

Establishment of body axes in the early *Xenopus* embryo: the importance of the “dorsal” side, inductive events, and the establishment of mesoderm

These notes and reading are for classes 7 and 8

Reading: 8th ed: Chp 10: 291-295, 302-312 9th ed: Chp 7: 252-267

Learning goals: Be able to:

1. Explain how the D-V axis is established in *Xenopus*, including the timing of this event and the molecules involved.
2. Explain how the organizer was shown to be capable of inducing the dorsal axis.
3. Compare the role and origin of the Nieuwkoop center to the role and origin of the organizer
4. Design experiments that prove the role of mesoderm-inducing factors.
5. Propose outcomes of experiments that prevent normal mesodermal patterning.
6. Relate the concept of morphogens to the determination of mesoderm and the role of the organizer in *Xenopus*

Our goal in this unit is to understand the logic of how animal body plans can arise from a one-cell embryo. Not long ago, this process seemed miraculous. At present, particularly in *Drosophila* and amphibians, we have a pretty good idea of how it works, and we have identified many of the molecules that are involved. In *Drosophila*, most of what we know has come from a genetic approach; in *Xenopus*, from experimental embryology. Most of the same mechanisms appear to be used in all embryos in similar ways. We'll focus on the *Xenopus* embryo for this section, and return to *Drosophila* when we discuss developmental biology from the perspective of genetics in Unit 3.

Establishment of embryonic polarities in amphibians

In most organisms (except mammals), axis determination relies upon maternally contributed factors, deposited directly into the oocyte. As we've already discussed, cleavage then partitions these factors into different cells, sometimes giving adjacent cells different capabilities. In addition to the cytoplasmic determinants, signaling is a major component of cell fate determination as well. Thus, the establishment of the axes (we'll consider mostly D-V and A-P) often begins with segregation of a transcription factor, and continues with signaling.

In amphibia, the animal-vegetal axis is the first to be established. A TGF- β ligand, Vg-1, is localized to the vegetal half of the embryo at the formation of the oocyte, along with numerous additional factors (including another factor called VegT, a transcription factor). The animal half seems to have very few maternal mRNAs. The cleavages are displaced towards the animal pole due to the high concentration of yolk at the vegetal pole.

Sperm entry specifies the dorsal side via cortical rotation. The mature amphibian egg is radially symmetric around the animal/vegetal axis. Early experiments showed that the sperm can enter the egg anywhere in the animal hemisphere, and that the future dorsal side where the blastopore forms is almost always on the meridian opposite the point of sperm entry toward the vegetal pole. After gastrulation, the blastopore will become the posterior most part of the dorsal side of the body, the anus. John Gerhart and coworkers found in the early 1980's that several treatments disruptive to microtubules – UV, cold, drugs like nocodazole – during the first cell cycle (which is much longer than subsequent cycles) “ventralized” the embryo; no dorsal lip appeared, gastrulation was prevented, and no dorsal structures formed. However, these embryos could be rescued by briefly tilting them toward the point of sperm entry, which causes yolky vegetal cytoplasm to move relative to the underlying cortex, as the cytoplasm flows to the lowest point in the cell. Furthermore, untreated embryos tilted away from the point of sperm entry midway through the first cell cycle formed a second blastopore and a second set of ectopic dorsal structures on the ventral side. These results suggested that establishment of the dorsal/ventral (D/V) axis might depend on some kind of internal rotation of material within the oocyte. In elegant marking experiments, they showed that such a rotation, of about 30 degrees does occur during the first cell cycle. The rotation is of the cortex of the cytoplasm, with respect to the internal cytoplasm.

Subsequent work has shown that the microtubules in the cortex become aligned with their “+” ends toward the direction of rotation, probably initially through interaction with astral microtubules growing outward from the sperm centrosome (in the opposite animal hemisphere) after fertilization. Normal rotation is an active process, acting against gravitational forces on the yolky cytoplasm, and is probably driven by microtubule associated motor proteins such as kinesins. During rotation, there are also cytoplasmic rearrangements resulting in some mixing of animal and vegetal cytoplasm (endoplasm), particularly on the dorsal side. Somehow, movement of a region of cortex out of the endoplasm into a different cytoplasmic environment and mixing and activation of cytoplasmic components generates a specialized region on the dorsal vegetal side that confers unique properties to the future cells that will be in this region. Below, we discuss the molecules that seem to be involved in this very early patterning event.

What are the possible factors involved in these early events?

Initially, no one knew what the actual molecules were that determined the dorsal side. The assays for “dorsal fate determinants” were basic: take a region of cytoplasm or a cell and move it around. If this resulted in an additional dorsal axis, that cell, or that cytoplasm contained the dorsalizing factors. Because cytoplasm could be transplanted and create a twinned embryo, the idea arose that the determinants were probably transcription factors (which of course could also then activate important signaling ligands).

Testing of candidate factors: To be considered a likely participant in normal development, a dorsalizing factor must satisfy at least two other criteria in addition to inducing dorsal structures when moved: 1) Candidate molecules must be present in the embryo at a concentration required for activity. 2) Blocking their action *in vivo* must interfere with normal dorsalization.

Over more than a decade, a variety of different proteins were isolated from the early embryo and tested for this “dorsalizing” activity. Both transcription factors and signaling ligands were identified, and most fall into two categories of molecules: members of either the Wnt pathway or the TGF β family. In all cases, the presence of a particular transcription factor early on can lead to the production of a signaling molecule which can then exert its influence on neighboring cells (the induction part).

The initial dorsalizing determinant appears to be β -catenin, the transcription factor activated in the Wnt signaling pathway. However, β -catenin is actually expressed uniformly in the fertilized egg, so how does it later become activated in only one location before embryonic transcription is activated? The mechanism is fascinating! The egg contains kinesin, a motor protein, and of course, microtubules used for transport. At fertilization, the sperm enters the egg and the sperm centriole begins to organize the microtubules of the egg into parallel arrays in the cytoplasm. The outer portion of the cytoplasm, called the cortex, is tethered by this array, and physically rotated with respect to the inner cytoplasm by about 30 degrees. Because there are maternally contributed components in the cortex, these molecules move along the microtubule tracks, with the cortical rotation and are displaced. Disheveled (Dsh), a member of the Wnt pathway, is one of the maternally contributed proteins that is present in the vegetal cortex. It moves via the cortical rotation, and diffuses into the cytoplasm. Because Dsh inhibits GSK-3 kinase (also maternally contributed, and originally distributed evenly throughout the egg), GSK-3 is deactivated on this side of the embryo. The deactivation of GSK-3 on this side allows for the accumulation of β -catenin on this side, while on the other side, β -catenin is degraded by the active GSK-3. So, now one half of the embryo has active β -catenin, and this is the future dorsal side of the embryo.

These dorsal cells have inductive properties later in development

The German embryologist Hans Spemann was among the first to recognize the phenomenon of induction in the 1920's, when he found that one tissue could influence the fate of another in contact with it. Spemann noted that the commitment of a tissue to its normal fate could change with time in transplantation experiments. When he transplanted a piece of salamander ectoderm normally fated to become neural tube to another location where epidermis normally forms, the outcome was dependent on age of the embryo. If the experiment was done with an early gastrula, the transplant would form epidermis like its new neighbors (“regulation”), but if it was done with a late gastrula the transplant would form ectopic neural tube in the new

location (G,10.20). He called this progressive determination, meaning that gradually become determined, that is, irreversibly committed to a specific fate. This is referred to as regulative development: 1) blastomeres are pluripotent, with developmental potential greater than their normal fate, and 2) interactions between neighboring cells cause them to become less pluripotent and more determined. These cell interactions are called inductions, and as we now know, they involve the juxtacrine and paracrine signals we have talked about.

In the course of the determination experiments described above asking what happened to transplanted tissues in the early gastrula stage embryo, Spemann's graduate student Hilde Mangold found one region that seemed to be not only completely determined, but also able when transplanted to induce changes in the new tissue around it. This region was the dorsal lip of the blastopore—the cells that initiate gastrulation. These cells are dorsally located (obviously), but occupy just a small region. In the next class we'll see why only these cells are capable of induction and initiating gastrulation. Anyway, when Mangold cut out a piece of the dorsal lip of an early salamander gastrula and implanted it into the ventral side of another "host" embryo, the host formed a second primary axis (that is, a second notochord, and above it, a second neural tube). Had the new organs arisen from the implanted donor tissue, which was already determined? Or had the implanted donor cells somehow interacted with the surrounding host ventral tissue and caused it to do something it would not otherwise do? Using a species of newt with unpigmented cells as donors and another darkly pigmented species for hosts, she was able to show that at least some of the tissue in the new secondary axes was derived from host; that is, tissue normally destined to become belly was instead caused to develop into dorsal structures. Although this may not seem surprising to us now, at the time, this was something new: the transplanted tissue was changing other adjacent cells. Spemann called this phenomenon "primary induction". He termed the cells of the dorsal lip the "organizer" because of their abilities to change the fates of other cells. Spemann received the Nobel Prize in 1935 for this work. (Sadly, Mangold died in a kitchen fire in 1926, the year her work was published, and thus could not be awarded the Nobel Prize.) We know now that the organizer is not the first example of induction in the *Xenopus* embryo: there are several important signaling events that precede its formation.

Pre-Organizer: the formation of the Nieuwkoop center

In the 1970's, P. Nieuwkoop observed that neither a separated animal cap nor vegetal cap explanted into culture medium from an early blastula would form mesodermal tissues (e.g. muscle, blood, heart, notochord: remember, these are the cells that have to undergo gastrulation), based on histological assays. However, if the two were put back together in culture, mesoderm would form. Later experiments confirmed these results using cardiac actin mRNA as a molecular marker for mesoderm formation. Complementary experiments in the 1970's showed that explanted marginal zone cells from an early blastula would not form mesodermal tissues in culture, but that marginal zone cells from a 256-cell (8th cleavage, 4 cleavages before MBT) blastula would form mesodermal tissues. This important result showed 1) that mesoderm is not specified autonomously by determinants inherited from early blastomeres, and 2) that by mid-blastula the capacity to form mesoderm has already been induced in these cells, well before gastrulation onset. Later experiments showed that mesodermal molecular markers also are expressed in these cells before gastrulation.

Together, these and further marking experiments on the origins of the mesodermal cells made it clear that animal hemisphere cells are induced to become mesoderm during the mid- to late blastula stages by signals from vegetal hemisphere cells. However, the mesoderm is not uniform, but patterned as seen from the blastula fate map: intermediate mesoderm gives rise to somites and muscle while dorsal mesoderm gives rise to the organizer and later to heart, notochord, and head mesoderm. Several experiments indicated that the signals for inducing different mesodermal tissues were coming from the underlying vegetal blastomeres as early as the 32-cell stage. When individual vegetal blastomeres from the 32-cell stage were cultured with animal caps, *ventral* vegetal blastomeres induced ventral and intermediate mesodermal tissues, while *dorsal* vegetal blastomeres induced dorsal mesodermal tissues. So somehow, different parts of the vegetal tissue seem to send different mesoderm-inducing signals.

The Nieuwkoop center. More early evidence came from transplant experiments using blastula stage embryos (Gerhart and colleagues, 1980s). They showed that transplantation of dorsal vegetal blastomeres from the 16- or

32-cell stage to a UV-irradiated embryo (described below: all ventralized, no organizer formed), could restore dorsalization. Moreover, transplantation into the ventral side of an untreated host embryo caused twinning; i.e., a second organizer causing a second dorsal axis. By marking the transplants with a fluorescent dye, these researchers showed that the descendants of these cells did not give rise to any dorsal structures, but only to gut tissue. Therefore, these dorsal vegetal cells were causing dorsalization by induction. They named this inducing region of the early blastula the Nieuwkoop center, after the Dutch embryologist who first demonstrated the dorsalizing properties of these cells in the 1970's. In later stages, the cells with this inducing activity are those just below the equator, near the position of the future organizer. The Nieuwkoop cells actually induce the organizer.

Inducing mesoderm as a result of the induction events that result in the Nieuwkoop center

The 4-signal successive induction model. The model suggested by these findings so far is one of successive inductions: The Spemann organizer, which will initiate the movements of gastrulation at the blastopore lip, is induced to form from mesodermal precursor cells on the dorsal side near the equator. The inductive signals come from the dorsal vegetal cells of the Nieuwkoop center lying just below. The Nieuwkoop center is induced by the positioning of b-catenin in a particular location in the embryo (the future dorsal side).

The model for mesoderm patterning is progressive in nature; signals build on each other. The 1st signal is for the general specification of mesoderm: the vegetal cells secrete Vg1. On the dorsal vegetal side of the embryo, b-catenin is present as well. In these cells, another TGF β ligand called **Xnr** is activated by the overlap of b-catenin and Vg1. This ligand is present at high concentrations in the dorsal vegetal cells, and diffuses across the embryo so that it is present in all cells of the vegetal hemisphere in a gradient: this is the 2nd signal (for the induction of dorsal mesoderm). The 2nd signal emanates from cells in the Nieuwkoop center, inducing the organizer cells above them. After the organizer has been induced, the organizer itself acts as the 3rd signaling center, responsible both for the formation of "intermediate mesoderm" and formation of overlying neural ectoderm. The mesodermal cells that receive the organizer signal would differentiate into ventral mesoderm without that signal (ventral mesoderm is primarily blood; intermediate mesoderm is tissues like kidney and muscle). Signal 3 is a BMP-4 inhibitor, discussed in more detail in the next class. The 4th signal is actually not 4th in temporal order. It is present back at the beginning, after the initial mesoderm induction signal. Cells that are specified to make ventral mesoderm secrete BMP-4 (another TGF- β ligand!) in all directions.

Review Questions

- 1) What kinds of experiments are necessary to demonstrate that a tissue contains factors required for a particular developmental event?
- 2) What was the early evidence that different regions of the frog ectoderm become progressively determined during early embryogenesis?
- 3) What is the initial cue that determines which side of a frog egg will become the dorsal side of the embryo, and how is this cue transmitted?
- 4) What is the evidence that cortical rotation produces a dorsalizing signal? What additional experiments helped elucidate the molecules involved?
- 5) How was the organizer discovered, and why was its discovery important? What evidence did Spemann and Mangold need to conclude that the organizer was an inducer?
- 6) Where does the signal come from that induces animal cap cells in a frog embryo to become mesoderm?
- 7) Describe the experiments that showed the existence of the Nieuwkoop center, and the evidence that these cells induce the organizer.
- 8) How does the mesodermal layer become patterned prior to gastrulation? Outline the sequence of event involved in the specification of mesoderm, and in the specification of different kinds of mesoderm.