Antagonism within and around the organizer: BMP inhibitors in vertebrate body patterning

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The vertebrate organizer has been of intense interest since its discovery in amphibia by Spemann and Mangold in the early 1920s (Ref. 1). The organizer was operationally defined as a piece of tissue that can induce a duplicated dorsal body axis and head when transplanted into the ventral side of a host embryo. The original Spemann and Mangold organizer experiment provided the first convincing demonstration that intercellular, or inductive, interactions affect cell fates in vertebrate embryos. Organizer mesoderm, in addition to forming part of the dorsal axis and head, exerts two key inductive influences on adjacent tissues: the organizer redirects the adjacent ventrolateral mesoderm to form tissues with more dorsal characteristics (dorsalization), and the organizer induces the nervous system in adjacent ectoderm.

Organizers have been described in virtually all classes of vertebrate embryos. In general, organizers are located at the site where gastrulation movements begin, and in non-amphibian species organizers correspond to the Koller's sickle in birds (from which Henson's node is derived), the embryonic shield in fish and the node in mammals. The patterning actions of organizers are also well conserved among vertebrates, and this conservation is also underscored at the molecular level, as illustrated by the conserved expression of an array of organizer-specific genes among vertebrates. The functions of vertebrate organizers have been reviewed recently[2–4]. Here, I focus on recent data from the amphibian *Xenopus*, which reveal a rather unexpected twist on the mechanism whereby several organizer-specific factors mediate dorsalization and neural specification.

BMPs and ventral mesodermal patterning

A signature feature of a transplanted organizer is its dominance over the would-be fate of its neighboring cells. In amphibia, an organizer implanted on the ventral side of the early gastrula redirects the adjacent mesoderm (such as blood) to form tissues that are normally found at a more dorsal position, such as muscle. Similarly, ventral ectoderm exposed to the implanted organizer forms neural tissue instead of becoming epidermis. Organizer transplants in fish or bird embryos behave similarly. In amphibia, the dominant behavior of the organizer in mesodermal patterning has forged a general impression over the years that induction of ventral mesoderm is something of a 'ground state' developmental program.

This impression, however, has been substantially challenged by the recent accumulation of evidence pointing to an active role for bone morphogenetic proteins (BMPs) in the induction and patterning of ventral mesoderm (reviewed in Ref. 5). In *Xenopus* animal cap assays, BMPs induce ventral mesoderm, such as blood, when overexpressed. Strangely, homodimeric BMP proteins alone are weak mesoderm inducers, yet such proteins can 'ventralize' the response of animal caps to dorsal mesoderm inducers, such as activin (which, alone, induces muscle and notochord). Originally, it was uncertain whether the ventralizing effects of BMP4 were due to its mesoderm-inducing action during blastula stages, or whether BMP4 acted later in the gastrula when mesoderm is not induced, but when it can be patterned. Recently, strong evidence has been obtained that BMP4 can ventralize the fate of organizer mesoderm after its induction[6], but whether BMPs directly induce mesoderm in the blastula is still an open question.

At late blastula and early gastrula stages, BMP4 is expressed in the ventral and lateral regions of the marginal zone, consistent with its suggested role in ventral mesodermal patterning. In the mouse, BMP4 is similarly expressed in the ventral and posterior mesoderm, and it is essential for normal development[7]. BMP2 and BMP7 are expressed zygotically in *Xenopus* and ventralize embryos like BMP4, but BMP2 and BMP7 transcripts are uniformly distributed in ectodermal and mesodermal tissues (including the organizer) at blastula and gastrula stages[8–10] (S. Nishimatsu and G.H. Thomsen, unpublished).

Extracellular antagonism of BMPs

The capacity for BMP signals to override the dorsalizing influence of the organizer challenges the view that the organizer is the dominant force in embryonic patterning. The ventralizing capacity of BMPs and their expression nearby, and even within, the territory of the organizer make imperative some mechanism for the organizer to 'defend itself' against being ventralized. As it turns out, mustering a strong defense against BMPs is a key modus operandi of the organizer.

Findings over the past two years or so demonstrate that the organizer produces factors that antagonize BMPs, and this antagonism takes place in the extracellular and intracellular space. A recent surprise in the stories is that the antagonists act directly on BMP ligands, in contrast to triggering cellular responses directly (via receptors) that then prevail over BMP signals. At the extracellular level three molecules have been identified that act as BMP antagonists: noggin[11], chordin[12] and follistatin[13] (Fig. 1). Chordin and noggin were isolated in *Xenopus* on the basis of their ability to mimic partly organizer functions and each encode unique secreted factors. The *Drosophila short gastrulation* (*sog*) gene is the homolog of chordin and, in the fly, SOG affects dorsoventral patterning by antagonizing the Decapentaplegic (DPP) ligand, which is the homolog of vertebrate BMP2 and BMP4 (Ref. 14). In a similar fashion, *Xenopus* noggin can

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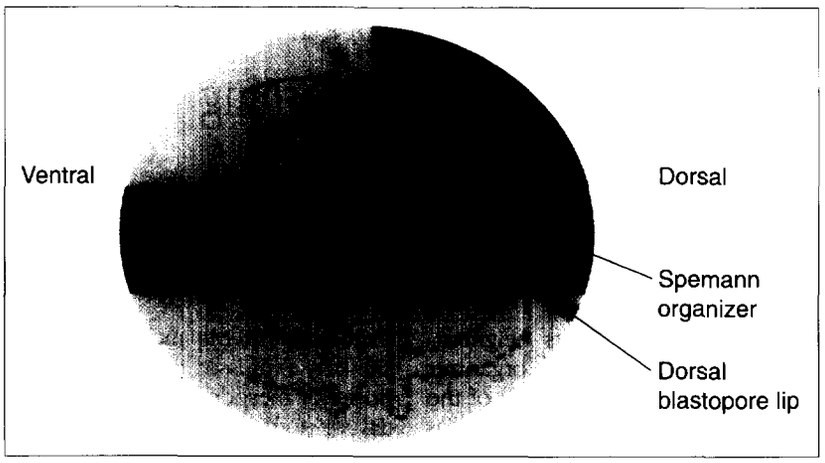

FIGURE 1. Depiction of a *Xenopus* embryo at early gastrulation, highlighting the location of the three germ layers and the action of ventralizing BMP signals (arrows) and their antagonists, noggin, chordin and follistatin (bar symbols). The ectoderm is colored light green and the neurectoderm is dark green. The mesoderm is orange, with the Spemann organizer highlighted in purple. The endoderm is yellow. The arrow and blocker symbols in the endoderm are dashed to indicate their less certain nature. A small loop represents the blocking action of Xnr3 on BMP production within the organizer.

antagonize DPP activity in *Drosophila*[15], but noggin homologs in *Drosophila* or other animals have not yet been identified.

Because noggin and chordin are secreted factors it was initially anticipated that they might exert their effects through some receptor-mediated signaling pathway. Recent reports, however, reveal that noggin and chordin bind BMP ligands directly, with affinities approximating those of cellular BMP receptors[16,17]. These findings do not completely rule out the possibility that noggin and chordin might also signal directly through their own cellular receptors but, as yet, no receptors for those factors have been identified.

The other BMP antagonist, follistatin, can dorsalize ventral mesoderm[18] and induce neural tissue[13]. Follistatin, however, was originally identified as a physiological agent that binds to and inactivates activin, another TGF-β-related ligand. In *Xenopus*, activin is not a ventralizing agent, it is a dorsal mesoderm inducer[19]. The observation that follistatin can dorsalize ventral mesoderm is more consistent with recent findings, which indicate that follistatin can bind heterodimeric BMP4/7 (Ref. 20). It has yet to be established, however, whether or not BMP heterodimers exist in *Xenopus*, but the finding provides a logical explanation for the effects of follistatin. Importantly, follistatin, like noggin and chordin, is expressed in the organizer and axial mesoderm of the *Xenopus* embryo[13], consistent with its ability to induce neural tissue and dorsalize ventral mesoderm. Within the prospective mesoderm of the marginal zone one can imagine that limited diffusion of chordin, noggin or follistatin from the organizer might form a gradient that trails off towards the ventral side. Because BMP4 is expressed rather evenly in most of the marginal zone, this inhibitor gradient might carve out an opposing, complementary gradient of BMP activity that acts to directly specify (via BMP receptor activation) different ventrolateral mesodermal fates.

Antagonism in the ectoderm and endoderm

The battle of antagonism between the BMPs and their rivals, noggin, chordin and follistatin, is also played out in the ectoderm where the decision to become skin or nervous system is at stake. Neurons self-differentiate from the ectodermal germ layer when endogenous BMP ligand synthesis or receptor signaling is blocked or when ectodermal cells are dispersed in single cell culture. Re-supplying BMP4 ligand to dispersed cells or restoration of either BMP ligand synthesis or receptor signaling prevents neuralization and maintains epidermal cell fates. Genes encoding BMP2, 4 and 7 are all expressed in the *Xenopus* ectoderm, so inhibition of BMP activity in the ectoderm by follistatin, chordin and noggin is the most likely mechanism that triggers neural differentiation in the ectoderm (reviewed in Refs 2, 4).

A recent set of experiments also point to a role for noggin and chordin in the induction of endodermal tissues. By monitoring a pan-endodermal marker gene (endodermin), Sasai *et al.*[21] showed that noggin and chordin can induce endoderm in animal caps during the time of gastrulation, when the organizer acts. Endoderm induction by noggin and chordin in the animal cap can be inhibited by BMP4, suggesting that the same kind of ligand antagonism that occurs in the mesoderm and ectoderm to alter cell fates is operating in endoderm development. Evidence that BMP4 inhibits endoderm formation in vegetal cells would help substantiate this hypothesis. The findings that noggin and chordin trigger endoderm differentiation in animal cap ectoderm are very puzzling, however, when considered alongside the evidence that *Xenopus* ectoderm follows a 'default' neural differentiation pathway when BMP signals are removed[2]. How can BMP antagonists trigger endodermal and neural fates in animal caps? This question needs resolution. Of further interest is the possibility that endodermin protein might inhibit the actions of BMPs or related molecules, because this factor is 49% identical with human α2 macroglobulin (α-2M), which can bind mammalian TGF-β2. Despite some uncertainties, these new findings are helping to shape a wider, more unifying view that dorsoventral patterning in all three germ layers might occur by a similar mechanism involving BMPs and their inhibitors.

Intracellular antagonism

The inactivation of BMPs by diffusible, secreted factors might provide an effective means to block BMP activity in the extracellular space, but recent studies suggest that a second mode of BMP antagonism can also occur at the intracellular level, directly within cells of the organizer. This mechanism interferes directly with BMP ligand synthesis. Several TGF-β-related factors related to nodal proteins are expressed in the Spemann organizer: Xnr1 and Xnr2 are expressed in the mesodermal layer of the organizer[22], but another, Xnr3, is expressed in the superficial epithelial layer only that eventually lines the anterior gut[23]. Xnr1 and

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A new player on defense – FRZB

The tally of Spemann organizer defense mechanisms continues to rise with the recent discovery that a WNT-binding protein, named FRZB (pronounced 'frizbee'), is expressed in the organizer at gastrulation (Refs 1, 2; reviewed in Ref. 3). FRZB is a secreted protein with homology to the ligand-binding domain of the WNT receptor, FRIZZLED. FRZB protein was originally discovered as a component of bovine articular cartilage[4], where it might function in limb patterning. The *Xenopus* FRZB protein binds to soluble XWNT8 ligand and, in embryos, FRZB neutralizes XWNT8 activities. Ectopic expression of FRZB in the embryo's ventral marginal zone dorsalizes the mesoderm. This is vital because although a WNT signal in the early blastula acts in dorsal axis specification, during gastrulation XWNT8 can ventralize dorsoanterior mesoderm, much like BMP4. XWNT8 is, in fact, expressed in the ventrolateral mesoderm in a pattern that almost matches BMP4. Thus, FRZB made by the organizer fends off the ventralizing activity of XWNT8 by a mechanism analogous to that used by noggin, follistatin and chordin to antagonize BMPs.

1 Leyns, L. *et al.* (1997) *Cell* 88, 747–756
2 Wang, S. *et al.* (1997) *Cell* 88, 757–766
3 Moon, R.T., Brown, J.D., Yang-Snyder, J.A. and Miller, J.R. (1997) *Cell* 88, 725–728
4 Hoang, B., Moos, M., Vukicevic, S. and Luyten, F.P. (1996) *J. Biol. Chem.* 271, 26131–26137

Xnr2 can induce mesoderm and pattern ventral mesoderm to form dorsal tissues. Xnr3 can also dorsalize ventral mesoderm when expressed in the ventral marginal zone, but it cannot induce mesoderm. Unlike Xnr1 and Xnr2, however, Xnr3 also induces neural tissue in animal caps, and its action is direct because it occurs in the absence of mesoderm induction[24].

Both activities of Xnr3 suggest that it interferes with BMP signals, and animal cap assays support this possibility[24]. Xnr3 can inhibit mesoderm induction by BMP4, and neural induction by Xnr3 can be rescued by BMP4 or by an activated BMP4 receptor. Xnr3 does not, however, inhibit mesoderm induction by the related factor Xnr2. The structure of Xnr3 and mutagenesis experiments further suggest that BMP inhibition happens at the ligand synthesis or secretion step, rather than some downstream point, such as receptor binding. Structurally, Xnr3 conforms fairly well to the typical structure of a TGF-β growth factor, and it has a typical secretion signal sequence, a predicted ligand-processing site and a C-terminal ligand domain with a set of conserved cysteine residues. The Xnr3 protein deviates from the canonical TGF-β format at its C-terminus, where two conserved cysteine residues, required for activity in most TGF-βs, are absent. However, even when these missing cysteines are replaced, the adjusted Xnr3 molecule still behaves like wild-type Xnr3. So far, the activities and specificities of Xnr3 have been assessed only by expressing it from mRNA injected in animal cap cells. Whether or not Xnr3 protein can be secreted as a processed ligand has yet to be shown, so the question of whether Xnr3 protein applied to animal caps can induce neural tissue remains unanswered. Nonetheless, the structure, activity and expression patterns of Xnr3 altogether predict it operates by interfering with BMP ligand synthesis, thereby preventing the production of ventralizing BMP signals within the organizer. Inhibition of BMP synthesis at the intracellular level might be of critical importance because BMP2, BMP7 and a BMP-related molecule, ADMP (Ref. 25), are expressed in the organizer, and all can ventralize dorsal mesoderm.

Perhaps it is time to acknowledge that a reasonable 'balance of power' exists between the dorsal and ventral domains of the amphibian embryo. The ventral side, rather than being passive, exerts a strong patterning influence on the mesodermal and ectodermal germ layers – an influence that forces the organizer to go on the molecular defensive. One might even be tempted to suggest that a 'ventral organizer', manifested as a BMP signal, exists in the embryo. A twist on an old American football adage perhaps makes a fitting motto for the Spemann organizer: 'The best offense is a strong defense'.

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