Development 124, 4179-4191 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV7602

Patterning the *Xenopus* blastula

Janet Heasman*

Institute of Human Genetics and Department of Cell Biology and Neuroanatomy, University of Minnesota School of Medicine, Box 206 Mayo, 420 Delaware St. SE, Minneapolis, MN 55455, USA

*(e-mail: heasman@lenti.med.umn.edu)

SUMMARY

This review starts from the classical standpoint that there are at least two separable processes acting with respect to axis formation and tissue specification in the early *Xenopus* embryo: a UV-insensitive event establishing a postgastrula embryo consisting of three concentric germ layers, ectoderm, mesoderm and endoderm, all of a ventral character; and a UV-sensitive event producing tissue of a dorsal type, including somites, notochord and neural tissue, and concomitantly establishing the dorsoventral and anteroposterior axes. The experimental evidence suggesting the molecular basis of the dorsal and ventral pathways is reviewed.

Key words: amphibian, blastula, pattern formation, gastrulation, *Xenopus*

(1) INTRODUCTION

Amphibian embryos have long been favoured organisms for the study of cell interactions in early morphogenesis, particularly with respect to the questions of axis formation and mesodermal tissue specification. Reviews of this subject have been numerous (Smith, 1989; Kimelman et al., 1992; Dawid, 1994; Sive, 1993; Kessler and Melton, 1994; Lemaire and Kodjabjchian, 1996), but recent studies have begun to suggest a shift from the text-book models of how this patterning is achieved and, therefore, require a fresh reappraisal of the data.

(2) THE THREE-SIGNAL MODEL

The pioneering work of Ogi (1967, 1969), and Nieuwkoop and colleagues (Nieuwkoop, 1969; Boterenbrood and Nieuwkoop, 1973), and more recently by Slack and Smith on *Xenopus* embryos (Smith and Slack, 1983; Slack et al., 1984; Smith et al., 1985; Dale and Slack, 1987), led to the three-signal model of mesoderm induction (Fig. 1). According to this model, the active signalling center causing mesoderm induction is the vegetal mass of the blastula-stage embryo. The evidence for this is that vegetal explants co-cultured with animal caps are able to divert animal cells from an epidermal to a mesodermal fate. In the model, the vegetal mass is further divided into dorsal and ventral halves, with the former inducing mesoderm of a dorsal character, including notochord and somites, while the latter induces ventral mesoderm, mesenchyme, lateral plate mesoderm and blood islands. These signals are considered to occur early in development, before the onset of zygotic transcription at the mid-blastula transition (MBT). The third signal in the model is viewed as a later event, passing horizontally from the dorsal equatorial region (known as the Spemann organizer region), in recognition of the finding that, when this region is transplanted to the ventral side of an early gastrula embryo, it is able to induce an ectopic axis (Spemann and Mangold, 1924). This signal expands the size of the area of dorsal mesodermal tissue.

Over the past decade, substantial effort has been made in many laboratories to characterize the molecules involved in mesoderm induction and axial patterning, and to extend the early observations on which the three-signal model was based. However, mesoderm-inducing molecules constituting the first two signals, with the characteristics suggested by this model, remain unidentified. Several modifications have been made to the original model (Kimelman et al., 1992; Sive, 1993; Watabe et al., 1995; Sakai, 1996). While we are still far from a complete understanding of the molecular mechanisms patterning the early *Xenopus* embryo, significant advances have been made. This review aims to summarize these advances. In doing so, the simplicity of the old model is lost, but what emerges is the framework of molecular and cellular interactions that pattern the early embryo.

(3) THE DORSAL PATHWAY

(a) UV-irradiation experiments

Classical UV irradiation experiments have indicated that there are at least two aspects to the patterning of early amphibian embryos (Fig. 2): a UV-insensitive event establishing a postgastrula stage, consisting of three concentric germ layers, ectoderm, mesoderm and endoderm, all of a ventral pattern; and a UV-sensitive event producing tissue of a dorsal type, including somites, notochord and neural tissue, and concomitantly establishing the dorsoventral and anteroposterior axes (Grant and Wacaster, 1972; Malacinski et al., 1974). UV irradiation is effective at blocking this dorsal pathway when it is applied to the vegetal pole at two different times: either to fer-

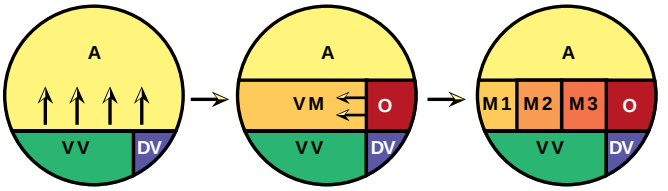

Fig. 1. The three-signal model of mesoderm induction as depicted by Smith (1989). Two mesoderm-inducing signals are considered to pass from the vegetal region of the early blastula. The dorsal signal induces dorsal mesoderm (the Spemann organizer), while the ventral one induces ventral mesoderm. The organizer produces a third signal probably during gastrulation that induces addititonal muscle (M3). Only the most ventral cells (M1) form blood-forming mesoderm.

tilized eggs shortly after sperm entry, or to the vegetal pole of full-grown ovarian oocytes (Holwill et al., 1987; Elinson and Pasceri, 1989). This latter finding suggests that a component/s of the dorsal pathway, or a factor indirectly influencing that pathway, must be established during oogenesis and localized to the cortex of the vegetal pole before maturation. Although there has been no molecular characterization of this oocyte dorsalizing activity, its vegetal localization has been confirmed by cytoplasmic transfer experiments (Holowacz and Elinson, 1995).

The second, postfertilization, UV-sensitive period has been intensively studied (Vincent et al., 1986, 1987; Yuge et al., 1990; Fujisue et al., 1993; Sakai, 1996). Several lines of evidence indicate that, at this time, the UV target is cytoskeletal; UV disrupts cortical cytoplasmic movements by disorganizing microtubules and prevents the normal displacement of a dorsalizing activity to the dorsal side of the embryo (Gerhart et al., 1989; Houliston, 1994). The nature of this dorsalizing activity or its relationship to the oocyte factor/s is not understood, although they are clearly separable, as UV-irradiation of the oocyte does not disrupt cortical rotation after fertilization (Elinson and Pasceri, 1989). One approach to this question has been to identify molecules that are expressed in early embryos and that are able to rescue the UV-ventralized phenotype. A surprisingly large number of molecules fit this description (Table 1), and current research is directed towards understanding their relative importance and their relationship to each other in the endogenous dorsal pathway. Most of these molecules (* in Table 1) are absent or at very low abundance as maternal mRNAs, and are only strongly expressed zygotically, after MBT. It seems likely therefore that these molecules are downstream of UV-sensitive maternal events.

Table 1. Molecules that rescue the UV phenotype

Complete axis	Incomplete axis	Reference
*Xwnt-8		Smith and Harland, 1991
*noggin		Smith and Harland, 1992
Vg1(only modified form)		Thomsen and Melton, 1993
Xwnt-8b		Cui et al., 1995
*Xnr1 & 2		Jones et al., 1995
*Siamois		Lemaire et al., 1995
*chordin		Sasai et al., 1994
	*gsc	Cho et al., 1991
	activin	Smith and Harland, 1991
	*Xbra	Smith et al., 1991
	Xdsh	Sokol et al., 1995
	Xwnt11	Ku and Melton, 1993
	dom. neg. BMP receptor	Graff et al., 1994
	*Xnr4	Joseph and Melton, 1997
	*Xnr3	Smith et al., 1995

One standard method towards this end has been to overexpress the candidate proteins shown in Table 1 by injecting mRNAs or dominant negative forms of those mRNAs into early cleavage-stage embryos. This has suggested roles in dorsal axis formation for several molecules, including activin, goosecoid (gsc), Siamois, Xwnt-8 and Vg1. However, *Xenopus* embryos consist of labile, pluripotent cells until the mid-blastula stage (Heasman et al., 1984; Wylie et al., 1987; Snape et al., 1987) and overexpression or ectopic expression of signalling components or receptors may reveal pathways that would not normally function in undisturbed embryos. Problems in interpretation of the results may also arise if the injected dorsalizing molecules can mimic, but are not necessarily the same as, endogenous molecules in these pluripotent cells, or in the case of dominant negative constructs, act promiscuously to block signalling by related molecules(Kessler and Melton, 1994; Schulte-Merker et al., 1994). A further complication is that the overexpressed protein may have activity at any time from the cleavage to the neurula stage, which may not reflect the timing of activity of endogenous proteins. Thus this approach has not resolved the question of which of the candidate molecules are active in the embryo, or when they take part in dorsal axis formation.

(b) β-catenin

An alternative method to identify the endogenous role of maternal gene-products is to deplete the protein or mRNA from the oocyte and study the effect of that depletion on development. This method uses antisense oligodeoxynucleotides to target-specific mRNAs, which are then cleaved by endogenous RNase H (Dash et al., 1987; Dagle et al., 1991; Heasman et al., 1994a,b). It is not used to test the function of zygotic gene products, as any degraded mRNAs would be replaced by new transcription after MBT. Recently, a combination of overexpression and depletion experiments have identified some of the essential ingredients of the dorsal, UV-sensitive pathway. Specifically, dorsal axis formation depends upon cytoplasmic components homologous to those of a signalling pathway in

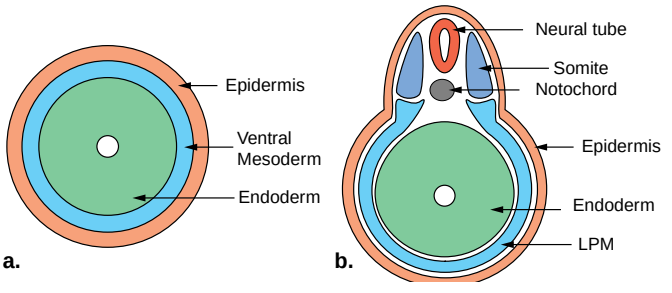

Fig. 2. Two events pattern the early *Xenopus* embryo. UV irradiation experiments reveal that at least two events pattern the embryo: (a) a UV-insensitive event establishes a postgastrula-stage embryo consisting of three concentric germ layers – ectoderm (epidermis), ventral mesoderm and ventral endoderm and (b) a UV-sensitive event produces tissue of a dorsal type, including neural tissue, somites, notochord and dorsal endoderm, and also produces dorsoventral and anterior/posterior axes in the embryo. LPM, lateral plate mesoderm.

Drosophila initiated by the Wingless secreted protein (Fig. 3). This *wingless*-initiated pathway has been shown to be of critical importance in the patterning of the *Drosophila* embryonic epidermis (DiNardo et al., 1988; Martinez-Arias et al., 1988; Bejsovec and Martinez-Arias, 1991), wing (Morata and Lawrence, 1977; Phillips and Whittle, 1993), leg (Diaz-Benjumea and Cohen, 1994) and midgut (Yu et al., 1996). Several *Xenopus* members of the Wnt family (*Xwnt* genes) are able to rescue the ventralized phenotype of UV-irradiated *Xenopus* embryos (see Table 1).

β-catenin is one of these essential ingredients. It was first identified as a cell membrane-associated protein in vertebrate cells, necessary for cadherin-mediated adhesion (Ozawa et al., 1989), and was later shown to be a vertebrate homolog of the *Drosophila* protein, Armadillo. Although *armadillo* was initially identified in a screen for segment polarity mutants, and is a component of the Wingless protein-initiated segment polarity signalling pathway (Peifer et al., 1992, 1994), several lines of evidence have shown that it has roles both in signalling and adhesion. In *Drosophila* ovary and in the early embryo, *armadillo* mutants have defects in cell-cell adhesion as well as signalling (Peifer et al., 1993; Cox et al., 1996). *Xenopus* embryos depleted of maternal β-catenin have a signalling defect: they develop without dorsal structures, including somites, notochord and neural tubes, and resemble the most severe cases of axis-deficient UV-irradiated embryos (Heasman et al., 1994b). In these experiments, cell-cell adhesion is not altered, but β-catenin is depleted only from the supernatant fraction, leaving membrane-associated β-catenin presumably available for an adhesive role (Kofron et al., 1997). Cytoplasmic β-catenin and Armadillo are thus essential for signalling, and the membrane-associated forms for adhesion. The distribution of β-catenin can be altered simply by overexpression of cadherin (Heasman et al., 1994b), which sequesters β-catenin at the cell membrane and depletes the cytosolic pool (Fagotto et al., 1996). However, it seems unlikely that endogenous cadherins regulate the cytosolic pool, as *Xenopus* embryos that have been depleted of maternal EP cadherin, and are disaggregated at the blastula stage, go on to develop with normal axes and dorsal structures when zygotic EP cadherin begins to accumulate (Heasman et al., 1994a). Recent studies suggest that cytoplasmic and membrane-associated Armadillo may be regulated separately by different kinases (Pai et al., 1997). The dorsal deficiencies of β-catenin-depleted embryos can be rescued by the injection of β-catenin mRNA, but not by Xwnt-8, even though Xwnt-8 has strong dorsalizing activity in wild-type embryos (Heasman et al., 1994b). This indicates that, as in *Drosophila*, β-catenin may lie downstream of a Wnt signal.

(c) Molecules upstream of β-catenin

The serine-threonine kinase zeste-white 3 *zw3*; (Peifer et al., 1994) acts as an inhibitor of the Wingless pathway in *Drosophila*, by altering the stability of soluble Armadillo (Pai et al., 1997). It also has a *Xenopus* homolog (glycogen synthase kinase, GSK3) that inhibits the dorsal pathway. A dominant negative form of GSK3 mRNA causes ectopic axis formation in *Xenopus* embryos, but is unable to rescue β-catenin-deficient embryos (Wylie et al., 1996), placing GSK3 in a similar upstream position to β-catenin, as its counterpart zw3 is to Armadillo in *Drosophila* (He et al., 1995; Pierce and Kimelman, 1996; Dominguez et al., 1995).

Unlike *Drosophila*, the components of the signalling pathway upstream of GSK3 and β-catenin have not been established in *Xenopus*. The *Xenopus* homolog of *dishevelled*, *Xdsh*, has the ability to induce complete ectopic axes (Sokol et al., 1995). A dominant negative form of this protein is active in blocking the signalling pathway stimulated by ectopically injected Xwnt-8 mRNA. Surprisingly, overexpression of this dominant negative protein alone does not prevent dorsal axis formation, suggesting that Xdsh is not an essential component of the endogenous dorsal pathway, although it may be important for convergence extension movements at the gastrula stage (Sokol, 1996).

β-catenin has many potential interactions. As well as its

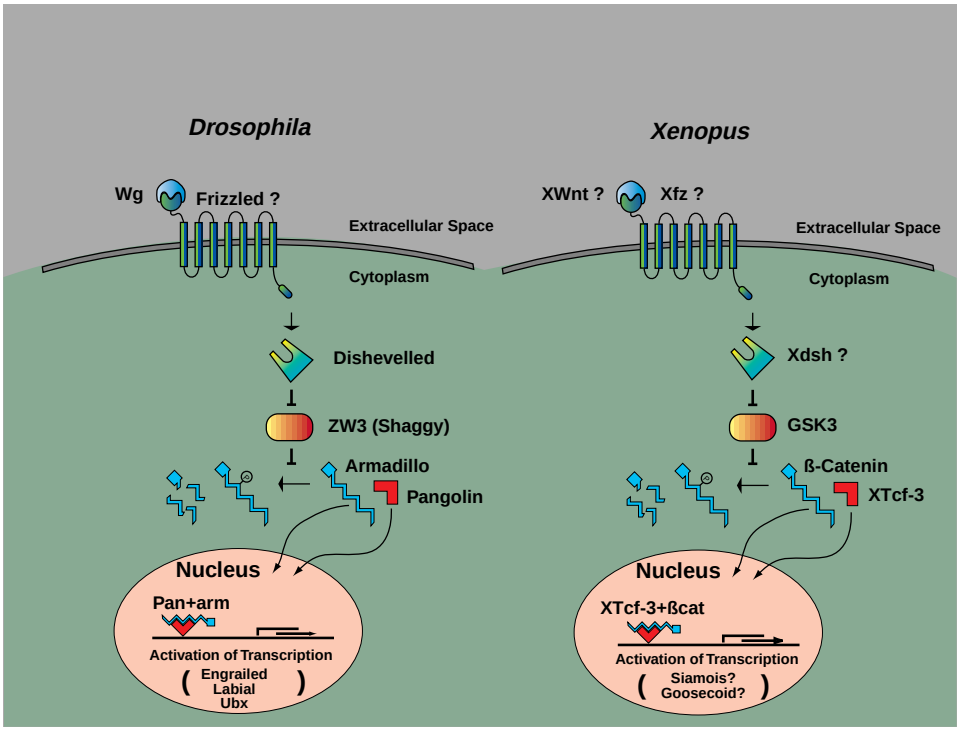

Fig. 3. A comparison of the *Drosophila* and *Xenopus* Wnt signal transduction pathways (adapted from Sokol, 1996). Wnt signalling promotes the inactivation of zw3/GSK3, which leads to the accumulation of Armadillo/β-catenin. Armadillo/β-catenin may then interact with HMG-box transcription factors which regulate the expression of specific genes. See text for details. ? indicates uncertain components. Although pangolin is depicted here in the nucleus and cytoplasm, its positioning remains hypothetical at this time.

association with cadherin and α-catenin, it has been shown to bind to EGF receptor (Hoschuetzky et al., 1994), c-erbB-2 (Kanai et al., 1995), fascin (Tao et al., 1996) and the cytoplasmic protein adenomatous polyposis coli (APC) protein. APC is expressed in early *Xenopus* embryos (Vleminckx et al., 1997), where it may be involved in regulating the levels of β-catenin, as it does in cultured cells (Munemitsu et al., 1994; Papkoff et al., 1996; Hayashi et al., 1997). APC is a substrate for GSK3, and its phosphorylation regulates binding of APC to β-catenin (Rubinfeld et al., 1996). In *Drosophila*, however, zygotic APC is not essential for Armadillo function (Hayashi et al., 1997) and overexpression of APC in *Xenopus* embryos causes dorsalization, rather than acting in a negatively regulatory fashion (Vleminckx et al., 1997). A second role for APC could be in associating β-catenin with the microtubule cytoskeleton during cortical rotation in the first cell cycle, as it is known to associate with microtubules in other cell types (Munemitsu et al., 1994; Smith et al., 1994).

There is also no evidence yet that defines which Xwnt, if any, initiates the pathway in *Xenopus*. At least two Xwnt mRNAs are present in the oocyte and early embryo and have dorsalizing activity when injected into embryos (Xwnt 11: Ku and Melton, 1993; Xwnt-8b: Cui et al., 1995). But the roles of the endogenous molecules have yet to be defined. Recent studies have indicated that homologs of the *Drosophila frizzled* gene, the putative Xwnt receptor, can interact with Xwnt family members (Yang-Snyder et al., 1996; He et al., 1997). Functional studies of these *frizzled* family members may help answer the question of whether dorsal signalling in *Xenopus* involves a signal transduction cascade homologous to the complete *wingless* signalling pathway or not.

Thus the steps upstream of β-catenin and GSK3 are not understood, and there is no clear link yet between them and the dorsalizing cytoplasmic localizations in the oocyte and 1- to 16-cell-stage embryos. Indeed, we do not know if UV treatment of the oocyte or embryo damages the Xwnt/β-catenin pathway directly or indirectly. Two lines of evidence point to the UV effects being indirect. Firstly, UV irradiation of the vegetal poles of fertilized eggs causes localization of Siamois, a transcription factor downstream of β-catenin, in the vegetal rather than the equatorial region of the embryo (Brannon and Kimelman, 1996). This supports the hypothesis that UV irradiation of the egg disrupts cytoplasmic movements, not the Xwnt/β-catenin pathway directly. Secondly, UV-irradiated oocytes can be dorsalized (form exaggerated dorsal and anterior structures) in the same way as wild-type oocytes and embryos, by lithium treatment at the 32-cell stage (see below), suggesting that the dorsalizing pathway is not irreparably damaged by UV treatment of the oocyte (Elinson and Pasceri, 1989).

(d) Lithium effects

Unlike UV treatment, the molecular basis of lithium treatment has been clarified. Classical experiments showed that exposure of embryos to a pulse of lithium ions at any time between early cleavage and MBT causes dorsalization (Kao et al., 1986). Lithium has been considered to act by inhibiting the phosphoinositol (PI) cycle, as it inhibits the enzyme, inositol monophosphatase. This hypothesis was supported by the observation that coinjection of myoinositol with lithium prevents dorsalization of *Xenopus* embryos (Busa and Gimlich, 1989). However, a direct inhibitor of inositol monophosphatase does not dorsalize embryos (Atack et al., 1993). Recently, the dorsalization effects of lithium have been shown to be due to its effects on the Xwnt/β-catenin pathway. Specifically, lithium inhibits the enzyme GSK3, which in turn causes the accumulation of β-catenin (Klein et al., 1996; Stambolic et al., 1996; Hedgepeth et al., 1997).

(e) Molecules downstream of β-catenin

While events upstream of beta catenin are still unclear, recent studies have succeeded in revealing the interaction immediately downstream (reviewed in Nusse, 1997). XTcf-3 is a transcription factor of the LEF/Tcf family whose association with β-catenin was first shown using the yeast two-hybrid system (Molenaar et al., 1996; Behrens et al., 1996). It is a ubiquitous protein in early *Xenopus* embryos. β-catenin bound to XTcf-3 is localized in nuclei, and binding of β-catenin to XTcf-3 is required for the activation of transcription in an in vitro assay. Furthermore, a dominant negative form of the mRNA coding for XTcf-3 causes ventralization of embryos similar to that caused by depletion of β-catenin (Molenaar et al., 1996). Thus the result of this dorsal signalling pathway is the localization of a specific transcription factor in nuclei. It is likely that this mechanism of signal transduction is conserved throughout metazoa. A *Drosophila* homolog of XTcf3, dTCF, has been shown to interact with Armadillo to affect expression of transcription factors, Engrailed and Ultrabithorax (van de Wetering et al., 1997); and a human family member hTcf-4 is expressed in colonic epithelium and transactivates transcription when bound to β-catenin (Korinek et al., 1997).

Recent studies have begun to define candidate zygotic genes that may be activated, directly or indirectly, by maternal XTcf3-β-catenin. One such candidate is the transcription factor, goosecoid (gsc). gsc is normally localized to the dorsal marginal zone, immediately zygotic transcription starts, and has head-organizing properties (Cho et al., 1991). β-catenin-deficient embryos have much reduced expression of gsc mRNA (Heasman et al., 1994b). Gsc has a proximal sensitive element in its promoter that is responsive to Xwnt signalling, defining this region as a possible target for interaction with XTcf3-β-catenin (Watabe et al., 1995). Other candidate downstream genes are suggested by mRNA overexpression experiments. β-catenin overexpression in animal caps causes the transcription of the transcription factor, Siamois, and the TGF-β class signalling molecule, Xnr3 (Carnac et al., 1996; Brannon and Kimelman, 1996; Fagotto et al., 1997). Recent dominant negative experiments also point to Siamois being downstream of β-catenin and essential for dorsal axis formation. (Fan and Sokol, 1997). Interestingly, UV irradiated embryos still express Siamois, but its expression is restricted to vegetal cells, suggesting the mislocalization of β-catenin-XTcf3 in these embryos (Brannon and Kimelman, 1996). Further studies are required to determine whether these interactions are direct or indirect, and whether the zygotic genes downstream of the dorsal pathway are the same or different in dorsal animal, equatorial and vegetal cells. In *Drosophila* development, the *wingless* signalling pathway has been shown to regulate the expression of several different genes in adjacent cell layers of the developing gut, ultimately determining the cell fate of endoderm and ventral mesodermal cells (Hoppler and Bienz, 1995; Yu et al., 1996). It seems likely that similar

complex interactions occur in the *Xenopus* gastrula to specify dorsal ectodermal, mesodermal and endodermal fates.

(f) Where does the dorsal pathway act in the embryo?

The site where the β-catenin/XTcf3 interaction happens has previously been suggested by numerous cytoplasmic and cell transfer experiments, indicating that, after the 16-cell stage, the dorsal side of the embryo contains the active dorsalizing activity. However, the exact localization of dorsalizing activity in vegetal, equatorial or animal cells remains controversial. The three-signal model emphasized the importance of the dorsal vegetal cells of the pregastrula embryo, the 'Nieuwkoop center'. They have the capacity to induce animal cells to form dorsal mesodermal tissue, while themselves differentiating into endodermal tissue (Ogi, 1967, 1969; Nieuwkoop, 1969; Nakamura et al., 1970; Sudarwati and Nieuwkoop, 1971; Gimlich and Gerhart, 1984; Dale and Slack 1987). However, the capacity to produce dorsalizing signals is not restricted to this zone. Cell transfer experiments show that the dorsal equatorial cells from the 32-cell stage onwards have the ability to rescue UV-ventralized embryos (Gimlich, 1986) and fate map experiments show that these cells are fated to form dorsal mesodermal derivatives in the undisturbed embryo (Vodicka and Gerhart, 1995). Furthermore, experiments involving depletion and overexpression of β-catenin demonstrate that animal cells, as well as equatorial and vegetal cells, secrete dorsal signals (Heasman et al., 1994b; Wylie et al., 1996). Specifically, animal caps that are either wild-type or overexpressing β-catenin dorsalize the equatorial regions and vegetal masses of β-catenin-depleted embryos in co-culture experiments.

Recently, immunostaining studies have confirmed that the site of nuclear localization of β-catenin is on the dorsal side. One study showed localization of the protein in nuclei from the 8-cell stage onwards (Larabell et al., 1997), and a second study indicated localization in a broad dorsal area of the mid-blastula, centered on the equatorial region and most conspicuous in superficial nuclei (Schneider et al., 1996). Thus the asymmetry established by the *wingless*-type pathway may be broadly across the dorsal region of the embryo and not restricted to the Nieuwkoop center.

(4) TGF-β FAMILY GROWTH FACTORS AND MESODERM FORMATION

(a) Family members expressed in early *Xenopus* embryos

Members of the TGF-β class of growth factors were first implicated in mesoderm formation by the discovery that a homolog of activin A was the active ingredient of mesoderm-inducing XTC culture medium (Smith et al., 1990). Further study suggested that activin is unlikely to play a role in mesoderm formation in vivo, as inhibition of activin activity with follistatin does not prevent mesoderm formation in early embryos (Schulte-Merke et al., 1994). Since then, two further types of TGF-β family members have been shown to be expressed maternally and have mesoderm-inducing activity; Vg1 and BMPs. Other TGF-β class growth factors, Xnr1-Xnr4 (see Table 1), are expressed after zygotic transcription starts. Only the maternal components most likely to be involved in early patterning events are considered further here. Vg1 is a vegetally localized maternal mRNA that becomes distributed in the vegetal half of the embryo after fertilization (Rebagliati et al., 1985; Weeks and Melton, 1987). While the processed, active form of the Vg1 protein cannot be detected in early *Xenopus* embryos, artificially processed Vg1 can rescue UV-ventralized embryos completely (Thomsen and Melton, 1993). The possibility therefore exists that localized processing of Vg1 in dorsal cells is important in dorsal axis formation. Vg1 has also been suggested to be involved in left/right axis and endoderm specification (Hyatt et al., 1996) although these experiments use overexpression of the artificially secreted form of the protein.

Several BMP (bone morphogenetic proteins) mRNAs are present in the early embryo as maternal transcripts (BMP2, BMP4 and BMP7), although no protein data are presently available (Koster et al., 1991; Nishimatsu et al., 1992; Dale et al., 1992; Jones et al., 1992). Overexpression studies show that they cause the formation of ventral mesodermal derivatives, including mesenchyme, blood islands and muscle but not notochord (Dale et al., 1992; Jones et al., 1992; Graff et al., 1994; Suzuki et al., 1994; Hemmati-Brivanlou and Thomsen, 1995). BMPs are implicated in ventral signalling events and are considered in a later section.

(b) TGF-β signal transduction

In contrast to the lack of information on signal reception in the Xwnt/ β-catenin dorsalizing pathway, a great deal is known about the interaction of TGF-β family members and their receptors (reviewed by Massagué, 1996; Fig. 4). Indeed, strong evidence for the involvement of TGF-β class molecules in mesoderm formation comes from studies involving dominant negative forms of the receptors. The first *Xenopus* TGF-β receptor to be cloned was an activin type II receptor, whose truncated dominant negative form disrupts embryonic development at the gastrula stage, reducing mesoderm formation (Hemmati-Brivanlou and Melton, 1992) and enhancing neural tissue (Hemmati-Brivanlou and Melton, 1994). More recently, this mutation has been shown to block not only activin, but also Vg1 and BMP signalling, suggesting that a number of growth factors can bind this receptor (Kessler and Melton, 1994; Schulte Merker et al., 1994). In general, interpretations of the relative importance of TGF-β molecules has been hampered by the promiscuity of activity of the growth factors and their receptors in overexpression experiments. Activin and BMP have heterodimeric receptors consisting of receptor serine/threonine kinases from two subclasses. In other systems, there is evidence (1) that several type II receptors bind more than one ligand: activin and BMP7 bind ActR11 (Yamashita et al., 1995) and BMP2 and BMP7 bind BMPRII (Ten Dijke et al., 1994a; Liu et al., 1995), and (2) that type I receptors may bind more than one type II receptor: ALK2, ALK3 and ALK6 all interact with both ActRII and BMPRII (ten Dijke et al., 1994b; Liu et al., 1995; Yamashita et al., 1995). So far, the type II receptor for BMP has not been isolated in *Xenopus*, but activin (XALK4) and BMP type I receptors have been characterized (Chang et al., 1997; Graff et al., 1994). Expression of constitutively active forms of these receptors induces mesoderm in animal caps.

Although the relative importance of these receptors in en-

dogenous mesoderm formation is still unclear, there is evidence that they are not functionally redundant. While dominant negative mutations of both type I and type II activin receptors block mesoderm formation in the equatorial regions of embryos, only the dominant negative ActRIIB receptor neuralizes ectodermal tissue (Chang et al., 1997). One model proposed recently to explain this difference is that activin signals through a dimeric receptor consisting of ActRIIB and ALK4 to cause mesoderm formation, while BMP2/BMP4 interact with dimers of ActRIIB and BMPR1 to induce mesoderm, and interacts with BMPR1 and an unknown type II receptor to signal ectoderm to differentiate as epidermis (Chang et al., 1997).

(c) Where and when does activin/Vg1 signalling occur in the embryo?

Although maternal as well as zygotic TGF-β family members and their receptors are implicated in roles in initiating zygotic gene expression, the extent to which they are essential for dorsal axis specification and mesoderm formation has not yet been shown, nor is their temporal and spatial activity understood. Only Vg1 is localized in the vegetal region as predicted by the three-signal model and no dorsal/ventral differences in its expression or activity have so far been detected. However, there is strong evidence that a maternal activin/Vg1 signalling pathway is important in regulating the transcription of a zygotic gene product, Mix2. Mix2 is a homeobox transcription factor that is expressed broadly across equatorial and vegetal regions at the gastrula stage (Vize, 1996). Although its function is not known, a highly related gene *Mix.1*, with a similar expression pattern causes ventralization of mesoderm, when overexpressed on the dorsal side of early embryos (Mead et al., 1996). An elegant series of experiments identified the signalling pathway that activates *Mix2* expression (Chen et al., 1996, Fig. 4b). Activin interaction with its receptor leads to phosphorylation of the type 1 receptor and the subsequent phosphorylation of a cytoplasmic protein, Smad2, in the MAD (mothers against dpp) family. Smad2 then becomes localized in nuclei and binds to a Winged helix group transcription factor FAST1 (forkhead activin-sensitive transcription factor). FAST1 is a maternal protein and activates zygotic transcription of *Mix2* in the absence of new protein synthesis.

A similar signalling cascade is also likely to exist for BMPs, as another MAD-related gene, Smad1 has been shown to be active in response to BMP signalling in *Xenopus* embryos (Graff et al., 1996; Fig. 4c). The transcription factors mediating BMP signalling are unknown, but the target genes are likely to include the zygotic transcription factors Xvent1 (Xom) and Xvent2 (Gawantka et al., 1995; Ladher et al., 1996; Onichtchouk et al., 1996; Papalopulu and Kintner,1996; Schmidt et al., 1996; Tidman-Ault et al., 1996) and Mix.1 (Mead et al., 1996).

(d) What is the relationship between the Xwnt/β-catenin/XTcf3 dorsal signalling pathway and the Smad2/FAST pathway?

While β-catenin/XTcf signalling is restricted to the dorsal segment of the early embryo, the localization of TGF-β type dorsalizing signalling pathways is not known. One important question concerns the relative roles of the dorsal β-catenin/XTcf3 signalling pathway and the activin/Vg1 pathway. Epistasis experiments have shown that BVg1 (the secretion competent-mutated form of Vg1) and activin can induce dorsal mesoderm formation in β-catenin-depleted embryos and animal caps (Wylie et al., 1996; Fagotto et al., 1997), suggesting that these embryos that lack the β-catenin/XTcf3 pathway have intact activin/Vg1 signal transduction pathways, presumably including an Smad2/FAST1 pathway. Even though the pathway is intact, no activin/Vg1 induction of dorsal mesoderm occurs in undisturbed β-catenin-depleted embryos, as they develop without forming this tissue (Heasman et al., 1994b). One model that would explain this is that, in the absence of Xwnt signalling, ventral (BMP) signals override throughout the embryo, and block or prevent the initiation of the endogenous Smad2 /FAST1 pathway.

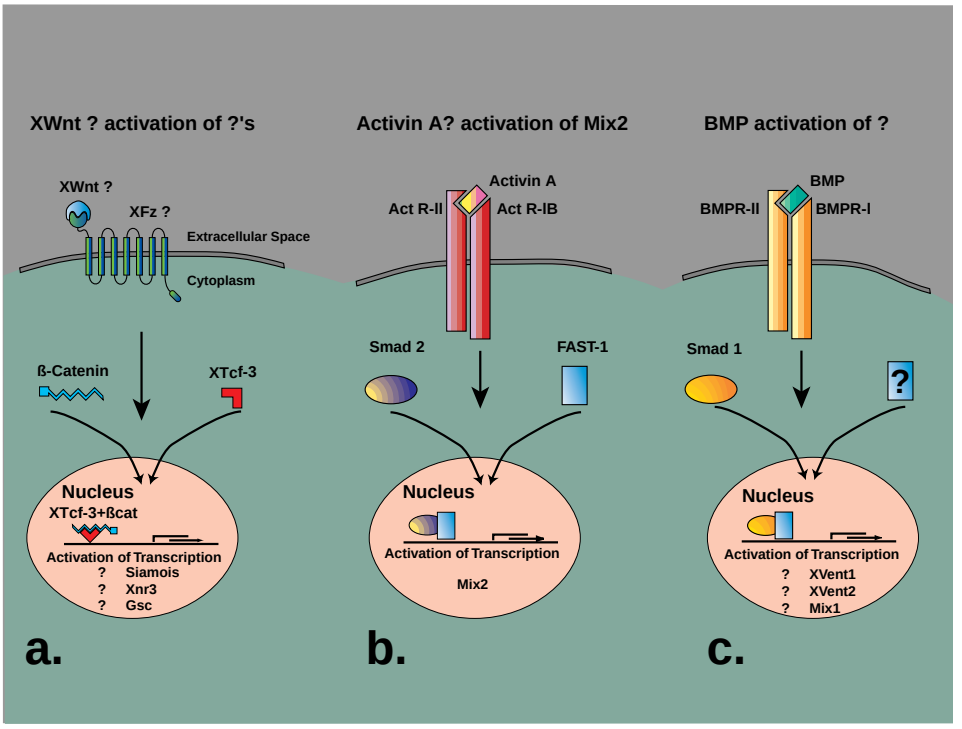

Fig. 4. Signal transduction pathways that pattern the early *Xenopus* embryo by activating localized zygotic transcription. Three signalling pathways are known to be involved. (a) The β-catenin/XTcf3 pathway, which is known to depend on maternal β-catenin. Upstream components are uncertain. (b) The pathway leading to the activation of zygotic Mix2. The transcription factor FAST-1 is a maternal component. Although the pathway has been shown to be activated by exogenous activin, the endogenous TGF-β growth factor activating the pathway has not been identified. (c) The activation of zygotic ventral gene products by BMP/Smad1 signal transduction. We do not know to what extent this is a maternal or zygotic pathway.

5. THE VENTRAL PATHWAY

(a) BMPs

β-catenin-deficient and UV-irradiated embryos form mesoderm that does not contain muscle tissue or notochord, but contains mesenchyme, (expressing fibronectin) mesothelial tissue and blood tissue (expressing globin) (Smith and Slack, 1983; Heasman et al., 1994a,b). The embryos lack dorsal/ventral and anterior/posterior axes. Several growth factors have been implicated in this ventral pathway, of which the best candidates are BMPS. BMP4 mRNA is first detectable by in situ hybridization at the gastrula stage, in animal and ventral equatorial cells. UV-irradiated embryos accumulate BMP4 transcripts prematurely and throughout the equatorial zone (Fainsod et al., 1994). Furthermore, suppression of BMP4 activity in the UV-irradiated embryo using antisense BMP4 mRNA leads to a restoration of axial structures, although not head structures (Steinbeisser et al., 1995), suggesting that the overactivity of the BMP pathway is at least partly responsible for the UV phenotype. Similarly, dominant negative BMP receptor expression rescues axial structures in β-catenin-depleted embryos (Wylie et al., 1996).

More is known about the effects of BMP signalling or of blocking BMP signalling than about the temporal and spatial localizations of those signals in the embryo. Activation of BMP signalling in mesodermal tissue causes ventral mesoderm to form (Dale et al., 1992; Jones et al., 1992; Fainsod et al., 1994) and inactivation of the pathway results in notochord and somite formation rather than ventral mesodermal tissue (Graff et al., 1994, Maeno et al., 1994, Suzuki et al., 1994). There is some evidence that dorsal signalling dominates in the marginal zone of the wild-type embryo, as ventral marginal zones co-cultured with dorsal marginal zones become dorsalized (form notochord and somite), while ventral marginal zones are unable to ventralize dorsal marginal zones (Smith and Slack, 1983; Dale and Slack, 1987).

As well as BMP4, two other maternal BMPs have been described in early *Xenopus* embryos (see above). The relative roles of the three BMPs is not known, nor is it clear when the pathway/s act. Further work is required to determine whether a maternal BMP is essential for ventral signalling, as the maternal β-catenin/XTcf3 is for the dorsal pathway. One possibility is that the activity of both the ventralizing and dorsalizing pathways is exclusively maternal and activates zygotic genes in a specific regional fashion. Alternatively, ventral signalling may occur only after MBT to modulate mesoderm formation. Depletion experiments targetting either maternal or zygotic BMPs specifically should resolve this issue.

As with dorsal differentiation, this ventralization pathway appears to be highly evolutionarily conserved (reviewed in deRobertis and Sasai, 1996). BMPs are homologs of the dorsoventral morphogen in *Drosophila*, the *decapentaplegic* gene, *dpp*. (Ferguson and Anderson, 1992a,b). *dpp* is essential in leg, wing and gut patterning (reviewed by Bienz, 1994; Massague et al., 1994; Rusch and Levine, 1996), as well as acting as a morphogen in dorsoventral patterning of the gastrula (Ferguson and Anderson, 1992; Wharton et al., 1993). Recent studies in *Xenopus* indicate that BMP4 can also act as a morphogen, in that different doses of BMP pattern three distinct domains in the gastrula marginal zone (Dosch et al., 1997).

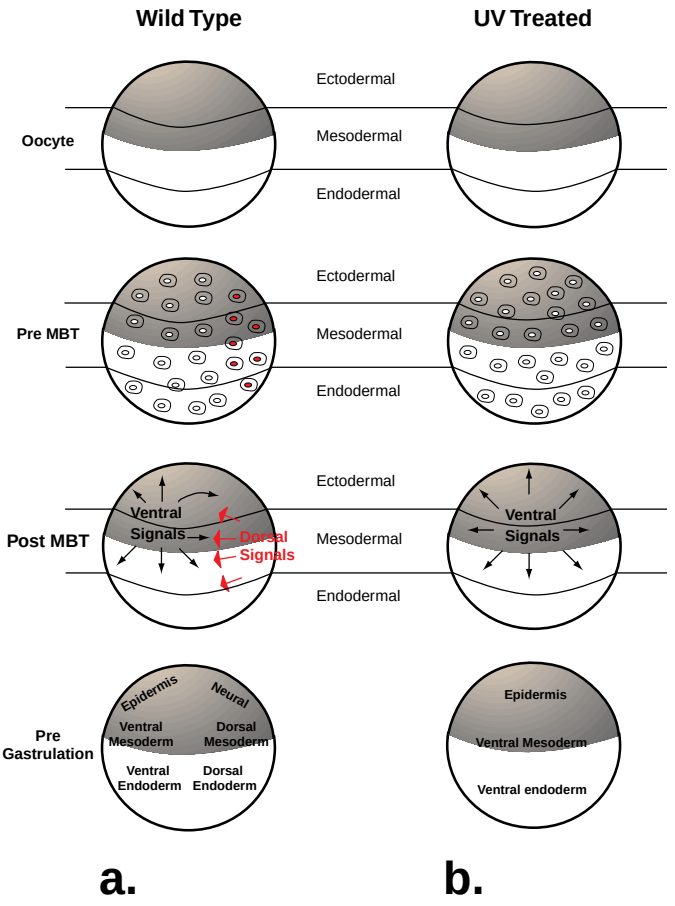

Fig. 5. A blueprint for *Xenopus* patterning. Patterning of the early *Xenopus* embryo is suggested to occur as a result of oocyte cytoplasmic localizations. These prepattern ectoderm, mesoderm and endoderm and are UV insensitive in their nature. Ventral signals (BMPs) are produced after MBT from either maternal or zygotic transcripts or both and cause ectoderm cells to form epidermis, and mesoderm and endoderm cells to form ventral derivatives (Fig. 5b). In wild-type embryos (Fig. 5a), symmetry is broken by fertilization, which sets up the localization of a dorsal segment in animal, equatorial and vegetal cells, whose nuclei contain XTcf/β-catenin (shaded in red). Animal and equatorial cells also accumulate mRNA (BMPs) for future ventral signalling functions. In the dorsal sector after MBT, maternal XTcf3/β-catenin directs zygotic transcription resulting in the expression of dorsalizing molecules (e.g. Siamois, gsc, Xnr3), which may differ in the animal, marginal and vegetal dorsal zones. One aspect of dorsal differentiation is the antagonism by dorsally secreted proteins (noggin, follistatin, chordin) of ventral BMP signals, in a germ-layer-specific manner. As well as these signals, individual cell fate may be directly or indirectly affected by the localized activity of other maternal transcripts (eg. Veg T, Xrel).

(6) THE INTERACTION OF THE DORSAL AND VENTRAL SIGNALLING PATHWAYS

(a) Antagonism between extracellular proteins

The patterning mechanism that has emerged in recent years consists of an interaction not between animal and vegetal cells, but between dorsal and ventral cells, as predicted by the third signal of the original three-signal model. When this interaction occurs is not known, because the timing of the ventral signalling pathway is not yet worked out, but it is likely to occur after MBT,

when β-catenin-XTcf3 activates transcription. The secreted proteins noggin and chordin are transcribed in the Spemann organizer region and are dorsalizing molecules, in that they can convert ventral mesodermal tissue to dorsal mesodermal types when overexpressed on the ventral side of the embryo (Smith and Harland, 1992; Sasai et al., 1994). Although there is no direct evidence as yet, these proteins are generally assumed to be downstream of the Xwnt/β-catenin/XTcf3 pathway. Interestingly, noggin and chordin can also alter the pattern of differentiation of animal cells. They induce both neural tissue and endoderm to form from animal cap tissue without causing mesodermal differentiation (Smith and Harland, 1992; Sasai et al., 1994; Bouwmeester et al., 1996). Recently, evidence for the mechanism of action of noggin and chordin has been provided by the demonstration that both proteins interact directly with BMP in vitro and can block the binding of BMP to its receptor (Zimmerman et al., 1996; Piccolo et al., 1996). Thus one hypothesis is that animal cells form neural tissue if BMP signalling is blocked by noggin and chordin, and marginal cells become notochord and somite if BMP signalling is blocked (deRobertis and Sasai, 1996). This suggests that the lineage specification of blastula cells may depend on two factors: (1) whether the cells are animal or equatorial in position (set up by maternal localizations in the fertilized egg or by cleavage-stage cell interactions) and (2) whether the BMP/Smad1 signalling cascade that results in ventralization is interrupted by dorsalizing molecules such as noggin and chordin. However, at present it is not possible to assign specific functions to these proteins.

(b) Antagonism between transcription factors

A second possible mode of regulation of the dorsoventral axis is at the level of antagonism between transcription factors. This is suggested by the finding that, in vitro, Siamois protein can heterodimerize with the similar Pax-like homeodomain protein, Mix.1 (Mead et al., 1996). Mix.1 is expressed in equatorial and vegetal cells immediately zygotic transcription starts, and overexpression causes the ventralization of mesoderm. Thus one attractive mechanism for Siamois' dorsalizing activity is that it heterodimerizes with and inactivates Mix1 in dorsal vegetal cells at the late blastula stage, and in Spemann's organizer at the gastrula stage, and thus suppresses the ventralizing activity of Mix1 homodimers (Mead et al., 1996).

(c) Regulation of the dorsal or ventral signal transduction pathways

While the previous two regulatory mechanisms are considered to act after zygotic transcription starts, a third possible control on the extent of dorsal or ventral differentiation may be regulation of the Xwnt/β-catenin/XTcf3 and/or the Smad1/BMP signal tranduction pathways themselves. Clearly all the cells of the blastula have the potential to follow a dorsal or a ventral fate (as evidenced by lithium experiments and β-catenin depletion experiments), suggesting that one or other pathway must normally be suppressed on each side of the embryo. The first suggestion of regulatory molecules that might act to inhibit axis formation on the ventral side is the *Xenopus* homolog of the mouse protein, Axin, the product of the *fused* locus (Zeng et al., 1997). Mouse Axin inhibits the Xwnt pathway upstream of β-catenin and downstream of GSK3. Furthermore, dominant negative Axin expressed on the ventral side causes axis duplication and the functional domain deleted in the dominant negative construct is an RGS domain (Regulation of G protein Signalling). The only other evidence at the present time for the involvement of G proteins in dorsoventral patterning is the fact that artificial activation of phospholipase C in dorsal blastomeres causes ventralization (Ault et al., 1996).

(7) HOW MUCH PATTERNING HAPPENS BEFORE MBT?

For the dorsal pathway, at least three early asymmetries are known to be important before MBT. In the full-grown oocyte an as yet unidentified, UV-sensitive dorsal determinant is restricted to the vegetal pole. The second asymmetry is the dorsal cortical cytoplasmic displacement of a dorsal determinant that is generated by microtubule-based activity in the subcortical cytoplasm during the first cell cycle. Neither of these asymmetries have been explained in molecular terms. The third is the dorsal nuclear localization of β-catenin. The mechanism by which this is set up is uncertain, but is likely to involve the two previous asymmetries. However, although Xwnts are present maternally in *Xenopus*, there is no evidence yet of interactions involving Wingless-like molecules secreted by one cell affecting adjacent cells, as is the case in *Drosophila*. One Xwnt (Xwnt8b) is maternally expressed and has potent axis-forming ability, but has not been shown to be involved in the dorsal pathway (Cui et al., 1995). A second Xwnt, Xwnt 11 is vegetally localized but does not have strong axis forming capacity (Ku and Melton, 1993).

For the ventral pathway, the only known asymmetry before MBT is the animal and equatorial localization of BMP 7 mRNA (Hawley et al., 1995). This, coupled with the dorsally placed β-catenin/XTcf3 could be sufficient to dictate the spheres of influence of the two pathways before MBT and once transcription starts. Apart from these two pathways, are the cells of the early embryo naive and equivalent to one another? Single cell transplantation experiments showed that early blastula cells are pluripotent (Heasman et al., 1984). However, there is evidence for cytoplasmic localization in the full-grown oocyte, that suggests that considerable prepattern is set up before cleavage starts. For example, ligation experiments on fertilized eggs showed that components required for the activation of muscle-specific actin genes are localized in the subequatorial region (Gurdon et al., 1985). mRNAs for a variety of molecules including secreted proteins (Vg1, Xwnt11), germplasm-associated proteins (Xcat2) (Forristal et al., 1995) and transcription factors (VegT) (Zhang and King, 1996; Stennard et al., 1996; Lustig et al., 1996) are restricted to the vegetal pole of the oocyte early in oogenesis and the *Xre*I transcription factor has been localized to nuclei in the animal hemisphere before MBT (Bearer, 1994). Recent studies on disaggregated cells suggests that there are also localizations in the equatorial zone (Lemaire and Gurdon, 1994). The ventral mesoderm marker, Xwnt-8, and the dorsal mesodermal marker, gsc, do not require cell interaction to be expressed in ventral and dorsal equatorial cells, respectively. Finally, animal cells cultured in isolation develop as neural cells, suggesting that they also have some prepattern that does not require cell interaction (Godsave and Slack, 1991). It is clear however, that for appropriate differentiation and axis formation, cell-cell interaction is essential. When do these interactions start?

(8) THE TIMING OF CELL-CELL INTERACTIONS IN DORSOVENTRAL PATTERNING

The current models of dorsoventral axis formation are based on the premise that cell interactions start early in embryogenesis (Smith and Slack, 1983; Kimelman, 1992; Sive, 1993) and, presumably, as there is no zygotic transcription until MBT, these interactions must cause post-transcriptional modifications to maternal mRNAs and proteins. Post-transcriptional regulation of expression has been shown for some maternal proteins including FGFR1 (Robbie et al., 1995) and Eg5 (Leguelle et al., 1991).

The early onset of cell interactions involved in axis formation has been suggested by at least four lines of reasoning, but none of these prove that this is the case. Nieuwkoop's original studies showed that isolated animal caps could be diverted from their epidermal fate to form mesoderm by co-culture with vegetal masses. This suggested that the mechanism for dorsoventral patterning was that vegetal cells produce mesoderm-inducing signals. However, it has also been shown that the equatorial zones of embryos will differentiate into mesodermal tissue autonomously even when they are isolated from the vegetal masses as early as the 32-cell stage (Nakamura et al., 1970). So, if vegetal signals are the mechanism of induction of equatorial cells those signals must pass from the vegetal cells before that time. The second line of evidence stems from heterochronic Nieuwkoop recombination studies (Jones and Woodland, 1987). Here, vegetal masses from as early as the 16-cell stage were cultured with early gastrula animal caps and induced the caps to form mesoderm. The evidence is indirect, as induction could occur at any time during the co-culture period, although animal cap responsiveness has been shown to be lost during the gastrula stage. Also, it is not possible to rule out in these experiments that some mesoderm arose from the vegetal masses, particularly when these pieces were dissected from 16-cell (stage 5) embryos. Thirdly, cell transplantation experiments have shown that,when wild-type dorsal cells are transplanted into UV-ventralized embryos at the 32-cell stage, the transplanted cells can rescue the UV phenotype (Gimlich, 1986). While this experiment shows that early vegetal cells can act as a signalling center, it does not address the timing of this inductive activity, which might be any time after transplantation. Finally, the mRNA for the TGF-β growth family member Vg1 is known to be localized in the vegetal hemisphere of early *Xenopus* embryos. While injected BVg1 is potent in axis formation, no active Vg1 protein has been identified in early embryos. It has been reasoned that the Vg1 precursor protein may be processed in a limited, localized region of the embryo to direct dorsal axis patterning, in quantities too small to be detected by the means available.

More recent experiments provide direct evidence that inductive signals activating zygotic transcription of *MyoD* (a dorsal mesodermal marker) do not pass from vegetal areas to animal caps placed in contact with them until after zygotic transcription starts at MBT. In heterochronic recombinants of animal and vegetal masses cultured together for a short, one-hour culture period, vegetal masses from late blastulae are able to induce competent animal caps to transcribe *MyoD*. In contrast, vegetal masses from early (preMBT) blastulae do not induce identical animal caps to express MyoD over the same period of time (Wylie et al., 1996). Thus cell interactions involving signals from the vegetal mass in this case occur only after zygotic transcription has started. Whether this is true for other markers and for equatorial cell-cell interactions has yet to be shown, although the localization of β-catenin/XTcf3 in equatorial nuclei at the time of MBT suggests that XTcf3 activates zygotic genes at this time and argues against the early onset of signalling events.

(9) CONCLUSION

In summary, the three-signal model envisioned early dorsal and ventral signals emanating from vegetal cells and being acted upon by naive animal cells before MBT. Recent evidence however suggests that animal cells are not naive and that dorsal animal cells as well as equatorial and vegetal cells produce dorsalizing signals. Also, no signals have been identified that are produced specifically by dorsal or ventral vegetal cells, while evidence is accumulating for dorsal signal/s emanating from a dorsal segment after MBT, and of ventral signals produced by animal and equatorial cells. An alternative model to the three-signal model is shown in Fig. 5.

Here, the oocyte is suggested to be prepatterned into at least three zones, presaging the three germ layers. Cells inheriting animal cytoplasm become ectodermal tissue, cells inheriting equatorial cytoplasm differentiate as mesodermal tissue and cells inheriting vegetal cytoplasm differentiate as endodermal tissue. These states are however labile as, at the early blastula stage, all cells are pluripotent and single cells placed in ectopic sites will differentiate according to their new positions. After fertilization, the cylindrical symmetry is broken and a dorsal segment is established in the form of animal, equatorial and vegetal cells whose nuclei contain XTcf/β-catenin. Animal and equatorial cells also accumulate mRNA (BMPs) for future ventral signalling functions. At MBT, animal, equatorial and vegetal cells respond to ventral signals by activating zygotic transcription factors (eg Xvent1, Mix1) which direct ventral differentiation, appropriate to each germ layer. In the dorsal sector, maternal XTcf3/β-catenin directs zygotic transcription resulting in the expression of dorsalizing molecules (eg Siamois, gsc, Xnr3), which may differ in the animal, marginal and vegetal dorsal zones. One aspect of dorsal differentiation is the antagonism by dorsally secreted proteins (noggin, follistatin, chordin) of ventral BMP signals, in a germ-layer-specific manner. As well as these signals, individual cell fate may be directly or indirectly affected by the localized activity of other maternal transcripts (eg. Veg T, Xrel).

In the case of a UV-irradiated or β-catenin-deficient embryo, ventral signals predominate and the embryo does not develop D/V or A/P axes, as the oocyte's symmetry is not broken and the dorsal sector is not established. The development of both dorsal and anterior structures depends on an intact maternal XTcf3/β-catenin signalling pathway.

This model is extremely fragile, leaving major questions unresolved including:

1. Is there a mesodermal state independent of the proposed dorsal and ventral pathways? If so, what is it?

2. Are maternal or zygotic BMPs (or both) essential for specifying ventral fates?

3. In the case of the dorsal signal, what is the primary activity

that causes asymmetry of XTcf3/beta catenin? In the case of the ventral signal, does a transcription factor similar to FAST1 bind to Smad2?

4. How do we account for the complexity of fine-grained expression patterns of transcription factors and secreted proteins in the Spemann organizer downstream of the dorsal and ventral signals?

5. What is the role of Vg1 and activin in embryonic patterning?

In conclusion, the patterning described here shows more similarities with pattern-forming mechanisms in *Drosophila* than were visible at the cellular level. In both *Drosophila* and *Xenopus*, maternal transcription factors specify regional activity of zygotic genes and, in both systems, similar signalling mechanisms are at work. We still do not understand how mesoderm forms in the early *Xenopus* embryo. Future emphasis will be placed not only on how the organizer organizes, but also on how, even without an organizer, the embryo generates three germ layers.

The financial support for this work has been provided by the Wellcome Trust, the Institute of Human Genetics, the Harrison Fund, and the NIH (1 RO1 HD33002-01). I am extremely grateful to Matt and Kelly Kofron for their help with the figures, to Steve Glaspey for assistance with references and to Chris Wylie for critical reading of the work, and essential intellectual contributions.

REFERENCES

Atack, J. R., Cook, S. M., Watt, A. P., Fletcher, S. R. and Ragan, C. I. (1993). In vitro and in vivo inhibition of inositol monophosphatase by the bisphosphonate L-690,330. *J. Neurochem.* **60**, 652-658.

Ault, K. T., Durmwicz, G., Galione, A., Harger, P. L. and Busa, W. B. (1996). Modulation of *Xenopus* embryo mesoderm-specific gene expression and dorsoanterior patterning by receptors that activate the phosphatidylinositol cycle signal transduction pathway. *Development* **122**, 2033-2041.

Bearer, E. (1994). Distribution of Xrel in the early *Xenopus* embryo: a cytoplasmic and nuclear gradient. *Eur. J. Cell Biol.* **63**, 255-268.

Behrens, J., von Kries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R. and Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* **382**, 638-642.

Bejsovec, A. and Martinez-Arias, A. (1991). Roles of *wingless* in patterning the larval epidermis of *Drosophila*. *Development* **113**, 471-485.

Bienz, M. (1994). Homeotic genes and positional signaling in the *Drosophila* viscera. *Trends Genet.* **10**, 22-36.

Boterenbrood, E. C. and Nieuwkoop, P. D. (1973). The formation of the mesoderm in urodelean amphibians. V. Its regional induction by the endoderm. *Wilhelm Roux' Arch. devl Biol.* **173**, 319-332.

Bouwmeester, T., Kim, S., Sasai, Y., Robertis, B. L. D. and deRobertis E. M. (1996). Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382**, 595-601.

Brannon, M. and Kimelman D. (1996). Rapid communication: Activation of *siamois* by the Wnt pathway. *Dev. Biol.* **180**, 344-347.

Busa, W. B. and Gimlich, R. L. (1989). Lithium-induced teratogenesis in frog embryos prevented by a polyphosphoinositide cycle intermediate or a diacylglycerol analog. *Dev. Biol.* **132**, 315-324.

Carnac, G., Kodjabachian, L., Gurdon, J. B. and Lemaire, P. (1996). The homeobox gene *Siamois* is a target of the Wnt dorsalisation pathway and triggers organiser activity in the absence of mesoderm. *Development* **122**, 3055-3065.

Chang, C., Wilson, P. A., Mathews, L. S. and Hemmati-Brivanlou, A. (1997). A *Xenopus* type 1 activin receptor mediates the mesodermal but not neural specification during embryogenesis. *Development* **124**, 827-837.

Chen, X., Rubock M. A. and Whitman, M. (1996). A transcriptional partner for MAD proteins in TGF-beta signalling. *Nature* **383**, 691-696.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and deRobertis, E. M. (1991). Molecular nature of Spemann's organizer: The role of the *Xenopus* homeobox gene *goosecoid*. *Cell* **67**, 1111-1120.

Cox, R. T., Kirkpatrick,C. and Peifer,M. (1996). Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during *Drosophila* embryogenesis. *J. Cell Biol.* **134**, 133-148.

Cui, Y., Brown, J. D., Moon, R. T. and Christian, J. L. (1995). *Xwnt*-8b: a maternally expressed *Xenopus Wnt* gene with a potential role in establishing the dorsoventral axis. *Development* **121**, 2177-2186.

Dagle, J. M., Weeks, D. L. and Walder, J. A. (1991). Pathways of degradation and mechanism of action of antisense oligonucleotides in *Xenopus laevis* embryos. *Antisense Res Dev.* **1**, 11-20.

Dale, L., Howes,G., Price, B. M. J. and Smith, J. C. (1992). Bone morphogenetic protein 4:a ventralizing factor in early *Xenopus* development. *Development* **115**, 573-585.

Dale, L. and Slack, J. M. W. (1987). Regional specification within the mesoderm of early embryos of *Xenopus laevis*. *Development* **100**, 279-295.

Dash, P., Lotan, I., Knapp, M., Kandel, E. R. and Goelet, P. (1987). Selective elimination of mRNAs *in vivo*:complimentary oligonucleotides promote RNA degradation by an RNaseH-like activity. *Proc. Natl. Acad. Sci. USA* **84**, 7896-7900.

Dawid, I. B. (1994). Intercellular Signaling and Gene Regulation during Early Embryogenesis of *Xenopus laevis*. *J. Biol. Chem.* **269**,

deRobertis, E. M. and Sasai, Y. (1996). A common plan for dorsoventral patterning in Bilateria. *Nature* **380**, 37-40.

Diaz-Benjumea, F. J. and Cohen, S. M. (1994). *Wingless* acts through the *shaggy/zeste-white 3* kinase to direct dorsal-ventral axis formation in the *Drosophila* leg. *Development* **120**, 1661-1670.

DiNardo, S., Sher, E., Heemskerk-Jongens, J., Kassis J. A. and O'Farrell, P. H. (1988). Two-tiered regulation of statially patterned engrailed gene expression during *Drosophila* embryogenesis. *Nature* **332**, 604-609.

Dominguez, I., Itoh K. and Sokol, S. Y. (1995). Role of glycogen synthase kinase 3beta as a negative regulator of dorsoventral axis formation in early *Xenopus* embryos. *Proc. Natl Acad. Sci. USA* **92**, 8498-8502.

Dosch, R., Gawantka, V., Delius, H., Blumenstock, C. and Niehrs, C. (1997). Bmp-4 acts as a morphogen in dorsoventral patterning in *Xenopus*. *Development* **124**, 2325-2334.

Elinson, R. P. and Pasceri, P. (1989). Two UV sensitive targets in dorso-anterior specification of frog embryos. *Development* **106**, 511-518.

Fagotto, F., Funayama, N., Gluck, U. and Gumbiner, B. (1996). Binding to cadherins antagonizes the signalling activity of β catenin during axis formation in *Xenopus*. *J. Cell Biol.* **132**, 1105-1114.

Fagotto, F., Guger, K. and Gumbiner, B. M. (1997). Induction of the primary dorsalizing center in *Xenopus* by the Wnt/GSK/beta-catenin signaling pathway, but not by Vg1, Activin or Noggin. *Development* **124**, 453-460.

Fainsod, A., Steinbeisser, H. and deRobertis, E. M. (1994). On the function of BMP-4 in patterning the marginal zone of the *Xenopus* embryo. *EMBO J.* **13**, 5015-5025.

Fan, M. J. and Sokol, S. Y. (1997). A role for siamois in Spemann organizer formation. *Development* **124**, 2581-2589.

Ferguson, E. L. and Anderson, K. V. (1992a). Decapentaplegic acts as a morphogen to organize dorsal-ventral pattern in the *Drosophila* embryo. *Cell* **71**, 451-461.

Ferguson, E. L. and Anderson, K. V. (1992b). Localized enhancement and repression of the activity of the TGF-beta family member, decapentaplegic, is necessary for dorsal-ventral pattern formation in the *Drosophila* embryo. *Development* **114**, 583-597.

Forristal, C., Pondell, M., Chen L. and King, M. L. (1995). Patterns of localisation and cytoskeletal association of two vegetally localised RNAs, *Vg1* and *Xcat-2*. *Development* **121**, 201-208.

Fujisue, M., Kobayakawa, Y. and Yamana, K. (1993). Occurence of dorsal axis-inducing activity around the vegetal pole of an uncleaved *Xenopus* egg and displacement to the equatorial region by cortical rotation. *Development* **118**, 163-170.

Gawantka, V., Delius, H., Hirschfeld, K., Blumenstock C. and Niehrs, C. (1995). Antagonizing the Spemann organizer:role of the homeobox gene *Xvent-1*. *EMBO J.* **14**, 6268-6279.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning B. and Stewart, R. (1989). Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development. *Development* **107 Supplement**, 37-51.

Gimlich, R. L. (1986). Acquisition of developmental autonomy in the equatorial region of the *Xenopus* embryo. *Dev. Biol.* **115**, 340-352.

Gimlich, R. L. and Gerhart, J. C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus* laevis. *Dev. Biol.* **104**, 117-130.

Godsave, S. F. and Slack, J. M. W. (1991). Single cell analysis of mesoderm induction in the *Xenopus* embryo. *Development* **111**, 523-530.

Graff, J. M., Bansal, A. and Melton, D. A. (1996). *Xenopus* Mad proteins transduce distinct subsets of signals for the TGF-beta superfamily. *Cell* **85**, 479-487.

Graff, J. M., Thies, R. S., Song, J. J., Celeste, A. J. and Melton, D. A. (1994). Studies with a *Xenopus* BMP receptor suggest that ventral mesoderm-inducing signals override dorsal signals *in vivo*. *Cell* **79**, 169-179.

Grant, P. and Wacaster, J. F. (1972). The amphibian grey crescent – A site of developmental information? *Dev. Biol.* **28**, 454-471.

Gurdon, J. B., Mohun, T. J., Fairman, S. and Brennan, S. (1985). All components required for the eventual activation of muscle-specific actin genes are localized in the subequatorial region of the uncleaved amphibian egg. *Proc. Natl. Acad. Sci. USA* **82**, 139-143.

Hawley, S. H. B., Wunnenberg-Stapleton, K. C., Hashimoto, C., Laurent, M. N., Watabe, T., Blumberg, B. W. and Cho, K. W. Y. (1995). Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction. *Genes Dev.* **9**, 2923-2935.

Hayashi, S., Rubinfeld, B., Souza, B., Polakis, P., Wieschaus, E. and Levine, A. J. (1997). A *Drosophila* homolog of the tumor supressor gene adenomatous polyposis coli down-regulates beta-catenin, but its zygotic expression is not essential for the regulation of Armadillo. *Proc. Natl. Acad. Sci. USA* **94**, 242-247.

He, X., Saint-Jeannet, J. -P., Woodgett, J. R., Varmus, H. E. and Dawid, I. B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* **374**, 617-622.

He, X., Saint-Jeannet, J. P., Wang, Y., Nathans, J., Dawid, I. and Varmus, H. (1997). A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* **275**, 1652-1654.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P. Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y. and Wylie C. (1994a). Overexpression of cadherins and underexpression of β catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell* **79**, 791-803.

Heasman, J., Ginsberg, D., Geiger, B. Goldstone, K., Pratt, T., Yoshida-Noro, C. and C. Wylie (1994b). A functional test for maternally inherited cadherin in *Xenopus* shows its importance in cell adhesion at the blastula stage. *Development* **120**, 49-57.

Heasman, J., Wylie, C. C., Hausen, P. and Smith, J. C. (1984). Fates and states of determination of single vegetal pole blastomeres of *Xenopus*. *Cell* **37**, 185-194.

Hedgepeth, C. M., Conrad, L. J., Zhang, J. Huang, H., Lee V. M. Y. and Klein, P. S. (1997). Activation of the Wnt signaling pathway: a molecular mechanism for Lithium action. *Dev. Biol.* **185**, 82-91.

Hemmati-Brivanlou, A. and Melton, D. A. (1994). Inhibition of activin receptor signalling promotes neuralisation in *Xenopus*. *Cell* **77**, 273-281.

Hemmati-Brivanlou, A. and Melton, D. A. (1992). A truncated activin receptor inhibits mesoderm induction formation of axial structures in *Xenopus* embryos. *Nature* **359**, 609-614.

Hemmati-Brivanlou, A. and Thomsen, G. H. (1995). Ventral mesodermal patterning in *Xenopus* embryos: expression patterns and activities of BMP-2 and BMP-4. *Dev. Genetics* **17**, 78-89.

Holowacz, T. and Elinson, R. P. (1995). Properties of the dorsal activity found in the vegetal cortical cytoplasm of *Xenopus* eggs. *Development* **121**, 2789-2798.

Holwill, S., Heasman, J., Crawley, C. and Wylie, C. C. (1987). Axis and germ line deficiencies caused by u. v irradiation of *Xenopus* oocytes cultured *in vitro*. *Development* **100**, 735-743.

Hoppler, S. and Bienz, M. (1995). Two different thresholds of wingless signaling with distinct developmental consequences in the *Drosophila* midgut. *EMBO J.* **14**, 5016-5026.

Hoschuetzky, H., Aberle, H. H. and Kemler, R. (1994). β catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. *J. Cell Biol.* **127**, 1375-1380.

Houliston, E. (1994). Microtubule translocation and polymerisation during cortical rotation in *Xenopus* eggs. *Development* **120**, 1213-1220.

Hyatt, B. A., Lohr, J. L. and Yost, J. (1996). Initiation of vertebrate left-right axis formation by maternal *Vg1*. *Nature* **384**, 62-65.

Jones, C., Kuehn, M., Hogan, B. Smith, J. and Wright, C. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121**, 3651-3662.

Jones, C. M., Lyons, K. M. Lapan, P. M., Wright, C. V. E. and Hogan, B. M. L. (1992). DVR-4 (bone morphogenetic protein 4) as a posterior ventralizing factor in *Xenopus* mesoderm induction. *Development* **115**, 639-647.

Joseph, E. M. and Melton, D. A. (1997). *Xnr4:* A *Xenopus* nodal-related gene expressed in the Spemann Organizer. *Dev. Biol.* **184**, 367-372.

Jones, E. A. and Woodland, H. R. (1987). The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. *Development* **101** 557-563

Kanai, Y., Ochiai, T., Shibata, T., Oyama, S. Ushijima, S., Akimoto S. and Hirohashi, S. (1995). *c-erbB-2* gene product associates with catenins in human cancer cells. *Biochem. Biophys. Res. Comm.* **208**, 1067-1072.

Kao, K. R., Masui, Y. and Elinson, R. P. (1986). Lithium induced respecification of pattern in *Xenopus laevis* embryos. *Nature* **322**, 371-373.

Kessler, D. S. and Melton, D. A. (1994). Vertebrate embryonic induction: mesodermal and neural patterning. *Science* **266**, 596-604.

Kimelman, D., Christian, J. L. and Moon, R. T. (1992). Synergistic principles of development: overlapping patterning sysyems in *Xenopus* mesoderm induction. *Development* **116**, 1-9.

Klein, P. S. and Melton, D. A (1996). A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. USA* **93**, 8455-8459.

Kofron, M., Spagnuolo, A., Klymkowsky, M., Wylie, C. and Heasman, J. (1997). The roles of maternal alpha-catenin and plakoglobin in the early *Xenopus* embryo. *Development* **124**, 1553-1560.

Korinek, V., Barker, N., Morin, P. J., Wichen, D. v., Weger, R. d., Kinzler, K. W., Vogelstein, B. and Clevers, H. (1997). Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC –/– colon carcinoma. *Science* **275**, 1784-1787.

Koster, M., Plessow, S., Clement, J. H., Lorenz, A., Tiedemann H. and Knochel, W. (1991). Bone Morphogenic Protein 4 (BMP4), a member of the TGF-beta family, in early embryos of *Xenopus laevis*: an analysis of mesoderm inducing activity. *Mech. Dev.* **33**, 191-200.

Ku, M. and Melton, D. A. (1993). *Xwnt-11*, a maternally expressed *Xenopus* Wnt gene. *Development* **119**, 1161-1173.

Ladher, R., Mohun, T., Smith J. C. and Snape, A. (1996). *Xom*:, a *Xenopus* homeobox gene that mediates the early effects of BMP-4. *Development* **122**, 2385-2394.

Larabell, C. A., Torres, M., Rowning, B. A., Yost, C., Miller, J., Wu, R. M., Kimelman, D. and Moon, R. T. (1997). Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. *J. Cell Biol.* **136**, 1123-1136.

Leguelle, R. Paris, J., Couturier, A., Roghi, C. and Philippe, M. (1991). Cloning by differential screening of a *Xenopus* cDNA that encodes a kinesin-related protein. *Mol. Cell Biol.* **11**, 3395-3398.

Lemaire, P., Garrett, N. and Gurdon, J. B. (1995). Expression cloning of *siamois*, a *Xenopus* homeobox gene expressed in dorsal-vegetal cells of blastulae and able to induce a complete secondary axis. *Cell* **81**, 85-94.

Lemaire, P. and Gurdon, J. B. (1994). A role for cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of the *goosecoid* and *Xwnt-8* genes along the dorsoventral axis of early *Xenopus* embryos. *Development* **120**, 1191-1199.

Lemaire, P. and Kodjabachian, L. (1996). The vertebrate organizer: structure and molecules. *Trends Genet.* **12**, 525-531.

Liu, F., Ventura, F., Doody J. and Massague, J. (1995). Human type II receptor for bone morphogenic proteins (BMPs): extension of the two-kinase receptor model to the BMPs. *Mol. Cell. Biol.* **15**, 3479-3486.

Lustig, K., Kroll, K. L., Sun, E. E. and Kirschner, M. W. (1996). Expression cloning of a *Xenopus* T-related gene (*Xombi*) involved in mesodermal patterning and blastopore lip formation. *Development* **122**, 4001-4012.

Maeno, M., Ong, R. C., Suzuki, A., Ueno, N. and Kung, H. (1994). A truncated bone morphogenic protein-4 receptor alters the fate of ventral mesoderm to dorsal mesoderm: the roles of animal pole tissue in the development of ventral mesoderm. *Proc. Natl. Acad. Sci. USA* **91**, 10260-10264.

Malacinski, G. M., Allis, C. D. and Chung, H. M. (1974). Correction of developmental abnormalities resulting from localized ultra-violet irradiation of an amphibian egg. *J. Exp. Zool.* **189**, 249-254.

Martinez-Arias, A., Baker, N. and Ingham, P. W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* **103**, 153-170.

Massague, J., Attisano, L. and Wrana, J. L. (1994). The TGF-beta family and its composite receptors. *Trends Cell Biol.* **4**, 172-178.

Massague, J. (1996). TGFβ signalling: receptors, transducers and Mad proteins. *Cell* **85**, 947-950.

Mead, P., Brivanlou, I., Kelley, C. and Zon, L. (1996). BMP-4-responsive regulation of dorsal-ventral patterning by the homebox protein Mix. 1. *Nature* **382**, 357-360.

Molenaar, M., van der Wetering, M., Oosterwegel, M., Perterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O. and Clevers, H. (1996). XTcf-3 transcription factor mediates β-catenin-induced axis formation in *Xenopus* embryos. *Cell* **86**, 391-399.

Morata, G. and Lawrence, P. A. (1977). The development of *wingless*, a homeotic mutation of *Drosophila*. *Dev. Biol.* **56**, 227-240.

Munemitsu, S., Souza, B., Muller, O., Albert, I., Rubenfeld, B. and Polakis, P. (1994). The APC gene product associates with microtubules *in vivo* and promotes their assembly *in vitro*. *Cancer Res.* **54**, 3676-3681.

Nakamura, O.,Takasaki, H. and Mizohata, T. (1970). Differentiation during cleavage in *Xenopus laevis*: Acquisition of self-differentiation capacity of the dorsal marginal zone. *Proc. Japan Acad.* **46**, 694-699.

Nieuwkoop, P. D. (1969). The formation of mesoderm in Urodelean amphibians. I. Induction by the endoderm. *Wilhelm Roux' Arch. EntwMech. Org.* **162**, 341-373.

Nishimatsu, S., Suzuki, A., Shoda, A., Murakami, K. and Ueno N. (1992). Genes for bone morphogenetic proteins are differentially transcribed in early amphibian embryos. *Biochem. Biophys. Res. Comm.* **186**, 1487-1495.

Nusse, R. (1997). A versatile transcriptional effector of wingless signaling. *Cell* **89**, 321-323.

Ogi, K. -I. (1967). Determination in the development of the amphibian embryo. *Sci. Rep. Tohuku Univ. Ser. IV (Biol.)* **33**, 239-247.

Ogi, K. -I. (1969). Regulative capacity in the early amphibian embryo. *Res. Bull. Dept. Gen. Ed. Nagoya University* **13**, 31-40.

Onichtchouk, D., Gawantka, V., Dosch, R., Delius, H., Hirschfeld, K., Blumenstock, C. and Niehrs, C. (1996). The *Xvent-2* homeobox gene is part of the Bmp-4 signaling pathway controlling dorsoventral patterning of *Xenopus* mesoderm. *Development* **122**, 3045-3053.

Ozawa, M., Baribault, H. and Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* **8**, 1711-1717.

Pai, L. M., Orsulic S., Bejsovec, A. and Peifer, M (1997). Negative regulation of armadillo, a wingless effector in *Drosophila*. *Development* **124**, 2255-2266.

Papalopulu, N. and Kintner, C. (1996). A Xenopus gene, *Xbr-1* defines a novel class of homeobox genes and is expressed in the dorsal ciliary margin of the eye. *Dev. Biol.* **174**, 104-114.

Papkoff, J., Rubinfeld, B., Schryver, B. and Polakis, P. (1996). Wnt-1 regulates free pools of catenins and stabilizes APC-catenin complexes. *Mol. Cell Biol.* **16**, 2128-2134.

Peifer, M. (1993). The product of the *Drosophila* segment polarity gene *armadillo* is part of a multi-protein complex resembling the vertebrate adherens junction. *J. Cell Sci.* **105**, 993-1000.

Peifer, M., McCrea, P., Green, K. J., Wieschaus, E. and Gumbiner, B. (1992). The vertebrate adhesive junction proteins β-catenin and plakoglobin and the *Drosophila* segment polarity gene *armadillo* form a multigene family with similar properties. *J. Cell Biol.* **118**, 681-692.

Peifer, M., Sweeton, D., Casey, M. and Weischaus, E. (1994). Wingless signal and zeste-white 3 kinase trigger opposing changes in the intracellular distribution of armadillo. *Development* **120**, 369-380.

Phillips, R. G. and Whittle, J. R. S. (1993). *Wingless* expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. *Development* **118**, 427-438.

Piccolo, S., Sasai, Y., Lu, B. and deRobertis, E. M. (1996). Dorsoventral patterning in *Xenopus*: Inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* **86**, 589-598.

Pierce, S. B. and Kimelman, D. (1996). Overexpression of Xgsk-3 disrupts anterior ectodermal patterning in *Xenopus*. *Dev. Biol.* **175**, 256-264.

Rebagliati, M. R., Weeks, D. L., Harvey, R. P. and Melton, D. A. (1985). Identification and cloning of localized maternal RNAs from *Xenopus* eggs. *Cell* **42**, 769-777.

Robbie, E., Peterson, M., Amaya, E. and Musci,T. (1995). Temporal regulation of the *Xenopus* FGF receptor in development: a translation inhibitory element in the 3' untranslated region. *Development* **121**, 1775-1785.

Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S. and Polakis, P. (1996). Binding of GSK3β to the APC-β catenin complex and regulation of complex assembly. *Science* **272**, 1023-1026.

Rusch, J. and Levine, M. (1996). Threshold responses to the dorsal regulatory gradient and the subdivision of primary tissue territories in the *Drosophila* embryo. *Curr. Opin. Genet. Dev.* **6**, 416-423.

Sakai, M. (1996). The vegetal determinants required for the Spemann organizer move equatorially during the first cell cycle. *Development* **122**, 2207-2214.

Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont L. K. and deRobertis, E. M. (1994). *Xenopus* chordin: A novel dorsalizing factor activated by Organizer-specific homeobox genes. *Cell* **79**, 779-790.

Schmidt, J. E., von Dassow, G. and Kimelman, D. (1996). Regulation of dorso-ventral patterning: the ventralizing affects of the novel *Xenopus* homeobox gene,*Vox*. *Development* **122**, 1711-1721.

Schneider, S., Steinbeisser, H., Warga, R. M. and Hausen, P. (1996). Beta-catenin translocation into nuclei demarcates the dorsalizing centers of frog and fish embryos. *Mech. Dev.* **56**, 191-198.

Schulte-Merker, S., Smith, J. C. and Dale, L. (1994). Effects of truncated activin and FGF receptors and of follistatin on the inducing activities of BVg1 and activin: does activin play a role in mesoderm induction? *EMBO J.* **13**, 3533-3541.

Sive, H. L. (1993). The frog prince-ss: A molecular formula for dorsoventral patterning in *Xenopus*. *Genes Dev.* **7**, 1-12.

Slack, J. M. W., Dale, L. and Smith, J. C. (1984). Analysis of embryonic induction by using cell lineage markers. *Phil. Trans. R. Soc. Lond. B* **307**, 331-336.

Smith, J. (1989). Mesoderm induction and mesoderm-inducing factors in early amphibian development. *Development* **105**, 665-677.

Smith, J. C., Dale, L. and Slack, J. M. W. (1985). Cell lineage labels and region-specific markers in the analysis of inductive interactions. *J. Embryol. Exp. Morphol.* **89 Supplement**, 317-331.

Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a *Xenopus* homolog of *Brachyury (T)* is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.

Smith, J. C., Price, B. M. J., Nimmen, K. V. and Huylebroeck, D. (1990). Identification of a potent *Xenopus* mesoderm-inducing factor as a homologue of activin A. *Nature* **345**, 729-731.

Smith, J. C. and Slack, J. M. W. (1983). Dorsalization and neural induction: properties of the organizer in *Xenopus laevis*. *J. Embryol. Exp. Morph.* **78**, 299-317.

Smith, K. J., Levy, D. B., Maupin, P., Pollard, T. D., Vogelstein, B. and Kinzler, K. W. (1994). Wild-type but not mutant APC associates with the microtubule cytoskeleton. *Cancer Res.* **54**, 3672-3675.

Smith, W. C. and Harland, R. M. (1991). Injected Xwnt-8 RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing centre. *Cell* **67**, 753-765.

Smith, W. C. and Harland, R. M. (1992). Expression cloning of *noggin*, a new dorsalizing factor localised to the Spemann organiser in *Xenopus* embryos. *Cell* **70**, 828-840.

Smith, W. C., McKendry, R., Ribisi, R. J. and Harland, R. M. (1995). A nodal-related gene defines a physical and functional domain within the Spemann organizer. *Cell* **82**, 37-46.

Snape, A., Wylie, C. C., Smith J. C. and Heasman, J. (1987). Changes in the states of commitment of single animal pole blastomeres of *Xenopus*. *Dev. Biol.* **119**, 503-510.

Sokol, S. Y. (1996). Analysis of Dishevelled signaling pathways during *Xenopus* development. *Curr. Biol.* **6**, 1456-1467.

Sokol, S. Y., Klingensmith, J., Perrimon N. and Itoh, K. (1995). Dorsalizing and neuralizing properties of *Xdsh*, a maternally expressed *Xenopus* homologue of *dishevelled*. *Development* **121**, 1637-1647.

Spemann, H. and Mangold, H. (1924). Uber Induktion von Embryonenanlagen durch Implantation artfremder Organisatoren. *Wilhelm Roux' Arch. EntwMech. Org.* **100**, 599-638.

Stambolic, V., Reul, L. and Woodgett, J. R. (1996). Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr. Biol.* **6**, 1664-1668.

Steinbeisser, H., Fainsod, A. C., Niehrs, C., Sasai Y. and deRobertis, E. M. (1995). The role of gsc and BMP-4 in dorsal-ventral patterning of the marginal zone in *Xenopus*: a loss-of-function study using antisense RNA. *EMBO J.* **14**, 5230-5243.

Stennard, F., Carnac, G. and Gurdon, J. B. (1996). The *Xenopus* T box gene *Antipodean* encodes a vegetally localized maternal mRNA that can trigger mesoderm formation. *Development* **122**, 4179-4188.

Sudarwati, S. and Nieuwkoop, P. D. (1971). Mesoderm formation in the Anuran *Xenopus laevis* (Daudlin). *Wilhelm Roux' Arch. EntwMech. Org.* **166**, 189-204.

Suzuki, A.,Thier, R. S., Yamaji, N., Song, J. M., Wozney, J. M., Murakami, K. and Ueno, N. (1994). A truncated bone morphogenetic protein receptor affects dorsal-ventral patterning in the early *Xenopus* embryo. *Proc. Natl. Acad. Sci. USA* **91**, 10255-10259.

Tao, Y., Edwards, R. A., Tubb, B., Wang, S., Bryan, J. and McCrea, P. (1996). β catenin associates with the actin-bundling protein fascin in a non-cadherin complex. *J. Cell Biol.* **134**, 1271-1281.

tenDijke, P., Yamashita, H. Ichijo, Franzen, H. P., Laiho,M., Miyazono, K. and Helden, C. H. (1994a). Characterization of type I receptors for transforming growth factor-β and activin. *Science* **264**, 101-104.

tenDijke, P., Yamashita, H., Sampath, T. K., Reddi, A. H., Estevez, M., Riddle, D. L., Ichijo, H., Helden, C. H. and Miyazono K. (1994b). Identification of type I receptors for osteogenic protein-1 and bone morphogenic protein-4. *J. Biol. Chem.* **269**, 16985-16988.

Thomsen, G. H. and Melton, D. A. (1993). Processed Vg1 Protein Is an Axial Mesoderm Inducer in *Xenopus*. *Cell* **74**, 433-441.

Tidman-Ault, C., Dirksen, M. L. and Jamrich, M. (1996). A novel homeobox gene PV. 1 mediates induction of ventral mesoderm in *Xenopus* embryos. *Proc Natl. Acad. Sci. USA* **93**, 6415-6420.

van derWetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro,J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M., & Clevers, H. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*. *Cell* **88**, 789-799.

Vincent, J., Oster, G. F. and Gerhart, J. (1986). Kinematics of gray crescent formation in *Xenopus* eggs:the displacement of subcortical cytoplasm relative to the egg surface. *Dev. Biol.* **113**, 484-500.

Vincent, J. P., Scharf, S. R. and Gerhart, J. C. (1987). Subcortical rotation in *Xenopus* eggs: A preliminary study of its mechanochemical basis. *Cell Motil. Cytoskel.* **8**, 143-154.

Vize, P. D. (1996). DNA sequences mediating the transcriptional response of the *Mix. 2* homeobox gene to mesoderm induction. *Dev. Biol.* **177**, 226-231.

Vleminckx, K., Wong, E., Guger, K., Rubinfeld, B., Polakis, P. and Gumbiner, B. M. (1997). Adenomatous Polyposis Coli tumor suppressor protein has signaling activity in *Xenopus laevis* embryos resulting in the induction of an ectopic dorsoanterior axis. *J. Cell Biol.* **136**, 411-420.

Vodicka, M. A. and Gerhart, J. C. (1995). Blastomere derivation and domains of gene expression in the Spemann Organizer of *Xenopus laevis*. *Development* **121**, 3505-3518.

Watabe, T., Kim, S., Candia, A., Rothbacher, U., Hashimoto, C., Inoue, K. and Cho, K. W. Y. (1995). Molecular mechanisms of Spemann's organizer formation: conserved growth factor synergy between *Xenopus* and mouse. *Genes Dev.* **9**, 3038-3050.

Weeks, D. L. and Melton, D. A. (1987). A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-β. *Cell* **51**, 861-867.

Wharton, K. A., Ray, R. P. and Gelbart, W. M. (1993). An activity gradient of decapentaplegic is necessary for the specification of dorsal pattern elements in the *Drosophila* embryo. *Development* **117**, 807-822.

Wylie, C., Kofron, M. Payne, C., Anderson, R. M. Hosobuchi, M., Joseph, E. and Heasman, J. (1996). Maternal beta-catenin establishes a 'dorsal signal' in early *Xenopus* embryos. *Development* **122**, 2987-2996.

Wylie, C. C., Snape, A., Heasman, J. and Smith, J. C. (1987). Vegetal pole cells and their commitment to form endoderm in *Xenopus*. *Dev. Biol.* **119**, 496-502.

Yamashita, H., tenDijke, P., Huylebroeck, D., Sampath, T. K., Andries, M., Smith, J. C., Helden, C. H. and Miyazono, K. (1995). Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J. Cell Biol.* **130**, 217-226.

Yang-Snyder, J., Miller, J. R., Brown, J. D., Lai C. -J. and Moon, R. T. (1996). A frizzled homolog functions in a vertebrate Wnt signaling pathway. *Curr. Biol.* **6**, 1302-1306.

Yu, X., Hoppler, S., Eresh, S. and Bienz, M. (1996). *Decapentaplegic*, a target gene of the *wingless* signaling pathway in the *Drosophila* midgut. *Development* **122**, 849-858.

Yuge, M., Kobayakawa, Y., Fujisue, M. and Yamana, K. (1990). A cytoplasmic determinant for dorsal axis formation in an early embryo of *Xenopus laevis*. *Development* **110**, 1051-1056.

Zeng, L., Fagotto, F., Zhang, T., Hsu, W., Vasicek, T. J., Perry 111, W. L., Lee, J. J., Tilghman, S. M., Gumbiner, B. M. and Constantini, F. (1997). The mouse *fused* locus encodes Axin, an inhibitor of the Wnt signalling pathway that regulates embryonic axis formation. *Cell* **90,** 181-192.

Zhang, J. and King, M. L. (1996). *Xenopus VegT* RNA is localized to the vegetal cortex during oogenesis and encodes a novel T-box transcription factor involved in mesoderm patterning. *Development* **122**, 4119-4129.

Zimmerman, L. B., Jesus-Escobar, J. M. D. and Harland R. M. (1996). The Spemann Organizer signal noggin binds and inactivates bone morphogenic protein 4. *Cell* **86**, 599-606.

(Accepted 18 August 1997)