Competition between noggin and bone morphogenetic protein 4 activities may regulate dorsalization during Xenopus development

Yael Re'em-Kalma*, Teresa Lamb†, and Dale Frank‡

*Department of Biochemistry, The Rapaport Family Institute for Research in the Medical Sciences, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel; and Department of Molecular and Cellular Biology, Division of Biochemistry and Molecular Biology, 401 Barker Hall, University of California, Berkeley, CA 94720

Communicated by John Gerhart, University of California, Berkeley, CA, September 15, 1995

ABSTRACT  Bone morphogenetic protein 4 (BMP-4) induces ventral mesoderm but represses dorsal mesoderm formation in Xenopus embryos. We show that BMP-4 inhibits two signaling pathways regulating dorsal mesoderm formation, the induction of dorsal mesoderm (Spemann organizer) and the dorsalization of ventral mesoderm. Ectopic expression of BMP-4 RNA reduces goosecid and forkhead-1 transcription in whole embryos and in activin-treated animal cap explants. Embryos and animal caps overexpressing BMP-4 transcribe high levels of genes expressed in ventral mesoderm (Xbra, Xwnt-8, Xpo, Mix-1, XMyoD). The Spemann organizer is ventralized in these embryos; abnormally high levels of Xwnt-8 mRNA and the levels of goosecid mRNA are detected in the organizer. In addition, the organizer loses the ability to dorsalize neighboring ventral marginal zone to muscle. Overexpression of BMP-4 in ventral mesoderm inhibits its response to dorsalization signals. Ventral marginal zone explants ectopically expressing BMP-4 form less muscle when treated with soluble noggin protein or when juxtaposed to a normal Spemann organizer in comparison to control explants. Endogenous BMP-4 transcripts are downregulated in ventral marginal zone explants dorsalized by noggin, in contrast to untreated explants. Thus, while BMP-4 inhibits noggin protein activity, noggin downregulates BMP-4 expression by dorsalizing ventral marginal zone to muscle. Noggin and BMP-4 activities may control the lateral extent of dorsalization within the marginal zone. Competition between these two molecules may determine the final degree of muscle formation in the marginal zone, thus defining the border between dorsolateral and ventral mesoderm.

In Xenopus embryos, mesoderm forms by a multistep inductive process (reviewed in refs. 1–3). During early development, vegetal pole cells exist in two states, either dorsal or ventral (4, 5). The most dorsal mesoderm (Spemann organizer) is induced in the subequatorial region (marginal zone, MZ) by underlying dorsal vegetal pole cells (Nieuwkoop center). Ventral mesoderm (blood, mesenchyme) is induced in the ventral MZ (VMZ) by underlying ventral vegetal pole cells. During gastrulation, a dorsalizing signal emanating from the Spemann organizer converts adjacent lateral mesoderm from ventral to more dorsal fates such as muscle, heart, and pronephros (6–8). Since noggin is expressed in the Spemann organizer during gastrulation and secreted noggin protein dorsalizes ventral mesoderm to muscle, noggin is a potential candidate molecule for acting as the endogenous dorsalizing signal (9, 10). Little is known about the regulation of the dorsalization process; it is not clear what factors limit the extent of dorsalization within the marginal zone, thus demarcating which cells will eventually make muscle and which cells will maintain a ventral fate. While it is clear that cells fated for ventral mesoderm have the potential to differentiate into muscle when placed near the source of the dorsalization signal (6–9), it is not clear how distinct axial borders are defined within the developing embryo. It is possible that cell borders are defined by a combination of positive and negative pathways which demarcate the dorsal/ventral border.

Bone morphogenetic protein 4 (BMP-4) is a member of the transforming growth factor β family (11). The gene has been isolated from Xenopus laevis, and ectopic expression of in vitro synthesized BMP-4 RNA in developing embryos causes developmental changes which suggest that BMP-4 protein induces ventroposterior mesoderm. Late-gastrula-stage animal caps isolated from BMP-4-injected embryos transcribe Xwnt-8 mRNA, a ventroposterior mesoderm marker (12). Histological analysis of animal cap explants treated with soluble BMP-4 protein as well as animal cap explants derived from BMP-4-injected embryos suggests that these cells resemble ventral mesoderm (12–14). BMP-4-injected animal caps also form posterior mesoderm in Einstecken experiments (13). Basic fibroblast growth factor (bFGF) also induces ventroposterior mesoderm in animal cap explants (15, 16), but the mode of action of bFGF differs from that of BMP-4. BMP-4 inhibits dorsal induction by activin in a dominant manner; animal caps injected with BMP-4 and treated with activin do not make muscle (12, 13). In contrast, bFGF does not antagonize activin’s induction of dorsal mesoderm in animal caps (17). Ectopic expression of BMP-4 RNA also inhibits dorsal axis formation in whole embryos; they fail to elongate and do not make detectable notochord or muscle (12, 13). In addition, ectopic expression of a dominant negative Xenopus BMP-4 receptor in embryos converts ventral mesoderm to dorsal mesoderm (18). Thus, while BMP-4 acts as a ventroposterior mesoderm inducer, it also inhibits dorsal mesoderm formation.

Previous studies did not determine by which signaling pathway BMP-4 inhibited dorsal mesoderm formation. We show that overexpression of BMP-4 inhibits two signaling pathways regulating dorsal mesoderm formation. Overexpression of BMP-4 RNA suppresses the initial induction of dorsal mesoderm in whole embryos and in activin-treated animal caps, thus inhibiting formation of the Spemann organizer. BMP-4 also strongly inhibits dorsalization of ventral mesoderm by the Spemann organizer or soluble noggin protein. Our results suggest that competition between noggin and BMP-4 activities may determine the eventual limit of muscle formation in the MZ.

MATERIALS AND METHODS

Xenopus Embryos and Inducing Factors. Ovulation, in vitro fertilization, embryo culture, and dissections were carried out as described (19). Embryos were staged according to Nieuwkoop and Faber (20). PIF/activin was prepared from the P33R1 cell line (21). Activin-treated animal cap explants

Abbreviations: BMP-4, bone morphogenetic protein 4; MZ, marginal zone; VMZ, ventral MZ; DMZ, dorsal MZ; LMZ, lateral MZ; bFGF, basic fibroblast growth factor; EF1α, elongation factor 1α; LCMR, low-Ca²⁺/Mg²⁺ modified Ringer’s solution; DA1, dorsal-anterior index.

*To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. 1734 solely to indicate this fact.
were grown in 0.5× modified Ringer’s solution with gentamicin from stage 8 until stage 11. Noggin was prepared from CHO-B5 cell conditioned medium (22). Noggin-treated (0.25× CHO-B5 conditioned medium) VMZ explants were grown in 1× low-Ca²⁺/Mg²⁺ modified Ringer’s solution (LCMR; ref. 7) with gentamicin from stage 10–10.25 until stage 13; at this stage, explants were transferred for further growth in fresh 1× LCMR lacking noggin. Control explants were grown in 1× LCMR with 0.25× conditioned medium from the non-noggin-producing parental CHO cell line. Recombinant explants were dissected and grown in 1× LCMR. MZ explants were removed at stage 10–10.25, a stage at which a pigment line is formed at the dorsal lip by inverting bottle cells, thus enabling identification of presumptive dorsal and ventral sides. A 45°–60° region of VMZ or dorsal MZ (DMZ) was dissected with an eyebrow knife. Lateral MZ (LMZ) represents the remainder of the MZ after removal of DMZ and VMZ. Recombinants were juxtaposed to one another with watchmaker’s forceps.

**RNA Injections.** Capped synthetic Xenopus BMP-4 RNA was prepared from the pSP64T-XBMP-4+-vector (12) according to Smith and Harland (23). Various concentrations of RNA (0.5–3.0 ng) were injected into embryos at the one-cell stage. In each experiment, 25–50 embryos were allowed to develop until stage 40. At this stage the dorsal–anterior index (DAI) was determined (24).

**Northern Blot Analysis.** RNA was extracted from embryos and prepared for Northern blot analysis (19). Electrophoresis, probe preparation, filter hybridization, and exposure were as described (19). Quantitation was performed with a Fuji phosphor-imaging system. Elongation factor 1α (EF1α) was used as a positive standard for comparing amounts of RNA loaded per well at any given stage (25).

---

**RESULTS**

**BMP-4 Induces Ventroposterior Mesoderm but Inhibits Formation of Dorsal Mesoderm.** We examined the inhibitory role of BMP-4 on activin induction of dorsal mesoderm. Previous studies showed that BMP-4 inhibited the ability of activin to induce muscle in animal cap explants (12, 13). It is not clear whether BMP-4 mediated this effect by inhibiting initial dorsal mesoderm induction or by inhibiting the dorsalization of ventral mesoderm to muscle. Experiments were carried out to elucidate whether BMP-4 inhibits initial formation of dorsal mesoderm (Spemann organizer).

We injected 2 ng of in vitro synthesized BMP-4 RNA into one-cell fertilized Xenopus embryos. Animal caps were removed from blastulae (stage 8) and grown until the mid-gastrula stage (stage 11). RNA was isolated for Northern analysis to determine whether BMP-4 prevented induction of markers characteristic of dorsal mesoderm. The Xenopus forhead-1 (XFKH-1), gooseoid (gcd), and Xlim-1 genes are expressed in the Spemann organizer and are induced by activin in animal caps (26–28). These markers were used to measure the formation of dorsal mesoderm. XFKH-1, gcd, and Xlim-1 (not shown) mRNAs were significantly reduced in BMP-4-injected, activin-treated gastrula-stage animal caps in comparison to un.injected, activin-treated controls (Fig. 1A). These effects were specific for BMP-4 RNA, since animal caps taken from embryos injected with 2 ng of proactin RNA behaved like the uninjectected controls (data not shown). At gastrula stages, BMP-4-injected whole embryos also expressed less gcd mRNA (Fig. 1B), as well as less XFKH-1 and Xlim-1 mRNA (data not shown), in comparison to control embryos. Analysis of dissected DMZs from stage 10–10.25 BMP-4-injected embryos showed a >10-fold reduction in gcd mRNA versus controls (Fig. 1B). In situ hybridization has also shown that gcd

---

**Fig. 1.** Ectopic expression of BMP-4 inhibits dorsal mesoderm formation and induces ventral mesoderm in gastrula- and neurula-stage embryos and embryonic explants. (A) Embryos at the one-cell stage were injected with 2 ng of in vitro synthesized Xenopus BMP-4 RNA. Animal cap explants were removed at stage 8 and treated with PIF/activin until stage 11. Total RNA was isolated from pools of five embryos (Emb) and 16 animal caps from each treatment at stage 11. One embryo equivalent of RNA was loaded per well for Northern analysis; all animal cap RNA was loaded per well. The filter was sequentially hybridized with Xenopus cDNA probes for gooseoid (gcd), forhead-1 (XFKH-1), Brachyury (Xbra), Xwnt-8, Xpo, Mix-1, and EF1α. (B) Embryos at the one-cell stage were injected with 2 ng of BMP-4 RNA. Total RNA was isolated from pools of five injected (+) and uninjectected (−) early gastrula (stage 10–10.25) embryos (Emb). Nine VMZ (V) and DMZ (D) regions were dissected and total RNA was isolated. One embryo equivalent of RNA was loaded per well for Northern analysis; all of the RNA from the dissected regions was loaded. The filter was sequentially hybridized with cDNA probes for gcd, Xwnt-8, Xbra, and EF1α. (C) Embryos at the one-cell stage were injected with 1, 2, or 3 ng of BMP-4 RNA. At stage 10+, DMZs were removed from injected and noninjected embryos. DMZ explants were recombined with VMZ explants taken from uninjectected embryos at the same stage. Ten recombinant explants from each of the three injection and control groups were grown in 1× LCMR until stage 10 and total RNA was isolated for Northern analysis. As controls, nine DMZ explants from each of the three injection groups as well as nine VMZ and DMZ explants from the noninjection group were grown to stage 20 for RNA isolation. Total RNA was also isolated from pools of five control and BMP-4-injected embryos (Emb, E). For Northern analysis, 1 embryo equivalent of RNA was loaded per well and 7 explant equivalents was loaded. Filters were sequentially hybridized with Xenopus cDNA probes for muscle-specific actin (m-actin) and EF1α. On all filters, muscle actin mRNA is the lower band; the upper band is cross-hybridization to cytoskeletal actin mRNA. Only explants from the uninjectected DMZs are shown; the BMP-4-injected DMZs did not express significant levels of muscle actin. Embryos injected with 1, 2, and 3 ng had a DAI of 0.1; muscle actin expression was inhibited by a factor of 25–50.
expression is inhibited in BMP-4-injected embryos (29). Initial activation of gpd transcription in BMP-4-injected embryos was similar to that observed by Hogan (30).

The Spemann organizer induces muscle in the VMZ region (6). To address the effect of BMP-4 on organizer dorsalization activity, recombinant explants were made between stage 10+ Spemann organizer regions (DMZs) from BMP-4-injected embryos and VMZs from noninjected control embryos. Explants were grown to stage 20 and RNA was isolated for Northern analysis (Fig. 1C). In recombinant DMZ–VMZ explants from normal embryos, high levels of muscle actin expression were observed (Fig. 1C). In recombinant explants comprising a DMZ from BMP-4-injected embryos (1–3 ng) and a VMZ from control embryos, muscle actin expression was reduced by a factor of 6–50 (Fig. 1C). In addition, BMP-4 injected DMZ–VMZ explants did not elongate in comparison to controls (data not shown). These results suggest that ectopic expression of BMP-4 inhibits the functional ability of a Spemann organizer to dorsalize ventral mesoderm to muscle.

Previous studies have suggested that BMP-4 can induce ventroposterior mesoderm, since Xho3-3 mRNA was expressed in BMP-4-injected neurula-stage animal caps (12, 13). We have extended these findings by examining a battery of inducible mesoderm markers expressed in gastrula-stage animal cap explants removed from BMP-4-injected embryos. By Northern analysis, we assayed expression of four additional inducible mesoderm markers, the Brachyury (Xbra), Xwnt-8, Xpo, and Mix.1 genes (31–34). Xbra is expressed in all gastrula mesoderm, including the Spemann organizer region. Xwnt-8 and Xpo are expressed mainly in ventroposterior/nonorganizer mesoderm (23, 32, 35). Mix.1 is an early inductive response gene expressed in the vegetal pole and marginal zone of gastrulae (34). BMP-4 induced expression of high levels of the Xbra, Xwnt-8, Xpo, and Mix.1 transcripts in animal caps (Fig. 1A). Xenopus MyoD (XMyoD), which is expressed in ventral mesoderm at gastrula stages (19), was also transiently transcribed in BMP-4-injected animal caps (data not shown).

In BMP-4-injected activin-treated animal caps, Xbra, Xwnt-8, Xpo, and Mix.1 were expressed at the same levels as in BMP-4-injected caps (Fig. 1A), suggesting that BMP-4 induces ventral mesoderm in a dominant manner. This effect was striking when Xwnt-8 and Xpo expression was examined; activin induced low Xwnt-8 and Xpo expression in animal caps; however, BMP-4 and BMP-4/activin-treated animal caps expressed high levels of Xwnt-8 and Xpo RNA (Fig. 1A).

BMP-4-injected whole embryos expressed mesodermal markers in an analogous pattern. In gastrula embryos, gpd, XFKH-1, and XIIm-1 transcripts were decreased by BMP-4 whereas Xbra and Xwnt-8 transcripts (Fig. 1B) as well as Xpo, Mix.1, and XMyoD transcripts (data not shown) were increased. DMZs isolated from stage 10–10.25 BMP-4-injected embryos were ventralized; they expressed high levels of Xwnt-8 RNA instead of mRNA; control DMZs expressed no detectable Xwnt-8 mRNA (Fig. 1B). Xbra expression was about 50% higher in the DMZ and VMZ of injected embryos than in controls (Fig. 1B). BMP-4-injected DMZ explants also failed to elongate after growth in culture to neurula stages in comparison to control explants, thus resembling VMZ explants (data not shown). These results suggest that BMP-4 can induce ventroposterior mesoderm while simultaneously inhibiting dorsal mesoderm formation (12, 13).

**BMP-4 Inhibits Dorsalization of Ventral Mesoderm to Muscle.** Embryonic muscle forms by the dorsalization of ventral mesoderm to a more dorsal fate by the Spemann organizer (6, 7). The noggin molecule mimics this signal; gastrula-stage VMZ explants differentiate to muscle after noggin treatment (9). The dorsal inhibitory activity of BMP-4 may not be exclusively restricted to induction of dorsal mesoderm. BMP-4 mRNA is expressed throughout the MZ at the onset of gastrulation (see Fig. 3); thus during gastrulation it could possibly act as an inhibitor of the dorsalization process. To address BMP-4’s role as a repressor of dorsalization, we examined whether noggin activity was inhibited by BMP-4. VMZ explants taken from BMP-4-injected embryos were removed at stage 10–10.25 and treated with noggin. VMZs were grown until stage 20 and RNA was isolated for Northern analysis. Dorsalization activity of noggin was completely inhibited in VMZ explants taken from embryos injected with 2 ng of BMP-4 RNA; muscle actin transcripts were not detected (decrease by a factor $\geq 25$) in comparison to VMZs dorsalized by noggin (Fig. 2A). Noggin and BMP-4 appear to compete with one another; by titration of injected BMP-4 RNA between 0.5 to 2.0 ng, it was shown that noggin could overcome BMP-4 inhibition and rescue dorsalization in VMZ explants (Fig. 2A). Noggin partially rescued dorsalization in VMZs taken from the 0.5- and 1.0-ng groups; muscle actin expression was inhibited by a factor of 7 in the 1.0-ng group and by a factor of only 2 in the 0.5-ng group in comparison to control VMZs treated with noggin (Fig. 2A). BMP-4’s inhibitory effect in VMZs is not simply repression of muscle actin transcription, since

![Figure 2](https://example.com/fig2.jpg)

**FIG. 2.** Ectopic expression of BMP-4 inhibits dorsalization of VMZ by noggin and Spemann organizer. (A) Embryos at the one-cell stage were injected with 2.0, 1.0, or 0.5 ng of BMP-4 RNA. Eighteen VMZ explants were removed from uninjected and injected groups of embryos (stage 10–10.25). Nine explants from each group were treated with noggin until stage 12.5–13, and nine untreated explants served as controls. Explants from each group were grown to stage 20 and total RNA was isolated. Total RNA was also isolated from pools of five whole embryos from the control and the three BMP-4 injected groups. For Northern analysis, 1 embryo equivalent of RNA was loaded per well and 5 explant equivalents were loaded. Filters were sequentially hybridized with Xenopus CDNA probes for muscle actin (m-actin) and EF1α. Of the untreated VMZs (−noggin) only the noninjected group is shown; the BMP-4-injected VMZ (−noggin) groups also did not express m-actin. In whole embryos injected with 1–2 ng of BMP-4 RNA, m-actin levels were suppressed by a factor of 25–50 (DAI = 0.1). In embryos injected with 0.5 ng of BMP-4 RNA, m-actin expression was inhibited by a factor of 6 (DAI = 0.4). (B) Embryos at the one-cell stage were injected with 2 ng of BMP-4 RNA. At stage 10+, VMZs were removed from injected and noninjected embryos. These VMZ explants were recombined with DMZ explants taken from uninjected embryos at the same stage. Twelve recombinant explants from BMP-4 injected and noninjected control VMZs were grown in 1× LCMR until stage 20 and total RNA was isolated. As controls, nine explants each of BMP-4 injected VMZ, control VMZ, and control DMZ were also grown until stage 20 and total RNA was isolated. Total RNA was isolated from pools of five control and BMP-4-injected embryos (Emb, E). For Northern analysis, 1 embryo equivalent of RNA was loaded per well and 9 explant equivalents of RNA was loaded. Filters were sequentially hybridized with cDNA probes for muscle actin (m-actin) and EF1α.
XM yoD expression was also inhibited in BMP-4/noggin-treated VMZs in comparison to noggin-treated controls (data not shown). In addition, BMP-4-injected VMZ explants did not extend to the same extent as dorsalized VMZ explants (data not shown). Inhibition of noggin by BMP-4 RNA is a specific effect; noggin-treated VMZs removed from embryos injected with other translatable RNAs, encoding a truncated FGF receptor (d50) or prolactin, underwent normal dorsalization after noggin treatment (data not shown).

Inhibitory effects were also observed in embryos zygotically expressing BMP-4 from the cytomegalovirus (CMV) promoter. DNA (100–150 pg per cell) was injected into the MZ of both blastomeres at the two-cell stage. Muscle actin expression in noggin-treated VMZs from CMV-BMP-4-injected embryos was reduced by a factor of 2 in comparison to embryos injected with plasmid DNA containing only the CMV promoter (data not shown). This result resembles effects observed when lower levels of BMP-4 RNA (0.5 ng) were injected, and it is most likely the result of mosaic expression from the CMV promoter.

To additionally address the effect of BMP-4 on endogenous dorsalization of ventral mesoderm within the embryo, recombinant explants were made between stage 10+ Spemann organizer regions (DMZ) from normal embryos and VMZs from BMP-4-injected and control embryos (Fig. 2B). Explants were grown until stage 20 and total RNA was isolated for Northern analysis. Neither organizer nor VMZ was specified to differentiate into muscle, since muscle actin expression was not observed in these explants (Fig. 2B). In recombinant DMZ-VMZ explants from normal embryos, high levels of muscle actin expression were observed (Fig. 2B). In recombinant explants comprising a VMZ from a BMP-4-injected embryo and a DMZ from a control embryo, muscle actin expression was reduced by a factor of 6 (Fig. 2B). These results suggest that ectopic expression of BMP-4 in the VMZ inhibits its ability to respond to the natural dorsalizing signal. When the DMZ used in the recombinant explant was specified to make muscle, the dorsalization inhibitory effect of BMP-4-injected VMZs was decreased; muscle actin expression was reduced by a factor of only 3 (data not shown). This phenomenon may be related to subtle variations in the time of DMZ removal or perhaps to differing sizes of organizer tissue.

**BMP-4 Expression Is Lowered in VMZ Explants Dorsalized by Noggin**. BMP-4 is equally expressed throughout the whole MZ at stage 10.5 (12) but is ventrally localized by stage 11–11.5 (29, 36). We extended these findings by examining the specification of BMP-4 expression in dorsalized explants grown in culture from stage 10–10.25 until stage 20. By dissection of the marginal zone and Northern analysis, we found that BMP-4 expression was expressed equally throughout the marginal zone at the onset of gastrulation (Fig. 3). Expression of Xwnt-8 and gcl served as controls for dissection accuracy (Fig. 3). VMZs removed from early gastrula and grown to late neurula expressed higher levels of BMP-4 mRNA relative to VMZs explanted dorsalized by noggin. Dorsalized VMZs downregulate BMP-4 mRNA in a noggin concentration-dependent manner; over an 8-fold noggin concentration range, BMP-4 expression was downregulated by a factor of ~25 (Fig. 3). In noggin-dorsalized VMZs, a correlation was seen between increases in muscle actin mRNA and decreases in BMP-4 transcripts (Fig. 3). Thus while BMP-4 inhibits noggin protein activity, noggin downregulated expression of BMP-4 mRNA by dorsalizing VMZ to muscle. In situ hybridization of mid-gastrula and neurula embryos with BMP-4 shows enriched expression in ventral versus doroanterior regions of the embryo (29, 36). Thus while BMP-4 expression starts out equally throughout the MZ at the onset of gastrulation, the dorsalization process restricts transcription to ventral areas. During normal development, competition between noggin and BMP-4 may define which cells eventually end up with ventral fates; noggin downregulates BMP-4 expression in LMZ fated for muscle. En-
state of inhibition by BMP-4. The major role of dorsalinization may be to unmask this repressed state by turning off BMP-4 thus enabling the VMZ to differentiate muscle. Graff et al. (18) suggest that while BMP-4 makes ventral mesoderm in a dominant manner, there must be an endogenous signal which can override its signaling. We suggest that the ability of noggin to shut down BMP-4 expression enables dorsalinization in dorsolateral mesoderm adjacent to the organizer. In contrast, the maintenance of high levels of BMP-4 in more ventrolateral regions farther away from the organizer suffices to produce ventral mesoderm while simultaneously inhibiting the dorsalinization process.

We thank Drs. R. Harland, J. Smith, I. Dawid, W. Smith, A. Hemmati-Brivanlou, M. Jamrich, E. Amaya, A. Fainsod, and J. Yisrael for plasmids. We thank D. A. Fainsod for sharing unpublished data. This research was supported by a grant from the Israel Science Foundation (Israel Academy of Sciences and Humanities) and by Bruce Rappaport and Technion research funds.


A three-signal model has been suggested to describe induction of mesoderm in Xenopus embryos (1). In this model, the dorsalinization signal is defined as a dominant signal emanating from the organizer which converts ventrally fated cells in the dorsolateral MZ to a more dorsal fate. Our data suggest that regulation of dorsalinization is more complex than was previously shown. It is likely that negative signaling molecules such as BMP-4 participate in the dorsalinization process in conjunction with positive regulating molecules such as noggin. Only by the proper combination of positive and negative signaling pathways is the correct pattern established in the mesoderm. VMZs removed from embryos injected with a truncated Xenopus BMP-4 receptor differentiate and elongate as muscle (18). This result suggests that elimination of BMP-4 signaling is sufficient to dorsalinize VMZ to muscle. It is possible that the VMZ has a latent potential to differentiate muscle which is in a constant