos injected with ΔBMPR RNA, *MyoD* expression was still absent from the dorsal midline and was still strongly expressed in paraxial mesoderm, although the region of expression appeared to be shortened along the anterior/posterior axis (Figs. 6O, 6P, and 6Q). In addition, a large band of *MyoD* expression was apparent on the ventral side of ΔBMPR-injected embryos, forming a continuous band around the ventral side of the closed blastopore (Figs. 6P and 6Q; Table 1), suggesting that the ventral region acquired a more dorsal specification in the absence of BMP-4 signaling.

If the hypothesis that the ventral region had acquired dorsolateral specification was correct, we would expect that the expression of genes localized to the ventral regions would be diminished. *Xwnt-8* is expressed in the ventral half of the embryo during gastrulation (Fig. 6G) and is a useful marker of ventral mesoderm. As shown in Fig. 6H, the expression of *Xwnt-8* was eliminated in embryos injected with ΔBMPR RNA (Table 1). Similarly, we examined by RNase protection the level of expression of the ventroposterior gene, *Xhox3* (Ruiz i Altaba and Melton, 1989), in stage 13 embryos injected with ΔBMPR RNA. The levels of *Xhox3* were reduced by the dominant-negative BMP receptor (Fig. 7A), whereas the levels of *MyoD* were increased in the same experiment (Fig. 7B). These results confirm the hypothesis that ventral mesodermal cells are respecified toward a dorsolateral fate by the dominant-negative BMP receptor. The extent of the mesoderm was not altered by the dominant-negative BMP receptor since the expression of the pan-mesodermal marker, *Xbra*, in stage 11.5 embryos, was not altered by the injection of ΔBMPR RNA (Figs. 6I and 6J; Table 1).

The expression of *Hairy II* was examined to determine the effects of the dominant-negative BMP receptor on the patterning of the neural plate. At all stages examined (11.5, 12, 13, and 15), we observed an expansion of the neural plate region to include the ventral side of the embryo (Figs. 6K–6N; Table 1; data not shown). Whereas *Hairy II* expression is normally confined to the dorsal side of the embryo, outlining the neural plate as described earlier (Figs. 2I, 6K, and 6M), embryos expressing the dominant-negative BMP receptor ex-

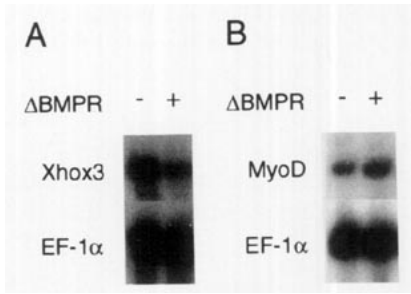

FIG. 7. The transcript levels of the ventroposterior gene, *Xhox3*, are reduced, whereas the levels of the dorsal paraxial gene *MyoD* are increased in embryos injected with ΔBMPR RNA. Embryos were injected into the marginal zone of all four blastomeres at the four-cell stage with 4 ng of ΔBMPR RNA. RNA was isolated at stage 13 and analyzed by RNase protection using *Xhox3* (A), *MyoD* (B), and EF-1α probes. The same sample of RNA was used to detect the levels of *MyoD* and *Xhox3*. In both panels, RNA from uninjected embryos is in lane 1 and RNA from ΔBMPR-injected embryos is in lane 2. EF-1α was used as a control for loading.

pressed *Hairy II* in a ring around the embryo, with strong staining on the ventral side (Figs. 6L and 6N; Table 1). These results demonstrate that the elimination of BMP-4 signaling causes large changes in the patterning of gastrula and neurula stage embryos, with a reduction of ventral mesoderm, and an increase and expansion of dorsolateral mesoderm and neuroectoderm.

DISCUSSION

BMP-4 Is a Ventralizing Signal That Acts after Primary Mesoderm Induction

We have shown that the expression and distribution of genes that are patterned during gastrulation are dramatically affected by the injection of BMP-4 RNA into early cleavage-stage embryos. Ectopic BMP-4 transforms the dorsal region of the gastrula-stage embryo to a more lateral or ventral fate, resulting in tadpoles in which ventral tissue has expanded and dorsal axial structures are diminished or absent. Genes that are normally expressed along the dorsal midline, such as *Xnot* and *gsc*, are suppressed by injection of BMP-4 RNA, whereas *MyoD*, which is normally expressed in the lateral (prospective paraxial) mesoderm, is observed along the dorsal midline in BMP-4 RNA-injected embryos. In addition, expression of the ventral/posterior genes *Xhox3* (Dale *et al.*, 1992; Jones *et al.*, 1992) and *Xpo* (our unpublished results), are enhanced in BMP-4 RNA-injected embryos, consistent with an expansion of ventral/posterior mesoderm. These results demonstrate that exogenous BMP-4 interferes with the normal patterning and expression levels of mesodermal genes during gastrulation. The resulting alterations in patterned gene expression account for the phenotype seen at later stages, namely diminishment of dorsoanterior structures and enlargement of ventroposterior structures.

Our results with a dominant-negative BMP receptor, which prevents signaling by BMP-4 (Suzuki *et al.*, 1994; our results), are reciprocal to the results of ectopic BMP-4 expression. The dorsal paraxial mesoderm is expanded at the expense of ventral mesoderm. These effects are due to changes in gene expression during gastrulation and neurulation, as shown by the altered expression of *MyoD*, *Xwnt-8*, and *Xhox3*. *MyoD*, which is normally expressed only adjacent to the dorsal midline during neurulation, is found in a thick band surrounding the ventral side of the closed blastopore. This effect is observable during gastrulation as an enhancement of *MyoD* expression on the ventral side of the embryo. Conversely, the expression of the ventral markers, *Xwnt-8* and *Xhox3*, is either eliminated or reduced. Dorsal midline gene expression, however, is not expanded in these "dorsalized" embryos, indicating that BMP-4 is not involved in regulating the extent of the dorsal midline along the dorsal/ventral axis. Gene expression along the dorsal midline appears to be shortened along the anterior/posterior axis when compared to controls, prefiguring the extreme shortened axis seen in tadpoles. These results demonstrate that endogenous BMP-4 functions prior to and/or during gastrulation to regulate the patterning and expression levels of mesodermal genes. Similar conclusions have been reached by RNase protection analysis (Graff *et al.*, 1994).

Further support for a role for BMP-4 in dorsal/ventral patterning comes from an analysis of its expression pattern during gastrulation. Prior to gastrulation, BMP-4 is present at low levels as a maternal transcript. BMP-4 is zygotically transcribed, reaching high levels during gastrulation, and then diminishing afterward. *In situ* hybridization reveals that BMP-4 transcripts are absent from the dorsal side of gastrula-stage embryos. This expression pattern is consistent with our functional studies using the dominant-negative BMP receptor, which show that endogenous BMP-4 does not regulate the equatorial size of the organizer region, but is important for regulating the pattern of lateral and ventral mesoderm. Thus, elimination of BMP-4 signaling on the ventral side of the embryo causes a conversion from ventral mesoderm to more dorsal mesoderm, but not to organizer-derived cell types, such as head mesoderm and notochord (Graff *et al.*, 1994; Suzuki *et al.*, 1994; our results). Similar results were observed in explants, although in this case, notochordal tissue and gene expression were observed (Graff *et al.*, 1994; Maeno *et al.*, 1994), suggesting that explants may not precisely reproduce the signaling environment of the whole embryo.

Specific levels of BMP-4 may be important for precise patterning of the ventral region of the embryo. Although BMP-4 expression is normally found on the ventral side of the gastrula-stage embryo, expression of the ventral mesodermal marker, *Xwnt-8*, was eliminated in BMP-4 RNA-injected embryos. Paradoxically, inhibition of BMP-4 signaling within the embryo by the dominant-negative BMP receptor also eliminated *Xwnt-8* expression. Therefore, BMP-4 signaling is required for the expression of *Xwnt-8*, but excess levels inhibit its expression. The level of BMP-4 expression appears higher in the presumptive ventral ectoderm than in the ventral marginal zone (Fig. 3A), where *Xwnt-8* is normally expressed. The levels present in the presumptive ventral ectoderm may repress *Xwnt-8* expression, whereas lower levels found in the marginal zone might be required for *Xwnt-8* expression. In addition, ectopic BMP-4 expression strongly enhances the expression of *Xhox3* (Dale *et al.*, 1992; Jones *et al.*, 1992), *Xbra* (Jones *et al.*, 1992), and *Xpo* (our unpublished results), but it eliminates the ventral expression of *MyoD* and *Xwnt-8* in gastrula-stage

embryos. Furthermore, at high doses, BMP-4 eliminated *Xbra* expression from the ventral marginal zone (unpublished observations). The apparent dose dependence leads to the hypothesis that BMP-4 may be acting as a morphogen. Future experiments will be necessary to test this hypothesis.

Both BMP-4 injections and uv irradiation "ventralize" embryos (Scharf and Gerhart, 1983; Dale *et al.*, 1992; Jones *et al.*, 1992). However, the ventralization differs in three important aspects. First, in gastrula-stage BMP-4-injected embryos, *Xwnt-8* expression is eliminated from the ventral marginal zone and remains in only two small patches on the dorsal side, whereas uv-irradiated embryos express *Xwnt-8* radially within the mesoderm (Smith and Harland, 1991). Second, there are phenotypic differences in partially ventralized embryos. BMP-4-injected embryos, which are only partially ventralized, have diminished dorsoanterior regions and correspondingly enlarged ventroposterior regions. In contrast, partially ventralized uv-irradiated embryos lose head structures without a corresponding enlargement of ventroposterior regions (Scharf and Gerhart, 1983; Elinson and Pasceri, 1989). Last, results presented here and in earlier work (Dale *et al.*, 1992; Jones *et al.*, 1992) suggest that BMP-4 regulates the patterning of the embryo during gastrulation, after primary mesoderm induction has occurred. Ultraviolet irradiation, on the other hand, disrupts primary mesoderm induction by interfering with the establishment of a dorsal mesoderm inducer(s) in the fertilized egg (Gimlich and Gerhart, 1984; Gerhart *et al.*, 1989).

In summary, we propose that localized expression of BMP-4 in the lateral and ventral regions is important for inhibiting the expression of dorsolateral genes in the ventral regions and, conversely, for permitting ventral gene expression on the ventral side of the embryo, as suggested by Graff *et al.* (1994) and Maeno *et al.* (1994). BMP-4 may act to oppose the action of dorsalizing signals, such as noggin (Smith and Harland, 1992), that originate in the dorsal organizer region. The interaction between dorsalizing and ventralizing factors during gastrulation is therefore likely to be important for establishing the dorsal/ventral pattern within the mesoderm of gastrula-stage embryos.

BMP-4 Limits the Extent of Neural Induction

In addition to dorsalizing the mesoderm, the organizer induces and patterns the neuroectoderm (reviewed in Slack and Tannahill, 1992). Our data reveal that BMP-4 has a role in limiting the extent of neural induction. Dorsal or ventral injection of BMP-4 RNA either eliminated the formation of the neural plate or greatly reduced it in size, as shown by the neural plate marker, *Hairy II*. Conversely, when BMP-4 signaling was blocked, the neuroectoderm became enlarged and extended continuously from the dorsal to the ventral side of the embryo. Intriguingly, the region on the dorsal side of the gastrula embryo that lacks BMP-4 transcripts appears to demarcate the territory of the future neural plate. This suggests that the localized expression of BMP-4 may help to define the size of the neural plate. It remains unclear whether BMP-4 limits the size of the neural plate directly, or indirectly through the mesoderm.

Relationships between BMP-4 and Decapentaplegic

The amino acid sequence of BMP-4 is closely related to that of the *dpp* gene (Padgett *et al.*, 1987; Koster *et al.*, 1991; Dale *et al.*, 1992; Nishimatsu *et al.*, 1992) which is involved in dorsal/ventral pattern formation in the *Drosophila* embryo. These genes are also functionally conserved since human BMP-4 is able to substitute for *dpp* in *dpp* mutant embryos (Padgett *et al.*, 1993). Our results suggest that there are significant similarities between the roles of these molecules in dorsal/ventral pattern formation in *Xenopus* and *Drosophila*.

In *Drosophila*, the expression of *dpp* is restricted to the dorsal side of the blastoderm-stage embryo and promotes the differentiation of dorsal tissues, such as the dorsal epidermis and the amnioserosa (St. Johnston and Gelbart, 1987; Wharton *et al.*, 1993). In mutants of those dorsal-group genes which show dorsalized phenotypes, expression of *dpp* is no longer restricted to the dorsal side of the embryo, but extends to the ventral side. Consequently, the neuroectoderm is reduced or eliminated as is the mesoderm (reviewed in Govind and Steward, 1991; St Johnston and Nusslein-Volhard, 1992). Similarly, when *dpp* RNA is injected into the dorsal or ventral side of wild-type syncytial blastoderm embryos, the amount of neuroectoderm is reduced (Ferguson and Anderson, 1992b). Because in these studies alterations in dorsal/ventral pattern formation were assessed by examination of cuticular structures or by the formation of the ventral furrow, the effect of overexpression of *dpp* on the formation of mesoderm in *Drosophila* has not been well characterized. Embryos lacking *dpp*, on the other hand, are ventralized and the neuroectoderm extends throughout the dorsal side of the embryo (Irish and Gelbart, 1987; Ferguson and Anderson, 1992a,b). Dorsal/ventral pattern formation in the early embryo is sensitive to *dpp* gene dosage; increased *dpp* copy number results in diminishment of ventral mesoderm and neuroectoderm (Ferguson and Anderson, 1992a), whereas elimination of one copy of *dpp* results in an expansion of ventral structures (Irish and Gelbart, 1987).

Similarly, in *Xenopus*, overexpression of BMP-4 leads

to the dose-dependent reduction or elimination of neural and mesodermal structures and gene expression (Dale *et al.*, 1992; Jones *et al.*, 1992; our results). Elimination of BMP-4 signaling throughout the embryo leads to the expansion of dorsal mesoderm and neuroectoderm at the expense of ventral/posterior tissue, comparable to the effects of a *dpp* null mutant. In addition, BMP-4 transcripts are absent from the dorsal side of the *Xenopus* embryo where neuroectoderm forms. We propose that BMP-4 and *dpp* may have analogous roles in dorsal/ventral pattern formation in the early embryo by limiting the extent of neuroectoderm formation and by patterning the region of the embryo which lies outside the neuroectodermal region.

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Note added in proof. Another study on the role of BMP-4 in *Xenopus* development has recently been published (Fainsod, A., *et al.*, 1994, *EMBO J.* **13**, 5015–5025). Recent work by K. Staehling-Hampton *et al.* (1994, *Nature* **372**, 783–786) further supports the analogy between *Xenopus* BMP-4 and *Drosophila dpp* in regulating dorsal/ventral pattern formation in early embryos.

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