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Synergistic principles of development: overlapping patterning systems in *Xenopus* mesoderm induction

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Summary

The first inductive event in *Xenopus* development establishes the mesoderm at the equator of the developing embryo. As part of this process, the dorsal-ventral and anterior-posterior axes of the embryo are initially established. A number of signalling molecules which may play a role in mesodermal induction and patterning have been identified in the last several years, including members of the FGF, TGF-β and *Wnt* gene families. A variety of experiments, using either purified factors or injection of RNA encoding these factors, have added to the wealth of classical embryogical experimental data collected over the last century.

We have synthesized some recent results with the classical data to provide a framework for examining the process of mesoderm induction, and to formulate putative roles for some of the different factors. We incorporate these ideas into a working model of mesoderm induction that provides a basis for future experimental directions. Finally, we suggest that mesoderm induction may not be a discrete set of well separated events, but instead may be a process involving partially overlapping signals that produce the same pattern.

Key words: mesoderm induction, *Xenopus*, FGF, *Wnt*, Activin B, noggin.

Introduction

Almost 70 years ago, Mangold and Spemann launched vertebrate embryology on a radical new course with the observation that transplantation of a small region of a gastrula stage amphibian embryo into a new location in a host embryo could induce the neighboring cells of the host embryo to form a second body axis (Spemann and Mangold, 1924). Spemann called this region, located above the dorsal blastopore lip, the "organizer" to indicate his belief that this small group of cells was able to determine completely the initial fate of the embryo. Almost 50 years later, Nieuwkoop and colleagues demonstrated that the vegetal hemisphere is able to signal the overlying animal hemisphere to convert prospective ectodermal cells to various mesodermal lineages by a process known as induction (Nieuwkoop, 1969a; Nieuwkoop, 1969b). This results in the formation of an annulus of mesoderm around the equator of the embryo in a region referred to as the marginal zone (Fig. 1). Careful fate mapping studies in Urodeles have shown that the mesoderm does not originate as a perfect ring around the equator, but instead a greater proportion of the mesoderm is formed from the dorsal side of the embryo (Vogt, 1929); a similar distribution of mesoderm may also occur in anurans such as *Xenopus laevis* (Keller, 1976; Dale and Slack, 1987a; Moody, 1987a; Moody, 1987b) although the current fate maps are ambiguous on this point. Since Nieuwkoop's experiments also demonstrated that the vegetal hemisphere could impart dorsal-ventral polarity on the animal hemisphere, he proposed that the mesoderm was formed and specified by a unique signal arising from the vegetal hemisphere with the maximal level of the signal emanating from the dorsal side (Boterenbrood and Nieuwkoop, 1973; Nieuwkoop, 1973).

Slack and colleagues re-examined the experiments of Spemann and Nieuwkoop and proposed a model in which three signals specify the early mesodermal pattern (Smith and Slack, 1983; Slack et al., 1984; Dale et al., 1985; Smith et al., 1985; Fig. 2). In this model, one mesoderm-inducing signal is released uniformly from the ventral and lateral vegetal hemisphere, creating a ring of ventral-type mesoderm in most of the equatorial zone. A second signal originating in the dorsal vegetal region induces the formation of the most dorsal type of mesoderm, which includes Spemann's organizer, in the overlying dorsal equatorial zone. Finally, the organizer sends a third signal across the mesoderm to convert the initial ventral mesodermal cells to a variety of different intermediate mesodermal cell types.

Although intercellular signals have long been thought to be important in inductive processes, only within the last five years has the molecular nature of some of these signals become known (Table 1). While there is still debate as to the exact signaling agents used in mesoderm formation and patterning, they are likely to include members of the fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), and *Wnt* gene families. Although most of these factors are likely to be secreted, they may remain tightly bound to the membrane or extracellular matrix of

the secreting cell. Thus unlike a freely diffusible morphogen, these factors may only be involved in signaling over short distances.

The mesoderm-inducing capacity of these factors has been tested primarily on cleavage-stage explants of the animal hemisphere (the "animal cap") which normally develop as undifferentiated ectoderm (Smith, 1987). For example, addition of basic FGF (bFGF) to these explants causes many of the cells to differentiate as ventral mesoderm (Kimelman and Kirschner, 1987; Slack et al., 1987). This has led to the speculation that bFGF is the vegetal ventralizing signal of the three-signal model (Slack et al., 1987). However, animal cap explants isolated from embryos injected with bFGF RNA form both dorsal and ventral mesoderm, demonstrating that bFGF has the capability of inducing most, if not all, mesodermal cell types (Kimelman and Maas, 1992).

Members of the FGF family have been suggested to be involved in mesoderm induction in vivo since bFGF transcripts and protein are present in the egg and early embryo (Kimelman et al., 1988; Slack and Isaacs, 1989) and both basic and acidic FGF have been shown by immunohistochemistry to be localized in the equatorial and vegetal regions of the cleavage stage embryo (Shiurba et al., 1991). A third FGF family member, XeFGF, has also been found in the early embryo (Isaacs et al., 1992). XeFGF, unlike bFGF or aFGF, contains a secretory signal sequence, and therefore may be regulated differently than the other two FGFs. A *Xenopus* bFGF receptor encoded by a maternal RNA has also been found in all regions of the cleavage stage embryo (Gillespie et al., 1989; Musci et al., 1990; Friesel and Dawid, 1991). Fertilized eggs injected with RNA encoding a dominant-negative mutant form of this FGF receptor develop into tadpoles lacking posterior and trunk regions, with reductions in the proportion of certain mesodermal cell types (Amaya et al., 1991). These results firmly establish a role for FGF-signaling in some aspects of normal mesoderm formation, with bFGF, aFGF, and XeFGF as possible candidates for endogenous signaling molecules.

Within the TGF-β family, activin is the most potent mesoderm inducing agent, although some other family members are also active in this system (Albano et al., 1990; Roberts et al., 1990; Smith et al., 1990; Thomsen et al., 1990; van den Eijnden Van Raaij et al., 1990; Dale et al., 1992). When added to animal caps, activin can induce both dorsal and ventral mesoderm with higher doses leading to the formation of more dorsal tissues (Green et al., 1990). Activin has also been reported to induce brain and eyes in explants, although this may be the result of secondary neural-inducing signals arising from the activin-induced mesoderm (Sokol et al., 1990; Kintner and Dodd, 1991). Furthermore, animal cap cells treated with activin behave as organizing centers when transplanted into ventral regions of the embryo (Cooke, 1989). With regard to the timing of expression of the activins, activin B transcripts are first detected at the midblastula transition (MBT), whereas activin A (also known as the XTC-mesoderm inducing factor (XTC-MIF) or as P38801-derived inducing factor (PIF)) RNA is not detected until later in embryogenesis (Thomsen et al., 1990). A maternal activin-like activity has also been detected in the pre-MBT embryo (Asashima et al., 1991). RNA encoding a *Xenopus* activin receptor has been found in the oocyte and early embryo, which like the FGF receptor appears to be uniformly distributed throughout the embryo (Kondo et al., 1991; Matthews et al., 1992). These results strongly suggest a role for activins in early *Xenopus* development.

A third class of signaling agents may be a member of a large family of developmentally expressed genes related to the proto-oncogene *Wnt*-1 (McMahon, 1992). Although not a mesoderm inducing agent itself (Christian et al., 1992), this factor appears to provide embryonic cells with positional information required for the formation of the body axes. Injection of RNA encoding at least two different *Wnt* proteins (*Wnt*-1 or *Xwnt*-8) into fertilized eggs or cells on the ventral side of a cleavage-stage embryo leads to the formation of an embryo with a duplicated axis (McMahon and Moon, 1989; Christian et al., 1991b; Smith and Harland, 1991; Sokol et al., 1991). The *Wnt* RNAs which can produce this phenotype are not detected until after the MBT (Noordermeer et al., 1989; Christian et al., 1991a), whereas the normal signaling involved in establishment of the embryonic axes occurs much earlier (see below). Unlike bFGF and activin, *Wnts*, when ectopically expressed prior to the MBT, do not appear to be capable of inducing mesoderm by themselves. Explants of animal caps from embryos injected with *Xwnt*-8 RNA do not form mesoderm, providing that only explants from the top of the animal hemisphere are used in the assay (Christian et al., 1992). In contrast, animal caps isolated from embryos injected with RNA encoding XbFGF or activin are induced to form a wide variety of mesodermal cell types (Sokol et al., 1991; Kimelman and Maas, 1992).

It has been suggested that these ectopically expressed *Wnt* proteins may be activating a receptor for an endogenous maternal *Wnt* protein which normally functions to establish the dorsal-ventral axis soon after fertilization (McMahon and Moon, 1989; Christian et al., 1991b; Smith and Harland, 1991; Sokol et al., 1991). Alternatively, activation of a *Wnt*-signaling pathway by injected *Wnt* RNA may fortuitously mimic an intracellular signaling pathway that can also be used by an unrelated ligand-receptor system such as the novel dorsalizing factor noggin (Smith and Harland, 1992), to establish the embryonic axes (Christian et al., 1991b; Olson et al., 1991; Smith and Harland, 1991). For this reason we will use the term "*Wnt*-like activity" throughout this essay to describe a factor with the same activity as *Wnt*-1, *Xwnt*-8, or noggin.

With the wealth of new molecular data on factors involved in mesoderm induction, it has been tempting to incorporate these findings into the three-signal model. Recent evidence from a number of laboratories, including our own, has suggested that a refinement and expansion of the three-signal model is now in order and we have therefore reexamined the current molecular data and classical embryological studies. We suggest that the formation and patterning of the mesoderm is the culmination of a number of overlapping signals rather than a discrete set of well separated events. As discussed below, this concept is based on a mixture of well-established experimental data and several of our speculative ideas. To distinguish widely accepted

Fig. 3. A synergistic model for mesoderm induction. *Dorsal-ventral patterning*. In step Ia, a *Wnt*-like activity (green) is activated by cortical rotation in a broad dorsal region of the egg, with maximum activity at the dorsal midline of the equatorial region. This activity is suggested to prepattern the competence of the animal hemisphere to respond to mesoderm inducing signals such that cells on the dorsal side of the embryo are biased to form dorsal mesoderm in response to mesoderm inducing signals. The mesoderm inducing factors, FGF and signal X (red and yellow arrows) are released from the vegetal hemisphere (or perhaps from within the marginal zone) and induce the mesoderm at the equator of the embryo. The result of these signals is shown in step Ib, corresponding to an embryo at the midblastula transition. The mesoderm is specified at the equator with perhaps a larger amount of mesoderm arising from the dorsal side. Signal X, together with maximum *Wnt*-like activity, establishes the late blastula organizer on the most dorsal side of the embryo, in the lower region of the marginal zone (purple). FGF also synergizes with the *Wnt*-like activity to establish other regions of dorsal mesoderm (magenta). By itself, FGF induces only ventral mesoderm (pink). In step II, a signal (red arrows), possibly activin B, is released from the late-blastula organizer to establish a region that will become the notochord. In step III, the notochordal region releases a signal (green arrows), possibly also activin B, which converts the adjacent mesoderm to muscle. The muscle might release a weaker activin B signal to pattern the mesoderm next to it to become lateral plate. Note that muscle cells nearer the animal hemisphere in the lateral and ventral regions will be brought next to the notochord during gastrulation and therefore the signals from the notochord will not need to travel across a long distance to induce these cells to become muscle. *Anterior-posterior patterning*. Step I, in the pre-MBT embryo, the *Wnt*-like activity (green arrow) together with Signal X (yellow arrow) signals a small dorsal region to become anterior mesoderm (A). This is the same area referred to in dorsal-ventral step Ib as the late blastula organizer. The uniform release of FGF (red arrows) signals the marginal zone to become posterior mesoderm (P). Regions of overlap between the *Wnt*-like activity and FGF might become specified to be mid-axial mesoderm (M). Step II, in the post-MBT embryo, a signal (blue arrows), possibly activin B, released from the late-blastula organizer region could instruct neighboring regions to adopt a mid-axial fate.

facts from our speculation, we have labeled the latter as "propositions". The facts and speculations have been incorporated into a model which combines Nieuwkoop's vegetal signaling model (Nieuwkoop, 1973), Slack and coworkers' organizer-dependent signaling (Smith and Slack, 1983; Slack et al., 1984; Dale et al., 1985; Smith et al., 1985), and Gerhart and colleagues concept of sequential organizing centers operating throughout early development (Gerhart et al., 1991). The propositions and the model are offered in the hope of stimulating future experimental and theoretical challenges, much as the three-signal model has stimulated research to this point.

Experimental evidence and speculation

Proposition: at fertilization, a Wnt*-like activity is locally activated in the dorsal vegetal region with maximum activity at the dorsal midline*

In addition to demonstrating that the vegetal hemisphere can induce animal hemisphere cells to become mesoderm, various investigators have shown that the dorsal-ventral axis is first established in the vegetal hemisphere and that this information can subsequently be transferred to the animal hemisphere. In a classic experiment, Nieuwkoop and colleagues combined non-equatorial portions of the animal and vegetal hemispheres at the 2000 cell stage (Nieuwkoop, 1969a; Nieuwkoop, 1969b; Boterenbrood and Nieuwkoop, 1973). Dorsal vegetal fragments induced animal hemisphere cells to differentiate as an extreme dorsal mesodermal tissue such as notochord, but did not induce the cells to form an extreme ventral mesodermal tissue such as blood. Conversely, ventral vegetal fragments induced blood cells but not notochord in the animal cap. Thus, vegetal cells can not only induce animal hemisphere cells to differentiate as mesoderm but they can also impart dorsal-ventral polarity on the mesoderm.

Further evidence that cleavage stage embryos contain a dorsal- and anterior-inducing capacity which can override the ventral state has been provided by several groups. When single dorsal blastomeres are transplanted into the ventral region of a host embryo they induce the formation of secondary dorsal axial mesodermal structures (Kageura and Yamana, 1984, 1986; Gallagher et al., 1991). This activity is broadly localized between the animal and vegetal poles at the 32-64 cell stage with its maximum at the equatorial and vegetal regions (Gimlich, 1986; Takasaki and Konishi, 1989; Kageura, 1990; Gallagher et al., 1991). By the mid-blastula stage, the dorsalizing activity becomes restricted to the dorsal equatorial region (Gimlich, 1986).

Gerhart and colleagues have proposed that the early dorsalizing activity is activated in a broad region on the dorsal side of the embryo by the sperm-mediated early rotation of the egg (Gerhart et al., 1991). This region, which they have named the "Nieuwkoop center", is proposed to emit signals prior to the MBT that induce a new center of dorsalizing signals within the dorsal equatorial cells, which they have termed the "late blastula organizer" (we have used the nomenclature of Gerhart et al. (1991) throughout this essay rather than the term "Spemann organizer", since, as

Table 1. *Partial list of the peptide factors and receptors identified in the early embryo*

Identity	Initial expression	Form	Localization
Factors			
aFGF	maternal	protein[1]	equatorial, vegetal
bFGF	maternal	mRNA, protein	equatorial, vegetal
XeFGF	maternal	mRNA	uniform
Activin A	stage 13	mRNA	?
Activin B	MBT	mRNA	?
Activin activity	maternal	protein	?
Xwnt-1	stage 16	mRNA	neural ectoderm
Xwnt-8	MBT	mRNA	ventral and lateral mesoderm
Vg1	maternal	mRNA, protein	vegetal
Noggin	maternal	mRNA	vegetal
BMP-4	maternal	mRNA	uniform
Receptors			
FGF receptor	maternal	protein, mRNA	uniform
Activin receptor	maternal	mRNA	uniform

[1]aFGF has only been detected by immunohistochemistry.

suggested by these investigators, there may be several organizing centers located in the region that Spemann had originally defined). Although it is perhaps easier to think of the Nieuwkoop center and the late blastula organizer as being spatially separated entities, they may instead be located in the same region of the embryo and represent temporally distinct inductive events rather than physically separated regions.

A maternal *Wnt*-like factor may be the signal which is released from the Nieuwkoop center by the rotation of the egg. Injection of RNA encoding *Wnt*-1 or *Xwnt*-8 into one ventral vegetal blastomere of an 8-to-16-cell embryo results in the production of a dorsal organizing center at the site of injection and consequently the development of a complete secondary axis (Smith and Harland, 1991; Sokol et al., 1991). Injection of these *Wnt* RNAs has also been shown to rescue dorsal-anterior development in a UV-irradiated embryo (Smith and Harland, 1991; Sokol et al., 1991; since *Wnt*-1 and *Xwnt*-8 produce the same effects we will not further distinguish between them). The *Wnt* protein appears to act intercellularly since tier 4 cells, which do not populate the mesoderm of the marginal zone, can rescue the dorsal-ventral and anterior-posterior axes of a UV-irradiated embryo when injected with *Wnt* RNA (Smith and Harland, 1991). These results, together with those mentioned above, suggest that the cortical rotation of the egg at fertilization activates a maternal *Wnt*-like signal within a large region of the egg, with the maximal activity centered in the dorsal vegetal region. The mechanism by which the maternal factor is activated is unknown but could include translational initiation of a maternal RNA, post-translational modification of an inactive protein, or secretion of a stored protein. In any event, the *Wnt*-like signal is suggested to be transmitted between several cells, with dorsal equatorial and dorsal vegetal cells receiving the strongest signal. Since the dorsalizing activity seems to be present in a large region of the embryo (Gimlich, 1986; Kageura, 1990), we expect that a large proportion of the cells in the animal hemisphere are influenced by the *Wnt*-like signal.

The Wnt-*like activity changes the responsiveness of animal hemisphere cells to mesoderm inducing agents*

The observation that ectopically expressed *Wnt* can restore the ability to form dorsal mesoderm to UV-ventralized embryos, but cannot itself induce mesodermal differentiation when expressed prior to the MBT (Christian et al., 1992), suggests that *Wnt* may be acting synergistically with mesoderm inducing agents. To test this hypothesis, animal caps isolated from embryos injected with *Xwnt*-8 RNA at the two-cell stage, and from uninjected embryos, were cultured in the presence of XbFGF. While explants from normal embryos form mainly ventral mesoderm in response to XbFGF (Green et al., 1990), animal caps isolated from *Xwnt*-8-injected embryos produce primarily dorsal mesodermal cell types in response to an identical concentration of XbFGF (Christian et al., 1992). Thus the *Wnt* protein, which by itself does not induce mesodermal differentiation, alters the response of the animal cap cells to the added bFGF, increasing the dorsal character of the mesoderm.

The effects of ectopically expressed *Wnt* in whole embryos and in animal caps are very reminiscent of those produced by treatment with lithium ion. As with injection of *Wnt* RNA, injection of lithium into single blastomeres of UV-treated embryos rescues dorsal-anterior development (Kao et al., 1986; Kao and Elinson, 1988, 1989). Lithium by itself can not induce mesoderm, but instead it enhances the induction of dorsal mesoderm when FGF is added to animal caps (Slack et al., 1988), suggesting that it, like *Wnt*, alters the response of cells to mesoderm inducing agents. Finally, both lithium (Nagajski et al., 1989) and *Wnt* (Olson et al., 1991) enhance gap-junctional permeability between ventral blastomeres of the early cleavage stage embryo, suggesting that these two agents may share similar pathways of action.

Proposition: mesoderm inducing agents are localized along the animal-vegetal axis but not along the dorsal-ventral axis

Since *Wnt* is incapable of inducing axial structures without the participation of mesoderm inducing agents, the differential responsiveness of cells within the 32-cell embryo to an ectopically introduced *Wnt* signal may provide some clues as to the normal distribution of endogenous mesoderm inducing factors. Injection of *Xwnt*-8 RNA into the top tier of a 32-cell stage embryo does not result in the formation of ectopic dorsal axial structures, whereas injection of this RNA into equatorial or vegetal tier cells produces a dorsal organizing center at the site of *Wnt* RNA injection (Smith and Harland, 1991; Sokol et al., 1991; Christian et al., 1992). Therefore the factor(s) cooperating with the *Wnt*-like activity to form an organizer must be present throughout the equatorial zone but are not at sufficient levels in the top of the animal hemisphere to be involved in organizer formation.

These inducing factors may not be precisely localized to the marginal zone, but may instead be present in a vegetal to animal gradient. For example, when small caps are isolated from the extreme animal pole of *Wnt* RNA-injected embryos, ectopic mesoderm is not formed (Christian et al., 1992). Larger caps from *Wnt* RNA-injected embryos, which include cells nearer the equator, differentiate autonomously into explants containing various mesodermal cell types (Sokol et al., 1991; Christian et al., 1992). In contrast, the same size caps from uninjected embryos form only atypical epidermis under identical culture conditions. These results suggest that endogenous mesoderm inducing signals are not sharply delimited along the animal-vegetal axis, but may instead be received by the animal hemisphere cells in a graded fashion with the lowest levels near the animal pole.

Proposition: competence prepatterning of animal hemisphere cells depends on the Wnt-*like activity*

Although the normal animal cap is frequently treated as a homogeneous group of cells, different regions of the animal hemisphere respond differentially to mesoderm-inducing signals. In conjugates of animal and vegetal hemisphere pieces, notochord is induced when the animal cap piece comes from the most dorsal side of the embryo but not if it is isolated from the ventral side (Sutasurya and Nieuwkoop, 1974). The bias of the dorsal animal hemisphere to produce dorsal mesoderm is also observed with

purified mesoderm-inducing agents; treatment of dorsal or ventral animal cap halves with bFGF or activin results in a stronger dorsal-type response in the dorsal half explants (Ruiz i Altaba and Jessell, 1991; Sokol and Melton, 1991; Kimelman and Maas, 1992). As the dorsal-type response can be eliminated by UV-treatment (Sokol and Melton, 1991; Kimelman and Maas, 1992), which eliminates the sperm-activated cortical rotation and consequently the formation of the Nieuwkoop organizing center (Grant and Wacaster, 1972; Malacinski et al., 1977; Scharf and Gerhart, 1980, 1983; Vincent and Gerhart, 1987), we suggest that the endogenous dorsal-type response of dorsal animal hemisphere cells is mediated by the maternal *Wnt*-like activity released from the dorsal vegetal and equatorial regions. In fact, the dorsal-type response can be mimicked in ventral half caps by ectopically introducing *Wnt* RNA into ectodermal cells (Christian et al., 1992). We will henceforth use the term "competence prepattern" to indicate that the embryo has an inherent dorsal-ventral bias in its response to mesoderm-inducing factors and suggest that the role of the *Wnt*-like activity is to regulate the degree of competence within each of the animal hemisphere cells.

Implicit in the concept of competence prepatterning of the animal hemisphere cells is the requirement that not all animal cap cells are equivalent. Previous studies have assumed that the animal hemisphere is a uniform field of cells, leading to conclusions that may be erroneous. For example, ventral vegetal cells combined with animal cap explants can induce some dorsal-type mesoderm even though they would normally not induce these types of cells in an intact embryo (Boterenbrood and Nieuwkoop, 1973; Dale and Slack, 1987b; Pierce and Brothers, 1988). This paradox may be due to the experimental juxtaposition of ventral vegetal cells with dorsal animal cap cells. In this experimental context, the ventral vegetal cells are able to induce some dorsal mesoderm because a portion of the animal cap cells had received the dorsal prepatterning signal. Therefore, to examine a uniform population of animal cap cells it will be necessary to use animal cap cells from a UV-irradiated embryo that has received no prepatterning information, or to use animal cap cells from a lithium-treated or *Wnt*-injected embryo which have the equivalent of a complete dorsal prepattern.

Proposition: the late-blastula organizer is created by the Wnt-*like activity and a factor other than bFGF*

Two lines of evidence argue that the *Wnt*-like activity needs another factor besides bFGF to create the late-blastula organizer. First, when RNA encoding a dominant negative mutant form of the FGF receptor is injected into the fertilized egg, the resulting tadpoles are deficient in posterior structures, but still form heads, eyes and brains, indicating the presence of a functional late blastula organizer (Amaya et al., 1991). Furthermore, in these embryos gastrulation begins on the dorsal side at the time that a normal dorsal lip organizer would begin involution. Second, when animal caps isolated from *Wnt* RNA-injected embryos are cultured in the presence of bFGF they differentiate into explants containing notochord, muscle and other mesodermal cell types, but lacking anterior neural structures such as eyes (Christian et al., 1992). These two results suggest that another factor (signal X) is likely to be involved in inducing the late blastula organizer.

Unlike bFGF, signal X may not be able to induce all mesodermal cell types since ventral mesoderm formation in embryos injected with RNA encoding the dominant-negative FGF receptor is eliminated while dorsal mesoderm formation is only partially decreased (Amaya et al., 1991). Signal X might be a mesoderm inducer present at a sufficiently low level such that it is able to induce mesoderm only in the presence of high levels of the *Wnt*-like activity. Signal X cannot be localized solely in the dorsal region, since injection of *Wnt* RNA into the ventral side of a normal embryo produces a new organizer on the ventral side (Smith and Harland, 1991; Sokol et al., 1991; Christian et al., 1992). An activin-like activity has been found in the egg and might serve as this additional factor (Asashima et al., 1991).

Proposition: the late blastula organizer induces notochord at stages 8-9

The upper region of the dorsal marginal zone is not specified to become notochord during the earliest cleavage stages but acquires this ability between stages 6 and 9 (Kaneda and Hama, 1979). Recombination experiments in *Xenopus* between normal stage-9 embryo halves cut between the upper and lower dorsal marginal zone and matching halves from UV-irradiated ventralized embryos demonstrated that the lower region (which includes the late blastula organizer) induces the upper region to become notochord (Gerhart et al., 1991). Similarly, prospective ectoderm is induced to differentiate as notochord when it is transplanted directly above the late blastula organizer (Kaneda, 1981). We suggest that this signal could be activin B, which is first transcribed at the MBT and is able to induce notochord in dorsal animal hemisphere explants (Sokol and Melton, 1991).

The gastrula organizer dorsalizes lateral mesoderm after stage 10

The experiments of Mangold and Spemann demonstrated that a transplanted piece of dorsal marginal zone, the gastrula organizer, can induce ventral mesodermal cells to adopt a more dorsal fate (Spemann and Mangold, 1924). In these experiments, the donor piece of tissue formed the notochord whereas the host tissue was induced to form the other mesodermal lineages. The lack of notochord induction by the donor piece was demonstrated more precisely using lineage labeled transplants (Smith et al., 1985) and combinations of dorsal and ventral marginal zone pieces at stage 10 (Slack and Forman, 1980; Dale and Slack, 1987b). Thus, while marginal zone cells can be induced to differentiate as notochord up to the late blastula stage (Kaneda, 1981), this capacity appears to be lost by the early gastrula stage. These results are similar to those obtained in the animal cap assay with the addition of exogenous activin. Specifically, while the mesoderm inducing potency of activin is not lost until stage 11.5, the ability of activin to induce the formation of dorsal mesoderm gradually declines between stages 8 and 11 (Green et al., 1990). In the embryo, cells receiving a high level of activin may differentiate as notochord before stage 10, but by stage 10 the same level

of activin may only induce the formation of muscle. By stage 11, the same activin dose may only elicit the formation of lateral and ventral mesoderm. We speculate that the gastrula organizer, which is located in the cells that will become notochord, may become a source of activin after stage 10, since this region has been shown to be able to dorsalize mesoderm (Yamada, 1938, 1950; Slack and Forman, 1980). As the notochordal region invaginates and extends during gastrulation it is brought into the proximity of the lateral and ventral mesoderm (Keller and Tibbetts, 1989; Wilson et al., 1989). By releasing activin it could convert mesodermal cells from the lateral and ventral regions to form more dorsal-type mesoderm such as muscle. In addition, cells that are specified as muscle could also release activin, at later times or in lower doses, converting their neighbors to a lateral mesodermal fate.

An FGF determines posterior mesodermal fate

Based on experiments with animal caps and homeobox genes as markers of anterior-posterior position, it has been proposed that bFGF determines posterior mesodermal fate and activin determines anterior mesodermal fate (Ruiz i Altaba and Melton, 1989; Cho and De Robertis, 1990). bFGF, for example, induces the expression of the relatively posterior genes, *Xhox*3 and *Xlhbox*6, to higher levels than does activin (Ruiz i Altaba and Melton, 1989; Cho and De Robertis, 1990). In contrast, activin induces the expression of *Xlhbox*1, which is expressed near the hindbrain region of the embryo, to higher levels than does bFGF (Cho and De Robertis, 1990). Further support for the view that bFGF is a posterior inducer comes from experiments with a dominant negative mutant of the FGF receptor. These embryos lack posterior and trunk regions but have a well-formed head (Amaya et al., 1991).

Proposition: the Wnt-*like activity and signal X establish the late blastula organizer to specify anterior fate*

Transplantation experiments have demonstrated that the early dorsal blastopore lip, which prior to gastrulation is the site of the late blastula organizer, will eventually form head mesoderm and will induce formation of anterior neural structures (Spemann and Mangold, 1924; Smith et al., 1985). Since injection of *Wnt* RNA into ventral cells of cleavage stage embryos can induce the formation of head mesoderm (Smith and Harland, 1991; Sokol et al., 1991), the *Wnt*-like activity must be involved in specifying the late blastula organizer to become anterior mesoderm. However, as discussed earlier, mesoderm induction cannot be accomplished by the *Wnt*-like activity alone but it appears to be dependent on another factor. An FGF family member does not appear to be the additional factor since animal caps from *Wnt* RNA-injected embryos do not form anterior structures after the addition of bFGF (Christian et al., 1992).

A better candidate for the second signal is activin, which previously was suggested to be the sole inducer of anterior mesoderm (Ruiz i Altaba and Melton, 1989; Cho and De Robertis, 1990). Two lines of evidence argue against the idea that activin alone can induce anterior mesoderm. Injection of activin RNA into the ventral side of an embryo produces a type of second axis containing muscle and occasionally notochord, but lacking any anterior structures (Thomsen et al., 1990; Sokol et al., 1991). This second axis may not have an anterior-posterior polarity, but may instead be simply formed from the convergence and extension of dorsal mesodermal cells. Secondly, addition of activin to the ventral half of an animal cap does not lead to the formation of anterior structures (Sokol and Melton, 1991). However, addition of activin to the dorsal halves of large animal caps, which have received the *Wnt*-like signal from the Nieuwkoop center, leads to the formation of anterior structures (Sokol et al., 1990; Sokol and Melton, 1991). Thus, activin may be the signal that in concert with the *Wnt*-like signal, establishes the late blastula organizer to become anterior mesoderm.

Proposition: anteriorizing signals are dominant over posteriorizing signals

Since we expect that FGF is released uniformly around the equator of the embryo it seems likely that the dorsal organizer region signals neighboring cells to override the FGF-mediated posterior specification of the mesoderm. Uniform treatment of cleavage stage embryos with increasing amounts of lithium converts increasing amounts of the marginal zone into organizer tissue (Kao and Elinson, 1989). The resulting embryos gain anterior structures at the expense of posterior tissue; in the most extreme case, the entire marginal zone becomes a dorsal organizer resulting in an embryo that is essentially a large head. These results demonstrate that anteriorizing signals, primarily released from the late blastula organizer, are dominant over posterior signals.

A synergistic model for mesoderm induction

We have incorporated the above facts and propositions into a model for induction and patterning of the mesoderm that derives from proposals of Nieuwkoop (patterning from the vegetal region; Nieuwkoop, 1973), Slack and coworkers (patterning primarily from the organizer; Dale and Slack, 1987b) and Gerhart and colleagues (a series of organizers moving from the vegetal toward the animal pole; Gerhart et al., 1991) with the newly acquired molecular data. Our model differs from the previous three-signal model in that we view the prepatterning of the animal cap by a *Wnt*-like activity, as one of the major determinants of the early embryonic pattern. We do not think that there is a specific organizer inducing factor and a vegetal inducing factor, but that two different factors work synergistically with the *Wnt*-like factor to induce and pattern the early mesoderm along both the dorsal-ventral and anterior-posterior axes. We also propose that the organizer signal partially overlaps some of the earlier signaling events so that cells on the dorsal side may receive redundant information. For the sake of simplicity, dorsal-ventral and anterior-posterior patterning are discussed separately, although the two processes might occur simultaneously using the same molecules.

Dorsal-ventral patterning

Four steps are envisaged in this process, with the first two occurring pre-MBT and the second two post-MBT. In the

first step, a *Wnt*-like activity is activated in a broad dorsal region of the embryo by the sperm-mediated cortical rotation. The maximum *Wnt*-like activity is likely to be centered on the future dorsal midline, near the equator, with a graded signal spread across a large region of the embryo (Fig. 3, dorsal-ventral, step Ia). We propose that this is the competence prepatterning signal which can also be mimicked by the addition of lithium.

We also propose that, during the early cleavages two signals are released circumferentially in the marginal zone. Both signals are shown here to emanate from the vegetal region, although either one of the signals could be released from within the marginal zone (Fig. 3, dorsal-ventral, step Ia). We suggest that signal X, which may be an activin-like activity, together with the maximum *Wnt*-like signal induces the formation of the late-blastula organizer in the lower part of the marginal zone with its center on the dorsal midline. The other signal in this model is a member of the FGF family, which is proposed to be uniform throughout the equator, but decreasing in strength toward the animal pole. The cumulative result of these three signals is the specification of a region of mesoderm at the equator which exhibits a rudimentary dorsal-ventral pattern (Fig. 3, dorsal-ventral, step Ib).

At the midblastula transition, activin B is first transcribed. We suggest that it may be the signal which is released by the late blastula organizer (Fig. 3, dorsal-ventral, step II) and which induces the region above the late blastula organizer to eventually become notochord. In the following step, which occurs throughout gastrulation, the notochordal region (the gastrula stage organizer) releases a signal, possibly activin B, which converts adjacent equatorial cells into muscle (Fig. 3, dorsal-ventral, step III). Some of the cells on the ventral side of the embryo, which were initially induced to form ventral mesoderm by FGF, may be converted to muscle as they are brought near the notochordal region during gastrulation. The prospective muscle cells may also be able to release activin B, albeit at a lower level than the notochordal region, converting their ventral neighbors to more lateral mesoderm. In this manner, a graded expression of activin release could be produced resulting in a dorsal-ventral gradient of mesoderm formation.

Anterior-posterior

The same polypeptide factors that produce the dorsal-ventral axis have also been proposed to establish the anterior-posterior gradient prior to gastrulation (Ruiz i Altaba and Melton, 1989; Cho and De Robertis, 1990), although it has recently been argued that the anterior-posterior axis is only established during gastrulation (Slack and Tannahill, 1992). This is in contrast to *Drosophila* where a different system is used for each axis (Nusslein-Vollhard and Roth, 1989). In accordance with the previous studies, we suggest that prior to the MBT, FGF specifies the entire marginal zone to have a posterior fate (Fig. 3, anterior-posterior, step I). The *Wnt*-like activity, together with signal X, specifies anterior fate in the region that will become the late blastula organizer. Lower amounts of the *Wnt*-like activity, together with signal X, might specify mid-axis positions in the pre-MBT embryo. After the MBT, a signal released from the organizer region, possibly activin B, might override the posterior-type specification induced by bFGF which would result in a region determined to be mid-axis (Fig. 3, anterior-posterior, step II).

Overlapping systems of induction

As suggested in the propositions and in the model, it appears that the embryo uses overlapping and partially redundant systems to pattern the early embryo. From the experiments of Mangold and Spemann it has been clear that the organizer can specify most of the mesodermal pattern (Spemann and Mangold, 1924). However, a combination of bFGF and the *Wnt*-like activity can also create almost all of the mesodermal cell types with the probable exception of head mesoderm (Christian et al., 1992; Kimelman and Maas, 1992). Therefore we suggest that prior to the MBT there may be one step of patterning involving a *Wnt*-like activity, an FGF family member, and a yet unidentified factor, which may be an activin. After the MBT, a partially redundant step involving signals such as activin and noggin is suggested to occur. There may be still other factors involved since TGF-β family members Vg1, BMP-4, and an activin-like activity, as well as at least two FGF family members, are present in the unfertilized egg (Rebagliati et al., 1985; Asashima et al., 1991; Koster et al., 1991; Shiurba et al., 1991; Dale et al., 1992). Whether all of these factors have distinct roles in early development remains to be determined.

The concept of overlapping systems may be a major theme in vertebrate development, although this overlap presents an unwelcome complication for experimental biologists. The targeted disruption of several mouse genes has also provided evidence for this type of functional redundancy. Elimination of the *src* gene causes relatively minor embryonic perturbation despite the widespread expression of this gene (Soriano et al., 1991). Deletion in mice of two of the antennapedia-type homeobox genes (Chisaka and Capecchi, 1991; Lufkin et al., 1991), the *Wnt*-1 gene (McMahon and Bradley, 1990; Thomas and Capecchi, 1990) or the *engrailed*-2 gene (Joyner et al., 1991) have shown effects only in a limited part of the region in which these genes are expressed. It seems likely that each factor used in the embryo has at least one role which is indispensable since it is difficult otherwise to rationalize a selection pressure to maintain these genes throughout evolution. However, each factor could also have dispensable functions that overlap synergistically with other embryonic factors. In this view, mesodermal induction and patterning may be thought of as involving at least four factors, each of which have both overlapping and distinct functions. An FGF, for example, may be unique in specifying ventral mesoderm, but may overlap signal X in determining the marginal zone and, together with the *Wnt*-like activity, may overlap activin in specifying mid-axial regions. For this reason, descriptions of the region in which a gene product is expressed may not correlate exactly with the effects caused by eliminating or overexpressing it. With this view in mind, it will be important to use multiple in vivo and in vitro approaches to study the role of developmentally important genes. Only

through such an approach will it be possible to decipher the unique as well as shared contributions of each factor.

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Fig. 1. Fate map of the early *Xenopus* embryo. A schematic fate map of the pregastrula embryo is shown with mesoderm forming at the equator (Vogt, 1929; Keller, 1976). The upper part of the animal hemisphere will form ectoderm and neurectoderm whereas the lower part of the vegetal hemisphere will become endoderm. The mesoderm will be patterned into different cell types along the future dorsal-ventral axis as shown, with, at least in Urodeles, a greater extent of mesoderm forming from the dorsal side of the embryo. The region labeled “head” is the late blastula organizer, whereas the notochord region will become the late-gastrula organizer (Gerhart et al., 1991). The regions comprising different mesodermal tissues are not drawn to scale, and some cell types have not been included in the drawing.

Fig. 2. Three-signal model (Smith and Slack, 1983; Slack et al., 1984; Dale et al., 1985; Smith et al., 1985). Initially, two signals are released from the vegetal hemisphere which has been divided into a ventral vegetal (VV) and a dorsal vegetal region (DV). The ventral vegetal signal converts the equatorial region above it to ventral mesoderm (M3). The signal from the dorsal vegetal region induces the organizer (O) in the most dorsal part of the mesoderm. The organizer then sends out signals which convert the ventral mesoderm into different types of lateral mesoderm (M1 and M2).