Embryonic Patterning: To BMP or Not to BMP, That Is the Question

Minireview

Jonathan M. Graff
University of Texas Southwestern Medical Center
Center for Developmental Biology
Department of Molecular Biology and Oncology
Dallas, Texas 75235-9133

Vertebrates begin life as a seemingly unpatterned cluster of cells. Rapidly, however, the shape of an embryo changes and begins to acquire an easily recognizable pattern. This formation of pattern occurs largely along two distinct axes, anterior–posterior and dorsal–ventral. The molecular mechanisms that underlie this transition from a radially symmetric egg to a patterned embryo remain largely unknown. Classical embryological experiments have provided many insights into the type of activities (signals) that may be important in pattern formation. For example, explants of dorsal tissues alter ventral tissues to dorsal fates while ventral cells have no effect on dorsal cells. Based on this ability of the dorsal cells, a model of dorsal–ventral patterning of the embryonic germ layers has been generated (reviewed by Slack, 1991; Harland, 1994). In this model, ventral tissue is thought to be the ground state and dorsal tissues to require the presence of additional or stronger signals. Recent molecular studies contradict this dorsal-dominant model. These studies indicate that an active BMP signal is required to form ventral tissues and that loss of this active ventral signal results in formation of dorsal tissues (reviewed by Harland, 1994; Hogan, 1996). This paradox appears to be reconciled by the recent discovery that dorsal signals bind BMP4 with high affinity and thereby block BMP signaling (Piccolo et al., 1996; Zimmerman et al., 1996). That is, the dorsal signals function by antagonizing the active ventral signal, BMP4, rather than by directly promoting dorsal fates.

In vertebrates, the three embryonic germ layers—ectoderm, mesoderm, and endoderm—are patterned. This review focuses on the dorsal–ventral patterning of the ectoderm and the mesoderm. The ectoderm produces the epidermis or skin as a ventral derivative and the nervous system as a dorsal derivative (Figure 1). The mesoderm forms mesenchyme and the hematopoetic system (blood) on the ventral side and head mesoderm, the notochord, and muscle on the dorsal side (Figure 1). How these germ layers are patterned has been an area of intense scrutiny. A model has been proposed in which dorsal tissue is thought to be the dominant state, requiring an active signal while the ventral side is believed to be the ground or default state (Slack, 1991; Harland, 1994). This popular model is largely based on three classical experiments.

Classical Embryological Experiments

In many embryological experiments, explants are analyzed after culture either in isolation or as recombinants of different tissues. These classical approaches were used to explore the underpinnings of dorsal–ventral pattern formation.

Initial insights into an active dorsal state are provided by the experiments of Spemann and colleagues first performed almost 75 years ago (reviewed by Smith, 1989). In these experiments, a small piece of the dorsal side of a donor embryo is grafted onto the ventral side of a host embryo (Figure 2B). The resulting host embryo develops two dorsal axes and a substantial part of the second dorsal axis is produced from the host's ventral tissue. That is, the dorsal donor graft redirects the fate of the host's ventral mesoderm into properly organized dorsal mesoderm with a notochord and muscle segments. The donor tissue also repatterns the host's ventral ectoderm, initially fated to become skin, into an organized neural tube, a dorsal ectodermal derivative. Therefore, in what appear to be parallel processes, the donor graft converts the fate of two germ layers from ventral to dorsal. This alteration in cell fate is often termed dorsalization in the mesoderm and neural induction in the ectoderm. Because of these properties, the small dorsal region of the amphibian embryo is called the Spemann organizer; the homolog in the chick and the mouse is called the node.

Additional support for a dorsal-determining signal is provided by studying ventral and dorsal explants (Figure 2C) (reviewed by Smith, 1989). When explants of ventral tissue are cultured in isolation, they heal and form an unpatterned ball of cells. Histological analysis reveals a loose mix of mesenchyme and blood-like cells; that is, only ventral tissues are present. In contrast, explants of dorsal tissue undergo dramatic movements and appear as organized, elongated structures; mimicking what occurs normally in the developing embryo. Histological analysis of these dorsal explants demonstrates the presence of appropriately patterned dorsal tissues, such as the notochord and muscle, and the absence of ventral tissues. These observations that ventral explants are unpatterned and that dorsal explants are organized were thought to suggest that the ventral tissue is in an ''unactivated ground state'' and that the patterned dorsal
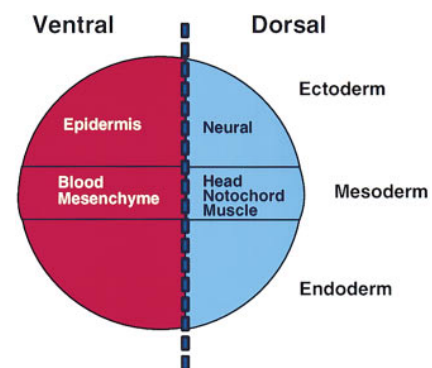

Figure 1. Schematic of Dorsal and Ventral Fates

The embryonic germ layers are patterned into distinct dorsal (light blue) and ventral fates (red). This cartoon represents the types of tissues that form from the dorsal and ventral regions of the embryo; however, it is not a fate map.

tissue results from an active signal (Cooke and Weber, 1983). Further support for this interpretation is provided by experiments in which the ventral tissue is juxtaposed with dorsal tissue. Analysis of these tissue recombinants reveals that the ventral part of the graft has been altered and now contains dorsal tissues such as muscle. That is, the dorsal tissue appears to instruct (send a signal to) the ventral tissue to change into a dorsal fate. In contrast, grafts of ventral tissue have virtually no effect on dorsal cell fate. Taken together, these data suggest the presence of an active dorsal state and a ground ventral state.

Further evidence for an unactivated ventral state is garnered from experiments done with ultraviolet (UV)-irradiation of amphibian eggs (Figure 2D) (Slack, 1991). When eggs are irradiated during the first cell cycle, development is dramatically perturbed. The resultant UV embryos are radially symmetric and lack a dorsal–ventral axis. Careful analysis of these UV embryos demonstrates that both ectoderm and mesoderm are present; however, the UV embryos lack all dorsal derivatives and only ventral tissues are present. The interpretation of these results was that an active dorsal signal was destroyed, leaving only the inactive ventral state.

Taken together, these classical embryological experiments were interpreted as follows (Figure 4). First, ventral tissue is a ground state. Second, formation of dorsal tissue requires an active signal. Third, this active signal dorsalizes both mesoderm and ectoderm (induces neural tissue). These classical studies have set the stage for modern experiments. However, a number of recent molecular studies seem to contradict the classical experiments. These studies suggest that ventral is the active state and that dorsal tissue is the result of the absence of an active ventral signal. These apparently contradictory results center around bone morphogenetic protein 4 (BMP4).

The Paradox: Bone Morphogenetic Proteins

BMPs are members of the transforming growth factor β (TGFβ) superfamily and were originally isolated by their ability to induce formation of bone. BMPs are important in a wide variety of biological processes in both vertebrates and invertebrates (reviewed by Hogan, 1996). BMPs appear to play roles in several aspects of development and an active BMP signal is required to form the ventral side of the body (Figure 4).

The Mesoderm

To explore a potential role for BMP in early development, a few groups added exogenous BMP4 to embryos (reviewed by Harland, 1994). They discovered that BMP4 actively induces the formation of ventral mesoderm. Remarkably, the induction of ventral mesoderm is observed even when BMP4 and dorsal-inducing molecules are added together. Furthermore, microinjection of *BMP4* mRNA in vivo leads to formation of ventralized embryos that lack dorsal structures such as the notochord and muscle. Taken together, these findings suggest that BMP4 is an active signal that induces ventral mesoderm and that this ventral-inducing signal blocks or overrides the endogenous dorsal-inducing signal. However, these studies were done with addition of exogenous BMP4, which raises the question of whether BMP4 performs these roles in vivo. Support for BMP4
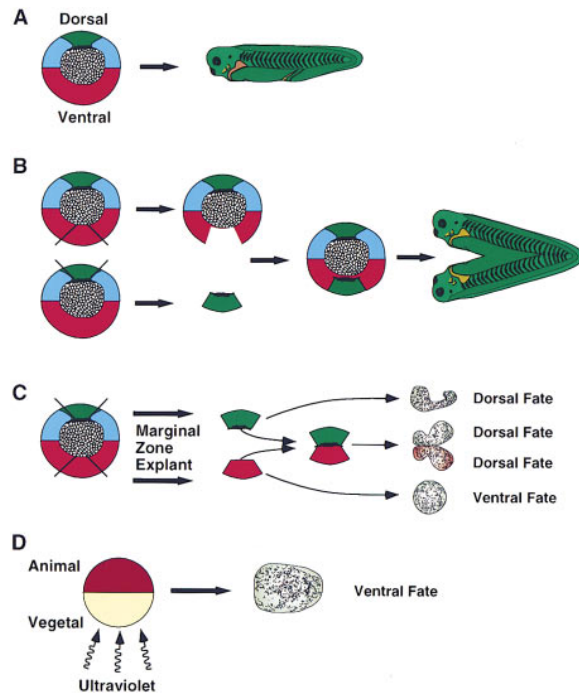

Figure 2. Classical Embryological Experiments

(A) An unperturbed embryo develops normally with a dorsal–ventral axis.

(B) Spemann organizer transplant. A graft of the dorsal region (Spemann organizer, green) of a gastrula-stage donor embryo to the ventral region (red) of a host embryo induces formation of a complete secondary dorsal axis. Therefore, the dorsal region of the embryo is able to reorganize the ventral side of the embryo into dorsal fates. This cartoon represents an idealized version of Spemann's classic experiment.

(C) Marginal zone (future mesoderm) explants and recombinants. Explants of the dorsal marginal zone (green), cultured in isolation, elongate in a fashion characteristic of dorsal tissue. Ventral marginal zone explants (red), cultured alone, form round balls of tissue. When dorsal and ventral marginal zone explants are juxtaposed and cultured together, dorsal explants elongate as if in isolation while ventral explants change dramatically and elongate like dorsal tissues. This suggests that dorsal tissues contain signals that can alter ventral tissues into dorsal fates.

(D) Ultraviolet irradiation of an embryo. When an embryo is irradiated from the vegetal region during the first cell cycle, the embryos fail to develop a dorsal axis, are radially symmetric, and adopt only ventral fates. This implies that destruction of an endogenous dorsal signal leads to a ventral ground state.

as an endogenous ventral-inducing signal stems from RNA in situ hybridization experiments. *BMP4* mRNA is expressed on the ventral side of the developing embryo at the appropriate time to play an essential role in ventral patterning (Schmidt et al., 1995). The critical issue, however, is to establish whether BMP4 actually functions in vivo in the processes that it is able to mimic by exogenous addition. This is essential, as classical embryological experiments never revealed the presence of an active ventral signal.

One approach to defining the endogenous role of BMPs is to eliminate BMP signaling in vivo and to determine if there are any phenotypic effects (Figure 3). This has been addressed in three separate ways: with dominant negative BMP-receptors that specifically block
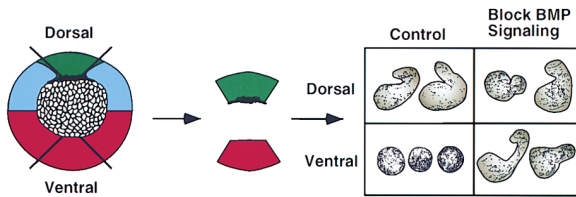
Figure 3. Blockade of BMP Signaling Converts Ventral Tissues to Dorsal Fates

Dorsal (green) and ventral marginal zones (red) are explanted from an early gastrula–stage embryo and cultured. Control dorsal explants elongate while ventral explants form round balls of tissue. Blockade of BMP signaling does not affect dorsal tissues. In contrast, ventral explants that lack BMP signaling are converted to dorsal fates and elongate. Thus, blocking BMP signaling mimics the effect of juxtaposing dorsal and ventral marginal zone explants (see Figure 2C).

BMP signaling, with antisense BMP4, and with dominant negative-forms of BMP4 ligand (Graff et al., 1994; Suzuki et al., 1994; Hawley et al., 1995; Sasai et al., 1995; Schmidt et al., 1995). All three approaches produce the same conclusions. First, active BMP signaling is required to form ventral mesoderm. Second, absence of this active BMP signal converts mesoderm from ventral to dorsal. Third, elimination of endogenous BMP signaling uncovers the presence of a dorsal-inducing signal on the ventral side of the embryo. Therefore, in vivo, this dorsal signal is overridden by an active ventral-inducing signal, BMP. Of course, this is a dramatic departure from the conventional view that dorsal is active and ventral is the ground state.

The Ectoderm
Similar experiments using dominant-negative BMP receptors and antisense and dominant-negative BMP4 have been performed to determine the role of BMP signaling in the ectoderm. The results from the ectodermal studies parallel the conclusions drawn from the experiments in the mesoderm (Hawley et al., 1995; Sasai et al., 1995). That is, dorsal–ventral patterning of the ectoderm requires an intact BMP signaling pathway. Blocking BMP signaling converts ventral ectoderm, epidermis, to dorsal ectoderm, neural tissue. The converse is also true; addition of BMP4 protein to cells fated to become neural tissue (dorsal) converts them to epidermal cells (ventral) (Wilson and Hemmati-Brivanlou, 1995).

Therefore, it is likely that dorsal–ventral patterning of

both the mesoderm and the ectoderm employ conserved mechanisms. Formation of both ventral mesoderm and ventral ectoderm requires an active BMP signal; inactivation of this signal leads to formation of dorsal mesoderm or dorsal ectoderm. This is in contrast to the models in which dorsal signaling is dominant. As reviewed earlier, the Spemann organizer produces secreted signals that appear in an active fashion both to dorsalize mesoderm and to induce the formation of neural tissue. To attempt to reconcile these divergent findings, the molecules produced by the organizer need to be better defined.

Reconciliation: BMP4 and the Spemann Organizer
Embryological experiments defined a small region of the embryo, known as the Spemann organizer, that has unique properties. The organizer can convert ventral mesoderm to dorsal mesoderm (dorsalization) and can convert ventral ectoderm to dorsal ectoderm (neural induction). This discovery set off a frenzied and intense search for molecules with these abilities. Recently, two unrelated secreted molecules, noggin and chordin, have been identified that are expressed in the Spemann organizer and possess both the dorsalizing and neural-inducing characteristics of the organizer (Sasai et al., 1994; Smith and Harland, 1992).

Two apparently contradictory influences can dorsalize both the mesoderm and the ectoderm; an active organizer (dorsal) signal or blocking BMP signaling. This apparent paradox might be reconciled if the molecular signals of the organizer antagonize BMP signaling. This is the conclusion of two recent papers that characterize the organizer proteins, chordin and noggin (Piccolo et al., 1996; Zimmerman et al., 1996). Using rigorous biochemical approaches, the groups of DeRobertis and Harland show that chordin and noggin bind to BMP4 and BMP2 with high affinity. The subnanomolar binding affinities are approximately the same as the affinity of the cognate signaling-receptors for the ligand. In addition, the DeRobertis group suggests that the concentration of chordin present in vivo is high enough to inhibit endogenous BMP function. Noggin and chordin not only bind BMP but also abolish BMP activity by inhibiting ligand binding to the signaling receptor (Holley et al., 1996; Piccolo et al., 1996; Zimmerman et al., 1996). Therefore, the organizer signals antagonize active ventral signals rather than actively promoting dorsal fates.

Conclusions
These studies have placed us in a much better position to understand patterning of two germ layers. In addition,
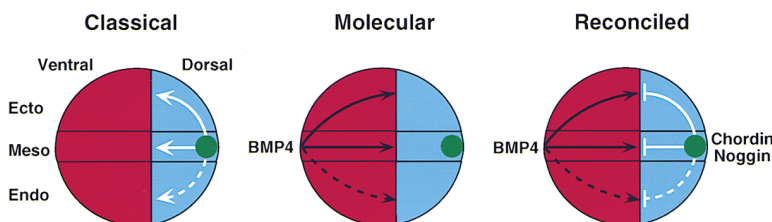

Figure 4. Models of Early Dorsal-Ventral Patterning

Classical embryological experiments suggest that the organizer (green) induces dorsal fates by sending an active inducing signal and that ventral fates are a ground state. Molecular experiments suggest that active BMP signals induce ventral tissues and that blocking BMP signaling leads to formation of dorsal fates.

Recent studies reconcile the differences between these two views as the organizer signals (chordin and noggin) function by blocking BMP signaling. The hatched lines indicate patterning effects in the endoderm that appear likely but have yet to be definitively proven. Ecto, ectoderm; Meso, mesoderm; Endo, endoderm.

the apparent paradox of formation of dorsal tissue, either the result of active signals defined by classical experiments or the result of blocking BMP signaling, is easily reconciled (Figure 4). First, it seems clear that active BMP signaling establishes ventral fates in both the ectoderm and the mesoderm. Furthermore, it is likely that the dorsal-inducing properties of the organizer are in part mediated by inhibiting BMP signaling. The organizer signals, noggin and chordin, function by binding to BMP4 and preventing BMP4 from activating its cognate receptor on the dorsal side of the embryo. Therefore, patterning of the germ layers is an interplay of active BMP signaling on the ventral side and blocking BMP signaling on the dorsal side. In other words, dorsal–ventral pattern formation may be simply due to the presence or absence of BMP signaling. The presence of active BMP signaling produces ventral fates, and the absence of BMP signaling generates dorsal fates.

Future Directions

Although some progress in understanding the molecular mechanisms of early embryogenesis has been made, a few outstanding issues remain to be clarified. Of particular interest is whether dorsal–ventral patterning of the endoderm is also controlled by BMP signaling. It appears quite likely that TGFβ signals are essential for endodermal patterning (Gamer and Wright, 1995; Henry et al., 1996; Sasai et al., 1996). Preliminary results suggest that BMP4, chordin, and noggin, the same molecules used in the ectoderm and the mesoderm, also pattern the endoderm (Sasai et al., 1996). This suggests that conserved mechanisms of patterning are employed in all three germ layers (Figure 4). BMPs are active in many other important biological processes, and it remains to be established whether noggin and chordin are important in BMP action at other times and places. It is unclear why two factors, noggin and chordin, with essentially identical activity are needed when only one might suffice. Relatedly, if there are two, there might be other proteins with similar activity either for BMPs or for other TGFβs. Although it appears that BMP signaling is essential for formation of the ventral side of the embryo, it is unclear what establishes the restricted pattern of BMP expression on the ventral side of the embryo. In addition, it remains unknown what signals establish formation of the organizer or restrict expression of chordin and noggin to the organizer. The best data supports a role for the Wnt signaling pathway in organizer formation, although how this is established is poorly understood (Carnac et al., 1996, and references therein). Finally, although we have begun to understand what patterns the ectoderm, mesoderm, and endoderm, we still do not know the nature of the molecule(s) that initially induces formation of the mesoderm.

Selected Reading

Carnac, G., Kodjabachian, L., Gurdon, J., and Lemaire, P. (1996). Development *122*, 3055–3065.

Cooke, J., and Weber, J. (1983). Nature *306*, 423–424.

Gamer, L., and Wright, C. (1995). Dev. Biol. *171*, 240–251.

Graff, J., Theis, R., Song, J., Celeste, A., and Melton, D. (1994). Cell *79*, 169–179.

Harland, R. (1994). Proc. Natl. Acad. Sci. USA *91*, 10243–10246.

Hawley, S., Wunnenberg-Stapleton, K., Hasimoto, C., Laurent, M., Watabe, T., Blumberg, B., and Cho, K. (1995). Genes Dev. *9*, 2923–2935.

Henry, G., Brivanlou, I., Kessler, D., Hemmati-Brivanlou, A., and Melton, D. (1996). Development *122*, 1007–1015.

Hogan, B. (1996). Genes Dev. *10*, 1580–1594.

Holley, S., Neul, J., Attisano, L., Wrana, J., Sasai, Y., O'Conner, M., DeRobertis, E., and Ferguson, E. (1996). Cell *86*, 607–617.

Piccolo, S., Sasai, Y., Lu, B., and DeRobertis, E. (1996). Cell *86*, 589–598.

Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L., and DeRobertis, E. (1994). Cell *79*, 779–790.

Sasai, Y., Lu, B., Steinbeisser, H., and DeRobertis, E.M. (1995). Nature *376*, 333–336.

Sasai, Y., Lu, B., Piccolo, S., and De Robertis, E. (1996). EMBO J. *15*, 4547–4555.

Schmidt, J., Suzuki, A., Ueno, N., and Kimelman, D. (1995). Dev. Biol. *169*, 37–50.

Slack, J. (1991). From Egg to Embryo (New York: Cambridge University Press).

Smith, J.C. (1989). Development *105*, 665–667.

Smith, W., and Harland, R. (1992). Cell *70*, 829–840.

Suzuki, A., Thies, R., Yamaji, N., Song, J., Wozney, J., Murakami, K., and Ueno, N. (1994). Proc. Natl. Acad. Sci. USA *91*, 10255–10259.

Wilson, P., and Hemmati-Brivanlou, A. (1995). Nature *376*, 331–333.

Zimmerman, L., De Jesus-Escobar, J., and Harland, R. (1996). Cell *86*, 599–606.