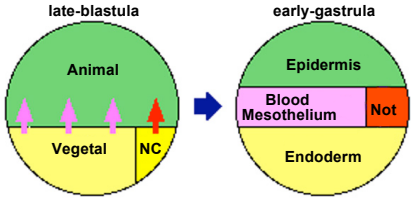
Dorsal-Ventral Patterning - The Spemann Organizer

Hans Spemann (1869-1941)

Hilde Mangold (1898-1924)

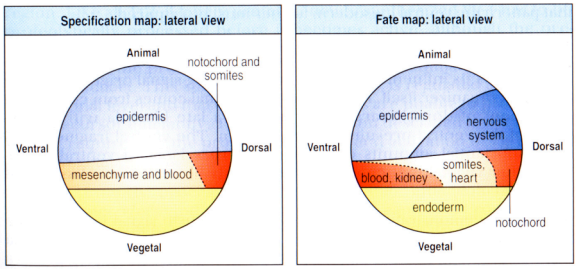
Dr L Dale (B2010) Lecture 3

Two mesoderm inducing signals



Signals from most of vegetal hemisphere induce ventral-type mesoderm in the marginal zone, while signal(s) from the Nieuwkoop centre (NC) induce dorsal-type mesoderm. Both signals are mediated by XNrs, high concentrations inducing dorsal mesoderm and low concentrations inducing ventral mesoderm. This simple model explains the specification map of early gastrulae, demonstrating that it is the result of mesoderm induction during blastula stages.

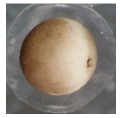
The nervous system and somites are not specified in early gastrulae

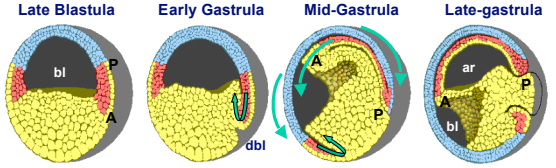


Wolpert, Principles of Development

Cells fated to become the notochord are already specified at the beginning of gastrulation but other tissues, such as the nervous system and somites, are not. Specification of these structures occurs during gastrulation.

Xenopus gastrulation

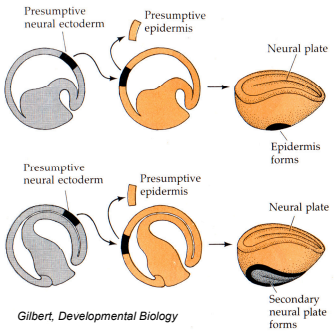




Wolpert, Principles of Development

Gastrulation (gut formation) is initiated by the formation of the dorsal blastopore lip (dbl), followed by involution of the dorsal mesoderm (red). Marginal zone endoderm (yellow) moves inside the embryo, forming the roof of the archenteron (ar). The mesoderm converges and extends along the anterior (A) - posterior (P) axis, pushing the blastocoel (bl) to anterior-ventral side where it eventually disappears. The blastopore lip, and involution of the mesoderm, spreads around the marginal zone, encircling the endoderm. At the same time the ectoderm (blue) spreads downwards (epiboly) until it covers the entire embryo. Note that the dorsal mesoderm moves along the blastocoel roof of the future nervous system, coming into very close contact.

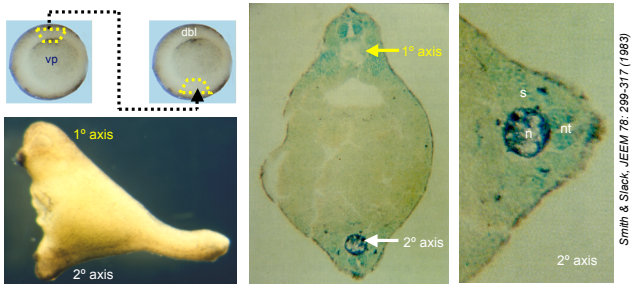
The nervous system is specified during gastrulation



Gilbert, Developmental Biology

Hans Spemann (1919) transplanted pieces of the animal hemisphere at the beginning of gastrulation. He used two closely related species of newt that had different levels of pigmentation, allowing him to discriminate between the transplant and host cells. He found that ectodermal transplants always differentiated according to their new location. Presumptive epidermis became neural plate and presumptive neural plate became epidermis. Transplants of the same cells at the end of gastrulation showed that they now retained their fate. Presumptive neural plate formed a second neural plate on the ventral side of the embryo. This experiment showed that the nervous system is specified during gastrulation.

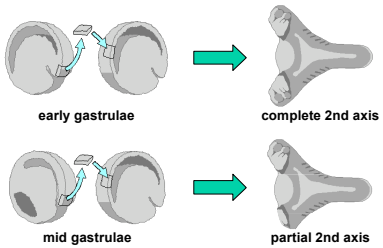
Spemann-Mangold Organizer Graft



Smith & Slack, JEEM 78: 299-317 (1983)

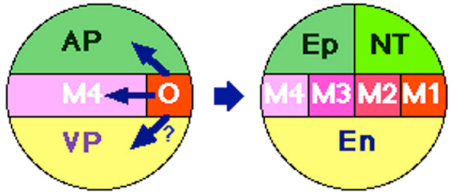
First performed by Spemann (1917) this graft induces a second dorsal axis on the ventral side of the embryo. However, Spemann used embryos of the same species and could not distinguish host from graft tissues. Mangold repeated the experiment (Spemann & Mangold, 1924) using different species of newt, with different levels of pigmentation, as donors and host. She showed that the notochord (n) was always derived from the graft, while the somites (s) and neural tube (nt) were largely derived from the host. This is illustrated by this *Xenopus* experiment, in which the donor embryo was labelled with horseradish peroxidase (this enzyme produces a dark pigment that allows donor cells to be detected). The transplant has "dorsalized" ventral tissues. Because of its ability to induce a correctly organized dorsal axis, Spemann named the transplant region "The Organizer".

The inductive properties of the dorsal blastopore lip change during gastrulation



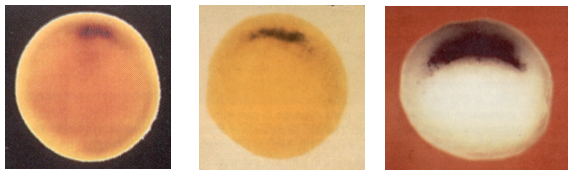
Dorsal lip grafts at the early gastrula stage produce a complete 2nd axis, while grafts from late gastrula stage produce only a partial 2nd axis that lacks a head. During gastrulation the mesoderm population at the dorsal lip changes, with anterior mesoderm being replaced by more posterior mesoderm. Only the former is capable of inducing a head. Suggests that there may be two organizers, a head organizer and a trunk organizer.

The organizer is the source of dorsalizing signals



Spemann suggested that the organizer is the source of dorsalizing signals that induce the nervous system (NT) in the dorsal ectoderm and somites, heart, pronephros in the mesoderm. It may also affect fates in the dorsal endoderm. This inspired biochemists to try and identify the factors responsible, using neural induction in newt gastrula stage animal caps as an assay. However, they form neural tissue in response to stress and even "dead organizers" induced neural tissue. The identity of the organizer factors remained elusive until molecular techniques became sufficiently powerful to clone their genes.

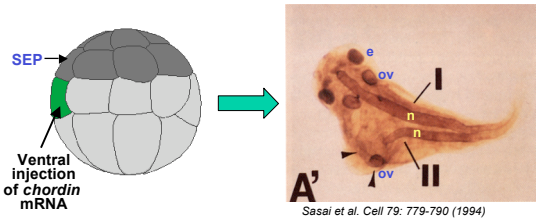
Genes encoding dorsalizing factors are localized to the Spemann organizer



follistatin *noggin* *chordin*

A number of genes have now been identified that are only expressed in the organizer of *Xenopus* gastrulae. *Noggin*, *chordin* and *follistatin* are shown here and all three genes encode secreted proteins. *Follistatin* was first cloned in mammals because of a role in the female reproductive cycle and "accidentally" discovered to be an organizer gene. *Noggin* was isolated in a screen for mRNAs that could rescue dorsal development in UV-irradiated embryos, while *chordin* was discovered in a screen for mRNAs specifically localized to the organizer. All three proteins were shown to have dorsalizing activity.

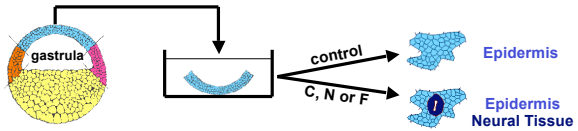
Ventral expression of dorsalizing signals induce a second dorsal axis



Sasai et al. Cell 79: 779-790 (1994)

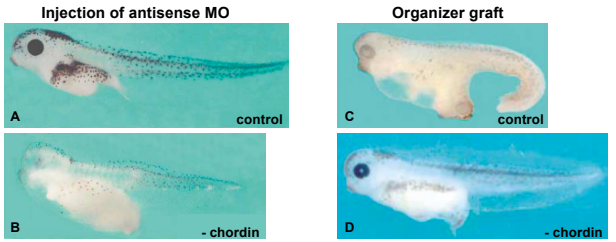
Injection of *chordin* mRNA into the ventral marginal zone produces embryos that have a second dorsal axis. This embryo was stained with an antibody that detects the notochord (n) and otic vesicle (ov), the eye (e) is naturally pigmented. We can see a second notochord with a single otic vesicle at the anterior end. The second axis does not possess more anterior regions of the head, thus Chordin only induces a partial dorsal axis. Similar results can be obtained with *noggin* but less so with *follistatin*.

Chordin, Noggin, and Follistatin will also neuralize *Xenopus* animal caps



Addition of Chordin, Noggin or Follistatin protein to gastrula stage animal caps induces neural tissue. Noggin is the most potent with Chordin being more potent than Follistatin. Thus all three proteins display inductive activities characteristic of the organizer.

Chordin is required for organizer function

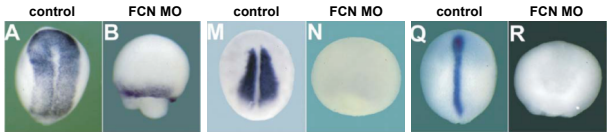


Oelgeschlager et al Dev Cell 4: 219-230 (2003)

Antisense morpholino oligonucleotides (MO) that specifically block translation of *chordin* mRNA were injected into *Xenopus* embryos. Injected embryos develop with head defects (B) but are otherwise normal. However, when injected embryos are used for an organizer graft a second axis is not induced (D). Thus Chordin is required for organizer activity.

Antisense MOs are oligonucleotides with a modified backbone that is resistant to degradation. MOs bind to their target mRNAs and prevent translation by dislodging the ribosome complex. They are proving to be a powerful tool for disrupting gene function in *Xenopus*, fish, sea urchins and cell lines.

Chordin, Noggin and Follistatin are required for the development of dorsal tissues

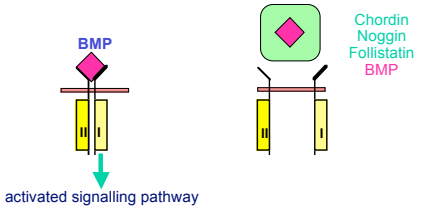


***Sox2* (neural plate)** ***MyoD* (Muscle)** ***Shh* (notochord)**

Khokha et al Dev Cell 8: 401-411 (2005)

In this study antisense MOs against *chordin* (C), *noggin* (N), and *follistatin* (F) were injected into *Xenopus* embryos. When injected alone these MOs had no discernible effect but when combined a dramatic loss of dorsal tissue types was observed. Here we can see that the nervous system, muscle and notochord are deleted. Thus all three proteins are required for normal organizer function - the remaining proteins can compensate for the loss of any single protein, indicating common function (this is called redundancy).

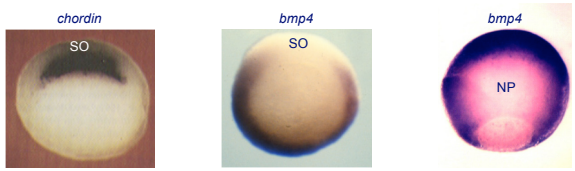
Chordin, Noggin and Follistatin are not signalling molecules



Embryological experiments showed that these proteins inhibited Bone Morphogenetic Proteins (BMPs), which are members of the TGFß family of extracellular signalling molecules. Biochemical experiments showed that they directly bind BMPs, preventing them from binding their receptors. Thus the BMP signalling pathway is blocked. This indicates that inhibiting BMPs is required for dorsal development and by inference that BMPs are required for specifying ventral fates.

Don't be confused by the name, BMPs have many different roles in vertebrate development. The name just reflects the first of these roles to be discovered.

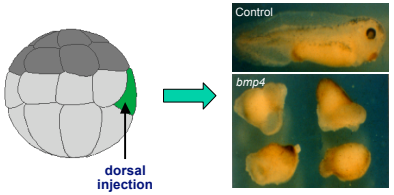
bmp4 is not expressed in either the Spemann Organizer or the neural plate



early-gastrula **early-gastrula** **mid-gastrula**

Bmp2, *bmp4*, and *bmp7* are all expressed in *Xenopus* gastrulae but *bmp4* has the most interesting expression pattern. It is activated in all cells at the mid-blastula transition but is lost from the Spemann Organizer (SO) of early gastrulae and the developing neural plate (NP) of mid-gastrulae. The expression pattern is therefore complementary to that of the dorsalizing signals (e.g. *chordin*) at these stages.

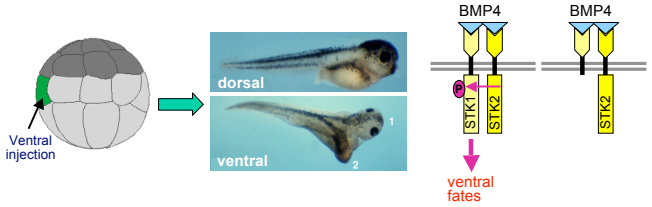
Dorsal injection of *bmp4* mRNA ventralizes *Xenopus* embryos



Dale et al Development 115: 573-585 (1992)

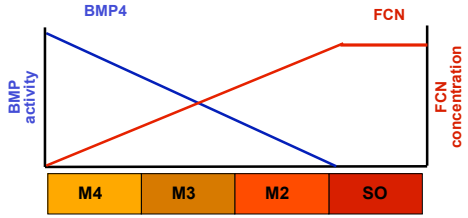
Injection of *bmp4* mRNA into the dorsal marginal zone sustains BMP synthesis in the organizer throughout gastrulation. Injected embryos are highly abnormal and lack all dorsal tissues. Most of the mesoderm in these embryos is of ventral character, such as blood. The same phenotype is observed with *bmp2* and *bmp7* so it is a characteristic phenotype of increased BMP signalling. The effect is concentration dependent, with lower concentrations permitting progressively more dorsal development. This suggests that high concentrations of BMPs specify ventral fates (blood) while lower concentrations specify lateral fates (muscle, pronephros).

Ventral injection of dominant-negative *bmpr1* mRNA induces a second dorsal axis



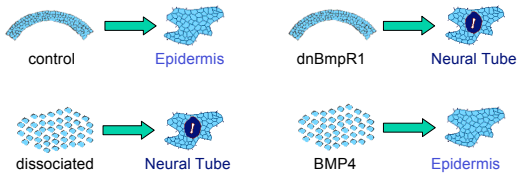
A dominant-negative type I BMP receptor (dnBmpR1) was created by deleting the intracellular kinase domain. This receptor specifically blocks BMP signalling. dnBmpR1 injected *Xenopus* embryos have expanded dorsal tissues and reduced ventral tissues, while a secondary axis is produced when the RNA is localized to ventral blastomeres. Just like injection of Chordin, Noggin and Follistatin the secondary axis does not have a complete head. This experiment demonstrates that the absence of BMP signalling is required for dorsal development. It provided a key piece of evidence that dorsalizing factors such as Chordin and Noggin might be inhibitors of BMP signalling.

Is BMP4 a morphogen?



A morphogen is any substance active in pattern formation that forms a concentrations gradient across a tissue, specifying different fates at different threshold concentrations. There is good evidence that different concentrations of BMPs specify different fates although a gradient of BMP protein has never been observed. It has been suggested that Chordin, Noggin and Follistatin are responsible for setting up a gradient of BMP activity across the marginal zone of *Xenopus* gastrulae. These three proteins (FCN) are synthesized in the organizer and diffuse throughout the marginal zone (once again this has not actually been observed), forming a gradient of BMP inhibition (high dorsal, low ventral). By binding and inhibiting BMPs they set up an apposing gradient (high ventral, low dorsal) of BMP activity.

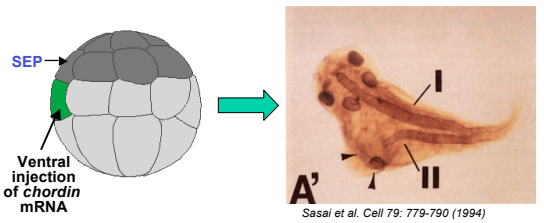
The Neural Default Model



Wilson & Hemmati-Brivanlou Nature 376: 331-333 (1995)

Whereas intact gastrula stage animal caps differentiate as epidermis, caps that have been dissociated form neural tissue. One explanation is that cap cells secrete an epidermalizing factor and that is diluted by dissociation and the absence of this factor cells become neural tissue. This suggests that neural is the "default state" of animal caps. To identify the epidermalizing factor dissociated caps were incubated in various growth factors, but only BMP4 was able to restore the epidermal fate. Animal caps isolated from embryos injected with dnBmp1 RNA are neuralized (as are FCN injected caps), demonstrating that BMP4 is the epidermalizing factor.

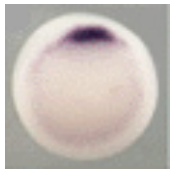
What, no heads?



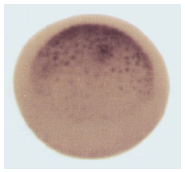
Sasai et al. Cell 79: 779-790 (1994)

Injecting RNA for BMP inhibitors induces a secondary axis that lacks head regions anterior to the otic vesicle, essentially the forebrain and midbrain. BMP inhibitors are therefore mimicking the trunk organizer. How then do you get a dorsal axis with a full head?

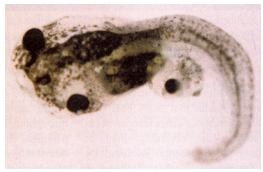
Genes encoding head inducing factors



frzb-1



cerberus



cerberus injected embryo

In addition to dorsalizing signals *Xenopus* gastrulae also express genes encoding head inducing factors. They are localized to the Spemann Organizer, but usually in the anterior endoderm and/or mesoderm. These genes include *cerberus* and *frzb-1*, above, and *dkk1*, which is not shown. They were identified in screens for organizer specific genes by the same group that discovered Chordin. All of these head inducing factors are inhibitors of the Wnt signalling pathway. Hence, the organizer is the source of inhibitors of both the BMP and Wnt signalling pathways.

Frzb-1 mRNA is localized to the head endomesoderm of late gastrulae

mid-gastrula | early-neurula | early-neurula

Leyns et al., Cell 88: 747-756 (1997)

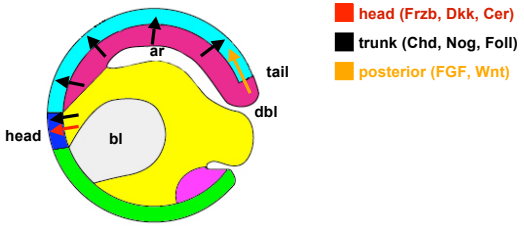
Whole mount *in situ* hybridization of *Xenopus* embryos using probes for *frzb-1* and *chordin*. In gastrulae *frzb-1* and *chordin* are both expressed in the organizer but in different populations of cells. *Frzb-1* is expressed in more anterior cells than *chordin*. This is more evident in neurulae where Frzb-1 is seen to be expressed in anterior endomesoderm that underlies the developing for- and midbrain. In contrast, *chordin* is expressed more posteriorly in the developing notochord. There is little or no overlap in expression.

Frzb induces a secondary head when coexpressed with Chordin

Glinka et al., Nature 389: 517-519 (1997)

As previously shown injection of *chordin* mRNA induces a partial second axis that lacks anterior head structures. Injection of *Frzb-1* RNA does not induce either a secondary axis or a head but it does induce a head when coinjected with *chordin* RNA. Thus while inhibition of BMP signalling is only sufficient for trunk development, inhibition of both BMP and Wnt signalling produces a complete axis.

Neural Induction



During gastrulation the dorsal mesoderm migrates anteriorly along the blastocoel roof, releasing "signals" that act on the adjacent dorsal ectoderm. Throughout the axis BMP inhibitors (Chordin, Noggin, Follistatin) block epidermal develop and promote neural development. Wnt inhibitors (Frzb, Dkk, Cer) released by the most anterior endomesoderm promote anterior neural development, thereby creating the head. The dorsal blastopore lip continues to express signalling molecules belonging to the FGF and Wnt families, which act on the posterior neural plate to promote posterior fates. In the absence of these signals embryos are anteriorized - the phenotype of embryos injected with a dominant-negative FGF receptor.