Organiser concept

What is an organizer?

The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence. The embryonic tissue which exerts such an influence is called an inductor and the chemical substance secreted by an inductor is known as evocators. The tissue on which evocator works and the tissue responses is known as responsive tissue. The action of the indicator through evocator is known as induction action or organizer action. This process of induction influences greatly the protein synthesis mechanism of responsive tissues as a result of which definite structure forming cells become very active.

Origin of the concept of the organizer

Spemann's experiment (1924): A German embryologist Hans Spemann and his student Hilde Mangold (1924) performed transplantation experiment on a newt *Triturus cristatus*, an Urodela of class Amphibia. Spemann grafted a piece taken from the dorsal lip of early gastrula of *Rana* sp. to the lateral lip region of the early gastrula of *Triturus cristatus*. The embryo of *Rana* sp. is donor and the embryo of *Triturus* is the host. They observed that the cells of the grafted piece enter into the gastrula and form notochord and somites. In this embryo its own dorsal lip of blastopore forms neural groove, notochord, mesoderm etc. Similarly the grafted tissue influences to form notochord, neural groove and mesoderm. That is in the same embryo double set of notochord, nerve cord and mesoderm are produced. In this case donor tissue has secreted some chemical substances which has induced to form neural groove, notochord etc. in the host embryo. The donor tissue had pigments and the induced neural groove has also coloured pigments. After the completion of the gastrulation they observed that a larva has developed with two heads. One head is due to normal development and the other head production has been induced by donor tissue.

They examined the larva under the microscope and found that notochord, renal tubules, gut etc. have been formed by the tissue of the host embryo as a secondary set. If the donor tissue would not have been grafted such secondary structures wound not develop. From this experiment they concluded that dorsal lip of the donor had influenced greatly the tissue and thus has brought about change in the host tissue development. If it is not the fact then how a head had developed in the abdomen of the host. This secondary head formation is due to induction effect of donor tissue. This process of influencing other tissue was termed as induction by Spemann and the tissue that induced the tissue was known as the inductor or organizer.

Primary organizer:

Spemann continued his grafting experiments taking tissues from different zones of the gastrula and observed that except dorsal lip of the early gastrula other zone of tissue can not create any induction effect but when dorsal lip is grafted a complete embryo is formed. He named the dorsal lip as organizer as this dorsal lip organizes the developmental process of the embryo. According to him this dorsal lip induces to form neural tube and the neural tube then induces to form the eyes. The dorsal lip is composed of chorda-mesoderm and as it primarily acts as inducer so he named the dorsal lip or chordamesoderm as primary organizers.

Secondary, tertirary and quaternary organizers:

As the gastrulation proceeds due to primary organizer's induction primary organs begin to form and the early stages of organ development are known as organ rudiments. These organ rudiments themselves may act as organizer and then they are known as secondary organizer. Tissues formed by the action of secondary organizer may in turn induce further development. Then they are known as tertiary organizer. These successive stages of organizer activities start from the primary organizer.

How these organizers act in succession can clearly be understood from the examples of the development of eye in amphibian, chick etc. First of all due to induction effect of the primary organizer forebrain and within the forebrain eye forming cells are produced. These cells push out as a vesicle outside the forebrain. These vesicles are known as optic vesicle. This vesicle grows through the lateral mesenchyme and reaches the epidermis.

As soon as the vesicle comes in contact with the epidermis the outer layer of the vesicle invaginates to form a double layered optic cup. The inner layer of the optic cup is formed of sensory cells and the outer layer is formed of pigmented cells. They two together form the retina. The chemical substances secreted by the optic cup induce to form the lens between the optic cup and the epidermis. The peculiar thing is that if the optic vesicle is prevented from coming in contact with the epidermis there will be no lens formation. So the optic cup acts as secondary organizer. Similarly lens and retina together induce to form cornea so lens and retina together act as tertiary organizer and so on.

Classification of induction:

Lovtrup (1974) classified induction into two principal classes.

Endogenous induction: Shapes and sizes of some of the embryonic cells changes after secreting inducting substances and this induction brings about differentiation of cells. As for example small cells of the dorsal lip carrying yolk granules act as endogenous induction.

1. Exogenous induction: When either by external influence or by contact any cell or tissue induces nearby tissue to differentiate, then it is known as exogenous induction. Exogenous induction may again be of two types. As-

1. Homotypic: When the contact induction induces to form same types of cells, it is known as homotypic.
2. Heterotypic: When the contact induction induces different types of cell differentiation, it is known as heterotypic induction.

Embryonic induction in vertebrates:

Spemann observing the induction effect of dorsal lip named it as primary organizer but Ebert and Sussex (1974) said the formation of secondary embryo is due to cell differentiation of both the donor as well as of the host. They preferred to call the primary organizer of Spemann as embryonic inductor. As the primary organizer induces the epidermis for the formation of neural tube so now a days the primary organizer has been renamed as primary inductor or neural inductor.

Morphology of Neural inductor:

Vogt (1924) has shown by vital staining technique that cells of the dorsal lip of blastopore of a newt's gastrula, move interior and form the roof of the archenteron. If a block of tissue from

archenteron roof is transplanted to the abdomen of another gastrula then from the abdomen created by the host gastrula tissue, a secondary larva is formed. All parts of the dorsal lip can not induce such induction. If only endodermal cells are grafted it will give rise to a partial embryo. If the anterior part is grafted it will induce to form the mouth, sensory organs head with the brain of the partial embryo. If the middle part is grafted it will give rise to eye and nasal cavities, lateral side induces to form posterior part of the head and if the posterior part is grafted then it will induce to form spinal cord, trunk and tail mesenchyme. From these experiments it can be concluded that the dorsal lip possess the regionality of its induction activity

Types of inductors:

On the basis of various experimental evidences Lehmon (1945) said that specific regionality of induction effects present in the dorsal lip of the blastopore. He further said that the roof of the archenteron definitely possess specific induction activities for the differentiation of head and trunk regions. On the basis of the regional specificity he classified the inductors into three groups. They are:

Archenocephalic inductor: Due to induction effect of this inductor partial head, fore-brain, eye, nasal cavities are formed.

1. **Deuterencephalic inductor:** By its induction effect posterior portion of the head, ear cavities etc. are formed.

As arechenocephalic and deuterencephalic inductors induce the formation of different parts in the head region so they together are known as cephalic or head inductors.

1. **c) Spino-caudal inductor:** Their inductive influence leads to the formation of spinal cord and different structures of the tail region.

Development of Eye in Chick

The first sign of the development of the eyes is a bulging at the lateral sides of the prosencephalon. These are the rudiments of the optic vesicles which lie beneath the head ectoderm. Meanwhile, the distal part of each optic vesicle (the future sensory layer) invaginates and presses against the proximal part (the future pigment layer of the retina, iris and ciliary body). This results in the formation of the optic cup, the elimination of the original lumen of the optic vesicle and the formation of a new lumen, the future vitreous chamber.

The lens is formed from the lens placode, a thickening of the ectoderm formed in response to an inductive signal from the optic cup. The lens sinks beneath the surface of the ectoderm, the latter becoming the cornea.

As the lens continues to grow, the cells in the thickened region lose their ability to divide and become converted into fibres that will become the core of the adult lens. New fibres are formed from the cells at the periphery of the lens which divide rapidly and become arranged in concentric circles around the original core. By the time of hatching there are three concentric layers of fibres, the core, the intermediate layer of irregularly arranged fibres, and the radial layers which continue to grow after hatching. The lens capsule, which is an extracellular material with a high collagenous component, starts to form about day 7. The ciliary body develops close to the lens, its role being to secrete the fluid of the vitreous chamber.

As the lens loses contact with the ectoderm a space is formed, the anterior chamber of the eye. The corneal epithelium develops from the ectoderm covering the anterior chamber, whilst the

corneal stroma forms from the mesenchyme and becomes visible on day 4 as a thin layer beneath the epithelium. It becomes thicker as mesenchyme cells migrate into it during day 7.

The iris arises from cells at the margin of the anterior chamber at about day 7. Removal of the lens results in disorganization of the components of the anterior chamber.

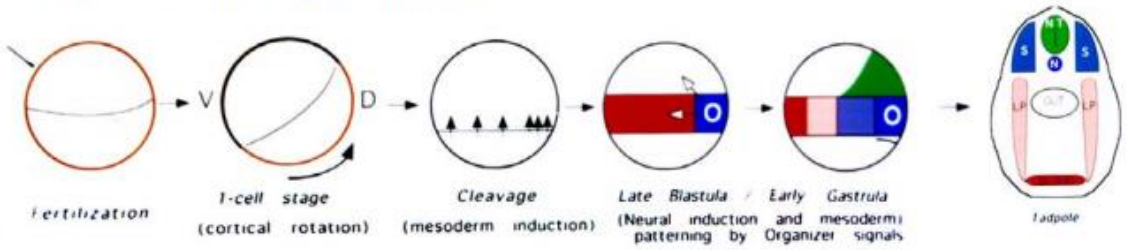
The retina is formed from the optic cup. Its inner layer becomes the neural retina and its outer layer the pigmented retina.

The choroid and sclera differentiate from the mesenchyme around the optic cup, forming the inner pigmented vascular layer, and the outer, fibrous layer, respectively. The melanophores of the choroids are derived from cells of the neural crest that reach the eye during day 2 and develop pigment on day 7. Cartilage starts to form in the sclera on day 8.

The eyelids start to form at about 7 days from a circular fold of skin surrounding the eye. The choroid fissure usually begins lo close in the region near the lens about day 4. At this time a ridge of mesoderm, carrying with it a blood vessel, migrates along the choroid fissure into the posterior chamber of the eye and enlarges during day 5 to form the pectin. The pigment cells of the pecten are derived from the pigmented retina. The pecten is a structure characteristic of birds, and it is thought that it acts not only by bringing oxygen and nutritive materials to the eye but that it may also play a role in vision.

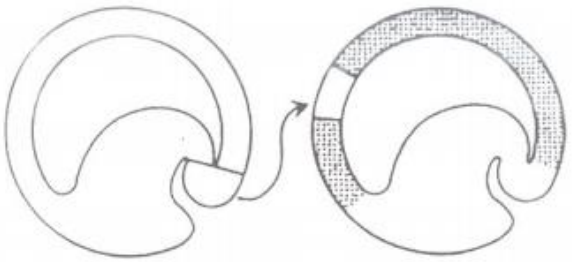
The vitreous humour is secreted by the cells of the optic cup.

Having discussed the early events in mesoderm induction, we now turn to signaling events that take place during gastrulation.

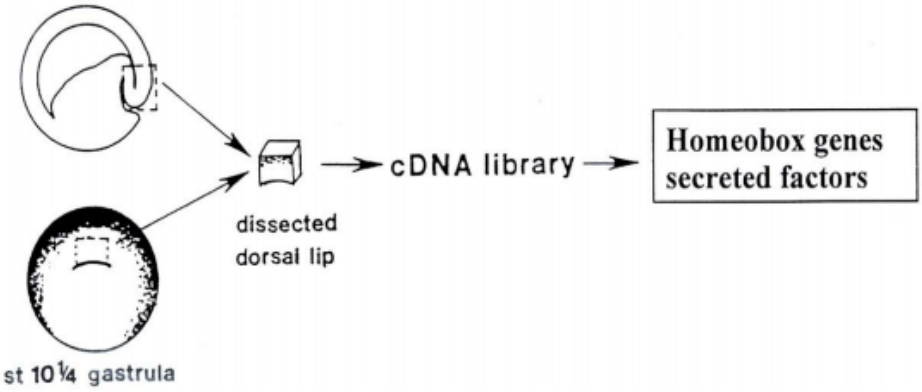


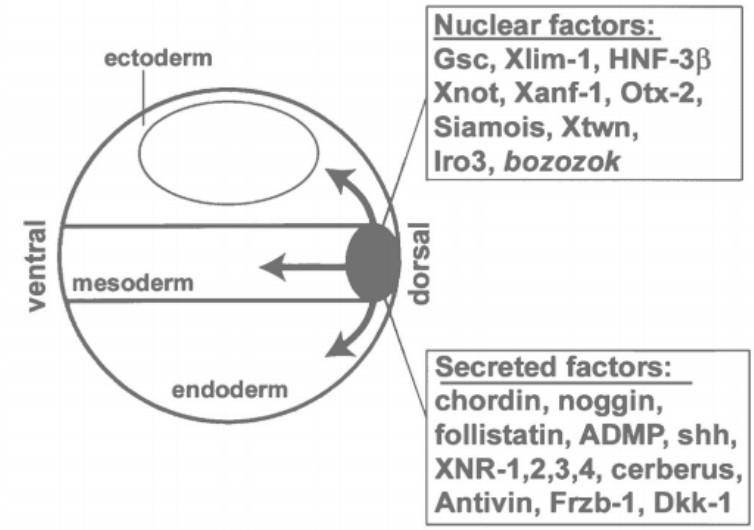
1. Dorsalization of mesoderm and neural induction by Spemann's Organizer during gastrulation

The "Organizer" experiment (Spemann and Mangold, 1924) is the best known experiment in embryology. It has led, more than any other, to the current view that development occurs through a cascade of cell-cell interactions. If the dorsal lip (the site where gastrulation starts) of the blastopore is transplanted to the opposite side of the embryo, it is able to recruit host cells organizing them into a secondary (twinned) body axis containing many histotypes and complex structures.



The organizer has three main properties: 1) it induces neural tissue on the overlying ectoderm, 2) imparts more dorsal characteristics to the mesoderm of the marginal zone (i.e., "dorsalizes mesoderm"), leading to the formation of somites and trunk muscles, and 3) it induces a secondary gut ("dorsalization of the endoderm"). This small region of the gastrula has been a goldmine for the isolation of new molecules involved in cell signaling. We made organizer-specific libraries and screened them for cDNAs expressed in the organizer (e.g., identification of *goosecoid*, *chordin*, *cerberus*, *Frzb-1*). Other labs used functional injection assays, injecting pools of synthetic mRNAs into the ventral side of embryos and then doing sib-selection until a single gene is identified (e.g., identification of *noggin*, *Siamois*, *Xtwn*, *dickkopf*).

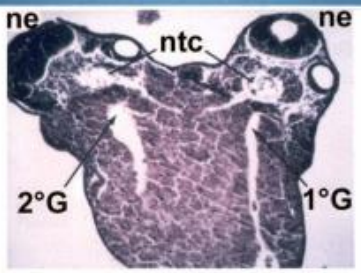


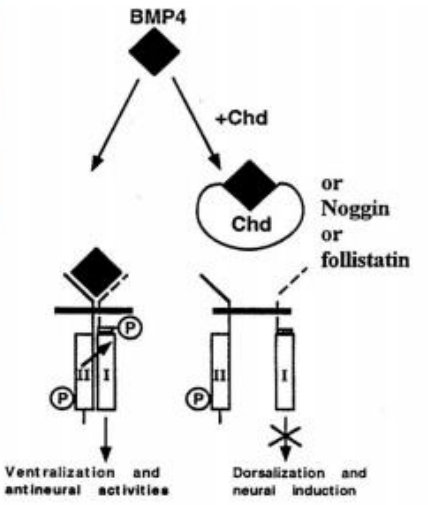


Most of the molecules isolated were transcription factors, especially homeobox genes, or secreted proteins. Ventral microinjection of these homeobox-containing mRNAs (*goosecoid, Siamois, Xtwn, Xlim-1, boz, Xnot*) can cause secondary axes and recruit neighboring uninjected cells into them, as in Spemann's experiments. Since they encode DNA binding proteins, the inductive effects on neighboring cells are mediated by changes in the expression of secreted proteins. The factors secreted by the organizer pattern the three germ layers. The effects of the organizer-specific factors are opposed by ventralizing genes that are expressed in ventro-lateral regions of the embryos. Notable among the latter are *BMP-2*, *BMP-4* and *Xwnt-8*.

2. Chordin, noggin and follistatin antagonize BMP ventralizing signals.

Microinjection of *chordin, noggin* or *follistatin* mRNA will induce secondary axes, rescue UV embryos, dorsalize mesoderm in ventral marginal zone explants and induce CNS differentiation in animal caps.





Model of the Molecular Mechanism by Which Chordin Antagonizes BMP-4 Signaling

To our surprise, co-injection of *BMP-4* abolished neural induction by chordin, by noggin, and by follistatin, which are secreted proteins of entirely different structures. This antagonism takes place in the extracellular space (as indicated by injecting different blastomeres). Production of the proteins in tissue culture showed that both noggin and chordin bind to BMPs and prevent their binding to BMP receptors. The lack of BMP signaling in turn leads to the dorsalization of mesoderm and neuralization of ectoderm. (The same result can be obtained using a DN-BMPReceptor construct). Thus, the surprising finding was that neural induction and the dorsalization of mesoderm (and also of endoderm) had the same molecular basis: antagonism of ventral BMP signals.

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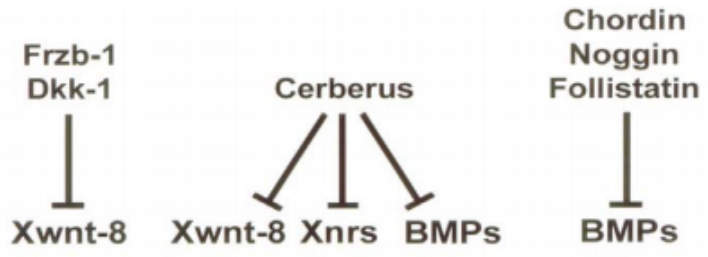
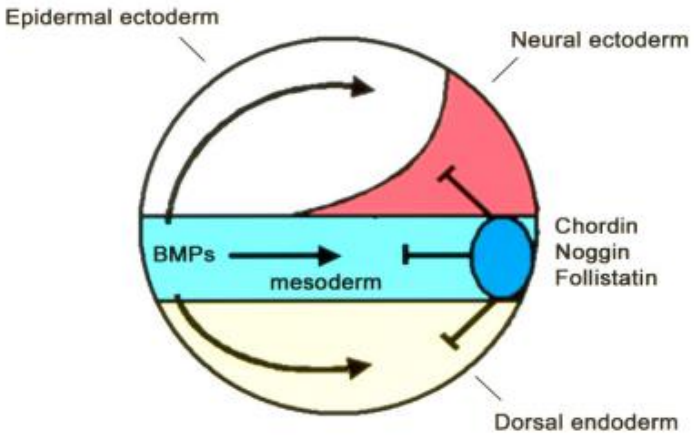
The biggest surprise from these studies on Spemann's organizer was that patterning by the organizer is effected through secreted antagonists of growth factors:





Model indicating that the same set of regulatory signals may provide the positional information that patterns ectoderm, mesoderm and endoderm in *Xenopus*. On the dorsal side (right), the organizer (oval) provides dorsal positional values to ectodermal (animal cap, top) and mesodermal (marginal zone, middle) tissues by secreting organizer factors such as *chd*, *noggin* and *follistatin*. On the opposite side (left), ventralizing factors such as *BMP-4* and presumably other signals give ventral positional values to the tissues, antagonizing organizer signals. High dorsal values promote neural differentiation in the ectoderm and formation of notochord and muscle in mesoderm, while high ventral values lead to epidermogenesis in ectoderm and differentiation of blood island and mesenchymes in the mesoderm.

Spemann's organizer is full of inhibitors. Some think that it may have been easier, perhaps, to produce inhibitors during the course of evolution to modulate pathways ("a spanner in the works") than to evolve entirely new signaling pathways. A large number of secreted antagonists have been discovered in various tissues:

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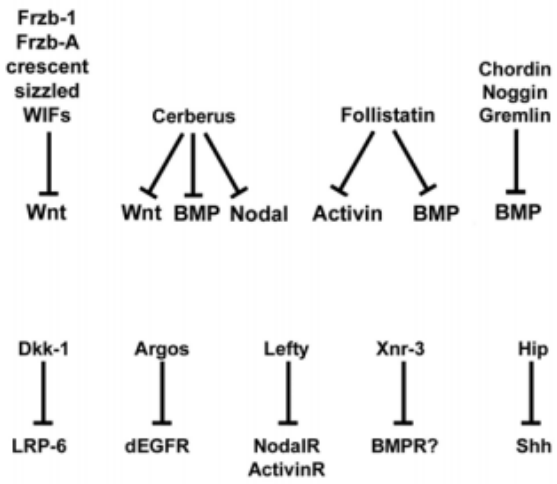


Organizing the Embryo: The Central Nervous System

In the embryonic development of a zygote, gradients of mRNAs and proteins, deposited in the egg by the mother as she formed it, give rise to cells of diverse fates despite their identical genomes.

But is the embryo fully patterned in the fertilized egg? It is difficult to imagine that the relatively simple gradients in the egg could account for all the complex migration and differentiation of cells during embryonic development. And, in fact, the answer is no. However, once these gradients have sent certain cells along a particular path of gene expression, the stage is set for those cells to begin influencing nearby cells to become increasingly diversified.

In other words,

- **cell-intrinsic signals** (established between a nucleus and the particular cytoplasmic environment that cleavage has placed it in) lay the foundation for
- **cell-cell interactions** to further guide the cells of the embryo to assume their proper position in the embryo and to differentiate into their final specialized form and function.

  Cell-cell interactions could — and probably do — occur in several ways:

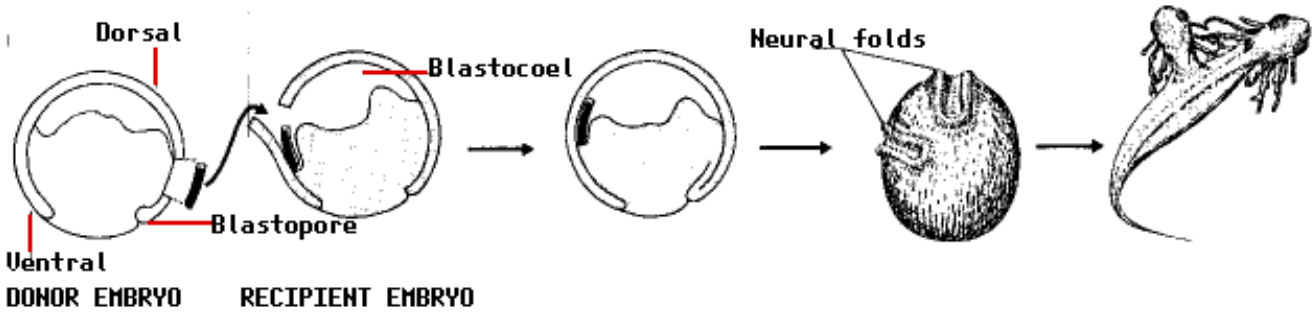
  - diffusion of a signaling molecule out of one cell and into other cells in the vicinity;
  - diffusion of a signaling molecule from one cell into an adjacent cell that then secretes the same molecule to diffuse to the next cell and so on (a "cell-relay" mechanism);
  - extension of projections from the plasma membrane of one cell until they make **direct contact** with nearby cells. This enables proteins embedded in the plasma membrane to serve as signaling molecules.

The Spemann Organizer
In 1924, the Ph.D. student Hilde Mangold working in the laboratory of German embryologist Hans Spemann performed an experiment that

- demonstrated that the pattern of development of cells **is** influenced by the activities of other cells and
- stimulated a search, which continues to this day, for the signals at work.

Spemann and Mangold knew that the cells that develop in the region of the gray crescent migrate into the embryo during gastrulation and form the **notochord** (the future backbone; made of **mesoderm**).

She cut out a piece of tissue from the gray crescent region of one newt gastrula and transplanted it into the ventral side of a second newt gastrula. To make it easier to follow the fate of the transplant, she used the embryo of one variety of newt as the donor and a second variety as the recipient.



The remarkable results:

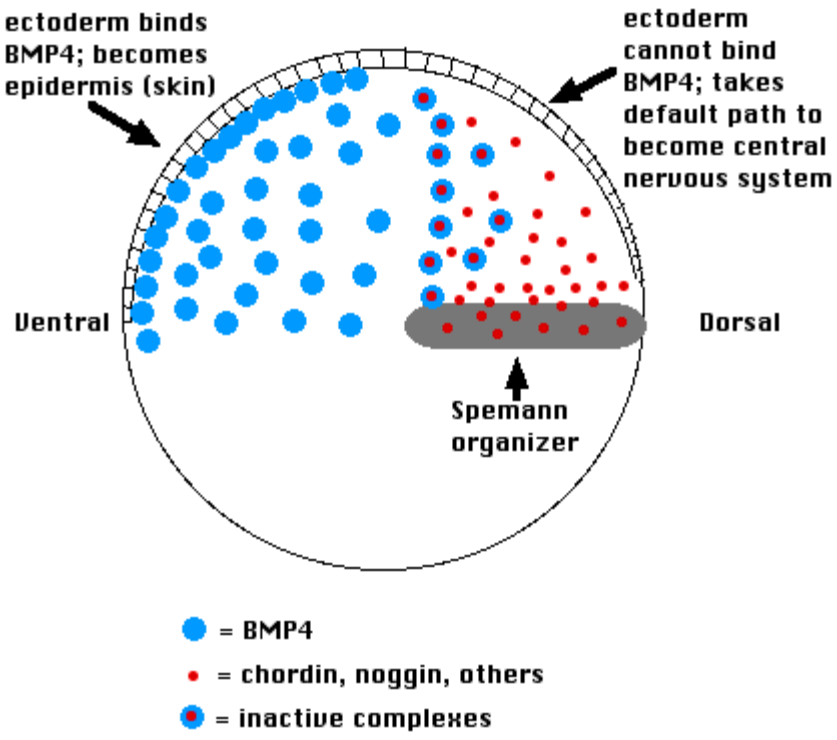
- the transplanted tissue developed into a second notochord
- **neural folds** developed above the extra notochord
- these went on to form a second central nervous system (portions of brain and spinal cord) and eventually
- a two-headed tadpole.

But the most remarkable finding of all was that the **neural folds were built from recipient cells, not donor cells**. In other words, the transplant had altered the fate of the overlying cells (which normally would have ended up forming skin [epidermis] on the side of the animal) so that they produced a second head instead!

Spemann and Mangold used the term **induction** for the ability of one group of cells to influence the fate of another. And because of the remarkable inductive power of the gray crescent cells, they called this region the **organizer**.

Ever since then, vigorous searches have been made to identify the molecules liberated by the organizer that induce overlying cells to become nerve tissue. One candidate after another has been put forward and then found not to be responsible. Part of the problem has been that not until just recently has it become clear that the organizer

- does **NOT** induce the central nervous system but, instead,
- it prevents signals originating from the ventral side of the blastula from inducing skin (epidermis) there.



This is how it works:

- Cells on the **ventral** side of the blastula secrete a variety of proteins such as **b**one **m**orphogenetic **p**rotein-4 (**BMP-4**)
- These **induce** the **ectoderm** above to become **epidermis**.
- If their action is blocked, the ectodermal cells are allowed to follow their **default pathway**, which is to become nerve tissue of the brain and spinal cord.
- The Spemann **organizer blocks the action of BMP-4** by secreting molecules of the proteins
    - **chordin** and
    - **noggin**
- Both of these physically bind to BMP-4 molecules in the extracellular space and thus prevent BMP-4 from binding to receptors on the surface of the overlying ectoderm cells.
- This allows the ectodermal cells to follow their intrinsic path to forming neural folds and, eventually, the brain and spinal cord.

In the Spemann/Mangold experiment, transplanting an organizer to the ventral side provided a second source of chordin. This blocked BMP-4 binding to the overlying ectoderm and thus changed the fate of those cells to forming a second central nervous system rather than skin.

What Organizes the Organizer?

Protein synthesis by the cells of the organizer requires transcription of the relevant genes (e.g., *chordin*). Expression of organizer genes depends first on Wnt transcription factors. Their messenger RNAs were deposited by the mother in the vegetal pole of the egg. After fertilization and formation of the gray crescent, they

- migrated into the gray crescent region (destined to become the organizer) where they were
- translated into Wnt protein.

Its accumulation on the dorsal side of the embryo unleashes the activity of **Nodal** — a member of the Transforming Growth Factor-beta (TGF-β) family. Nodal induces these dorsal cells to begin expressing the proteins of Spemann's organizer.

A Tail Organizer

One of the distinguishing features of vertebrates is their tail, which extends out behind the anus.

French researchers have reported (in the 24 July 2003 issue of **Nature**) their discovery of a tail "organizer", that is, a cluster of cells in the embryo that induces nearby cells to contribute to the formation of the tail.

They worked with the zebrafish, *Danio rerio* (which also has a head organizer like that of newts).

They removed tiny clusters of cells from the ventral part of the blastula (a region roughly opposite where the Spemann-like organizer forms) and transplanted this into a region of the host embryo that would normally form flank.

The result: a second tail.

Using a fluorescent label, they were able to show that the extra tail was made not only from descendants of the transplanted cells but also from host cells that would normally have made flank.

Three proteins were essential:

- a Wnt protein (establishes the anterior-posterior axis in all bilaterians)
- BMP (establishes the dorsal-ventral axis in all bilaterians)
- Nodal (establishes the left-right axis in all bilaterians)