Int. J. Dev. Biol. 38: 257-266 (1994)

Review

Control of cell differentiation and morphogenesis in amphibian development

AKIMASA FUKUI and MAKOTO ASASHIMA*

Department of Biology, Tokyo University, Tokyo, Japan

ABSTRACT We reviewed cell differentiation and morphogenesis by mesoderm-inducing factors during amphibian embryogenesis. Recently, two kinds of growth factors, activin and FGF, have been identified as influential candidates for natural mesoderm-inducing factor in amphibian development. These factors are present in early *Xenopus* embryos. In particular, activin has been shown to induce many kinds of mesodermal tissues in a dose-dependent manner. Activin-treated ectodermal sheet (animal cap) acts as an organizer causing gene expression, mesoderm formation and functional events such as secondary axis formation. Follistatin, an activin-specific binding protein, also present in the early *Xenopus* embryo, makes a complex with activin. Follistatin protein exerts no inducing activity of *Xenopus* animal cap. Endogenous follistatin may, however, play the role of an activin regulation factor. Endogenous actions of activin and FGF were studied using injection of their receptor mRNAs. Disruption of the FGF signaling pathway by its non-functional dominant negative receptors produced trunk and tail defects. In the case of activin, an embryo cannot form axial structures. Animal-half blastomeres from the late 8-cell stage *Xenopus* embryo respond to activin, and there are prepatterns in ventral and dorsal cells from very early stages. The timing of mesoderm induction during development and the relationship between the inducing factors and competent cells are discussed in this report. Differentiation of tissues and organized formation of organs can be understood as a system of serial inductive reactions originating from the organizer. We have attempted to construct a model of organizer formation based on the results of recent studies.

KEY WORDS: *activin, FGF, follistatin, mesoderm induction, competence, organizer*

Introduction

Since amphibian embryos develop externally their embryogenesis is easy to observe. Thus, the amphibian embryo has historically been one of the most important experimental materials in developmental biology. Recently, the most common amphibian used is the African clawed toad, *Xenopus laevis*. It is quite easy to breed and spawns a large number of eggs, which is advantageous for biochemical studies. Before fertilization, the egg is radially symmetrical around the animal-vegetal axis. That is, every meridian has the potential to form either future dorsal or ventral structures. Soon after fertilization, a cortical reaction occurs and the egg rotates. UV irradiation of the vegetal hemisphere or D_2O treatment of the egg can block that cortical rotation, and thus interfere with normal development (Gerhart *et al.*, 1989). Also, if after cortical rotation, the egg is rotated 90° from its normal gravity orientation, it will develop into a twin-headed embryo. Those effects suggest that the redistribution of cytoplasm by cortical rotation affects subsequent events, including mesoderm induction, axis formation and later morphogenesis. At very early stages, however, determination of future tissues is not yet definitive and can be changed by embryonic induction (Spemann and Mangold, 1924).

Mesoderm induction is the first inductive event of post fertilization development, and may occur in the early blastula. Mesoderm induction means that early animal half cells differentiate into mesoderm which is located at the marginal zone. It seems that an interaction occurs in which signals emanating from the vegetal hemisphere cells act on the overlying equatorial cells and induce them to form mesoderm characteristics (Nieuwkoop, 1969; Nakamura *et al.*, 1971; Asashima, 1975). Furthermore, recent studies have shown that mesoderm induction is a very important event. Not only is it the first induction for cell differentiation, but it is implicated in the regulation of morphogenesis as well (Gurdon, 1987; Tiedemann, 1990). Mesoderm induction seems to occur as a consequence of the process of expression of maternal information which is stored in the egg from oogenesis. After mesoderm induction, there is a drastic change in gene expression causing not

***Address for reprints:** Department of Biology, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153, Japan. Fax: 3-3485-2904.

0214-6282/94/$03.00
© UBC Press
Printed in Spain

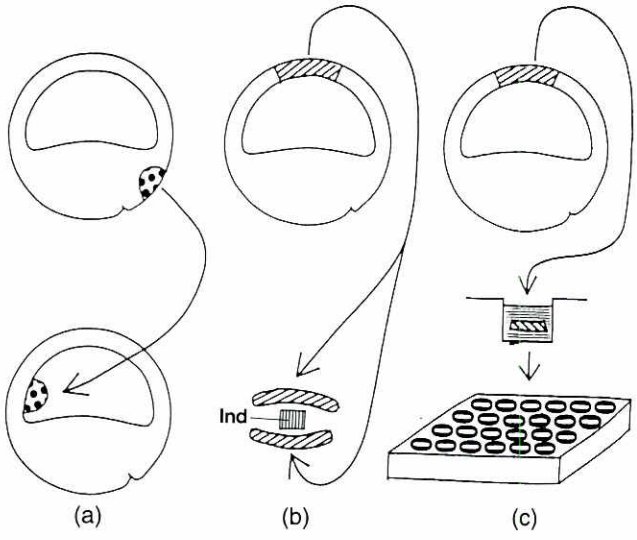

Fig. 1. Methods for bioassay of inducing factors. **(a)** *Implantation method or Einsteck method. Blastopore lip region or morphogenetic substance was inserted into blastocoel.* **(b)** *Sandwich method. Inducing substances like pellets (Ind) were wrapped in two ectoderm sheets taken from early gastrulae.* **(c)** *Piece culture method or animal cap assay. Presumptive ectoderm (animal cap) taken from blastula stage was cultured in saline solution.*

only maternal information but also paternal information. The coordinated initiation of transcription of genes and cell motility as well as the onset of an asynchronous and slower cell cycle occurs at the mid-blastula stage, termed midblastula transition (MBT) (Newport and Kirschner, 1982a,b).

For the bioassay of inducing substances three different methods have been developed (Fig. 1). The classical methods for testing are the implantation (Mangold, 1923) and sandwich methods (Holtfreter, 1933). The inducing activity of the pellets in both methods is measured as the percentage and size of resulting inductions.

The most common experimental procedure used for investigation of embryonic induction employs tissue culture (Becker and Tiedemann, 1961). Pieces of the presumptive ectodermal cell region (animal cap) are excised from amphibian blastulae and bathed in a culture medium containing the inducing factor to be investigated. This method is referred to as the "piece culture method" or "animal cap assay". This piece culture method is easy to use and suitable for testing large numbers of fractions of substances in solution, and can be employed to measure inducing activity quantitatively and qualitatively from the histological to molecular level. Without the inducer, the explant remains atypical epidermis. With inducers, the explants change their morphology and differentiation, showing different types of neural and mesoderm inductions. By using this method mesoderm-inducing factors have been identified in *Xenopus* embryos. Thus, the door to the most important problems in developmental biology has been opened for molecular biology research. A series of recent studies on induction has revealed that mesoderm-inducing factors are closely related to well known growth factors or peptide hormones.

FGF and activin are candidates for the natural mesoderm-inducing factor

In early studies of inducing factors extensive experiments were performed using extracts from different sources (heterogeneous factors), since the direct extraction of natural factors from *Xenopus* embryos is very difficult (Gurdon, 1987; Tiedemann, 1990). Although these factors often were inducers of mesoderm, their chemical structure remained unknown. Their action, however, seemed to "mimic" that of the natural mesoderm-inducing factors existing in the early embryo.

Recently, some biochemically well-characterized candidates for mesoderm-inducing factors have been discovered. These factors have effects on mesoderm cell differentiation, organogenesis, and even axis formation. Using the piece culture method, Smith (1987) characterized the factor responsible for mesoderm induction which is present in culture medium of *Xenopus* XTC cells. Slack *et al.* (1987) reported that basic fibroblast growth factor (bFGF), which has a high binding affinity for heparin, possesses mesoderm-inducing activity. Independent of this work, the research team of Tiedemann also reported, on the basis of their long-term studies, that a heparin-binding factor has mesoderm-inducing activity (Knöchel *et al.*, 1987). FGF is seriously considered to be one of the natural mesoderm-inducing factors because bFGF protein is present in the early *Xenopus* embryos (Kimelman *et al.*, 1988; Slack and Isaacs, 1989). Messenger RNA (mRNA) of bFGF has been detected in the oocyte and has also been localized in the vegetal hemisphere (Kimelman and Kirschner, 1987) of the fertile egg. And in unfertilized eggs, bFGF exists as a maternally inherited protein at a concentration of 7-200 ng/ml. That concentration of bFGF is sufficient to induce mesodermal tissues in the embryo. The piece culture method with FGF has demonstrated that induced explants differentiate mesoderm tissues such as muscle or mesenchyme, but not notochord, which is the most dorsal mesoderm tissue (Green *et al.*, 1990). *Xenopus* embryonal FGF (XeFGF), which has high homology to FGF-4 and FGF-6 members of the FGF family, has been isolated (Isaacs *et al.*, 1992). bFGF does not have the signal peptide sequence for secretion, but XeFGF does, and secretion has been confirmed. XeFGF mRNA is expressed just after fertilization and increases in amount up to gastrula. From its inducing ability, the FGF family is considered to be the natural ventral mesoderm inducer in the embryo.

Activin, a member of the transforming growth factor-ß (TGFß) superfamily (Fig. 2), was originally discovered as a peptide responsible for the activity of pituitary follicle-stimulating hormone (FSH). It is a dimer composed of two ß subunits (Ling *et al.*, 1986; Vale *et al.*, 1986). Because of the existence of two homologous, but nevertheless distinct ß subunits (termed $ß_A$ and $ß_B$), three isoforms of activin — activins A ($ß_A/ß_A$), AB ($ß_A/ß_B$), and B ($ß_B/ß_B$) — are possible. It has also been reported that activin A acts as erythroid differentiation factor (EDF) in a mouse erythroleukemia cell line (Eto *et al.*, 1987). Independent of those studies, we have been trying for a long time to isolate the mesoderm-inducing factor from carp swim bladder as well as culture fluids of human cell lines. The culture fluid of one strain (K-562) was partially purified by biochemical methods. We recognized that EDF activity and mesoderm-inducing activity remain associated through the purification process. We furthermore reported first that activin A (=EDF) has a potent ability to induce mesodermal tissues in early *Xenopus* embryos (Asashima *et al.*, 1989, 1990b; Nakano *et al.*, 1990).

Since then several mesoderm-inducing factors have been identified from various sources, such as the *Xenopus* XTC cell line (Smith *et al.*, 1990), murine myelomonocytic leukemia cells (Albano *et al.*, 1990), mouse macrophage cells (Mitrani *et al.*, 1990; Thomsen *et al.*, 1990), calf kidney (Asashima *et al.*, 1990a) and chicken embryos (Mitrani *et al.*, 1990; Asashima *et al.*, 1991c). They have been identified as homologs of activin A. Activin (XTC-MIF) treated ectoderm which is transplanted into the blastocoel of the early gastrula embryo (Einsteck method) elicits the formation of head structures with eyes and cement gland (Cho and DeRobertis, 1990). Activin mRNA is not expressed until the blastula stage (Dohrmann *et al.*, 1993). However, the presence of activin protein in unfertilized eggs and blastulae of *Xenopus* has been demonstrated (Asashima *et al.*, 1991b) even before the 16-cell stage of early embryos (Fukui *et al.*, 1994). Activin homologs are, indeed, contained in an egg of *Xenopus laevis* in a considerable amount (about 1 pg/egg) as a maternal protein. These activins appear to make a complex with follistatin, an activin-specific binding protein, remaining inactive until mesoderm induction. Thus, an endogenous activin may be one of the natural mesoderm-inducing factors acting in early *Xenopus* embryogenesis.

Follistatin was originally isolated from porcine follicular fluid based on its ability to suppress FSH secretion specifically from pituitary cultures (Ueno *et al.*, 1987). Later it was identified as an activin binding protein which suppresses the physiological activities of activin (Nakamura *et al.*, 1990). Follistatin is a single-chain, cysteine-rich protein, containing two glycosylated sites. It suppresses the mesoderm-inducing activity of activin (Asashima *et al.*, 1991a). In the presence of constant concentrations of activin, but with increasing concentrations of follistatin, using the piece culture method, we demonstrated that at higher follistatin concentrations cultures differentiated more into ventral mesodermal tissues than dorsal mesodermal tissues (Asashima *et al.*, 1991c; Fukui *et al.*, 1993). Follistatin is presently the only known physiological regulatory factor for activin.

Our most recent studies demonstrated the presence of both activin and follistatin protein before the 16-cell stage in the early *Xenopus* embryo (Fukui *et al.*, 1994). Three kinds of *Xenopus* activin isoforms, activins A, AB and B were observed in very early *Xenopus* (st 1-5). A sufficient amount of follistatin is also present at high enough concentrations to suppress the activin activity. In a cleavage stage embryo about 1 pg (total) of the activins, A, AB and B have been estimated, and about 40 pg of follistatin exists for making a complex in an egg. These activins and follistatin proteins can also be detected by immunohistological examination. Though the accumulation mechanism during oogenesis is not yet clear, the storage of these maternal proteins might be related to the polarity of the egg mentioned in the introduction.

Inducing potency of activin

Activin has been demonstrated to induce all mesodermal tissues at nanomolar concentrations (Asashima *et al.*, 1990b). A dose dependency is however observed; low levels of activin (concentration of approx. 0.1 ng/ml) cause presumptive ectoderm explants to differentiate into ventral mesodermal tissues, including mesenchyme, coelomic epithelium (same as mesothelium), and blood-like cells. Medium concentrations (approx. 1 ng/ml) of activin cause explants to differentiate into various mesodermal tissues such as mesenchyme, muscle, coelomic epithelium and secondary-induced neural tissues. At a high concentration (approx. 10 ng/ml) dorsal tissue such as notochord was induced. Activin thus induced all mesodermal tissues in a dose-dependent manner, indicating the presence of a gradient.

Low dose-induced ventral mesodermal structures and high dose-induced dorsal mesodermal structures (Green and Smith, 1990; Ariizumi *et al.*, 1991a,b; Nakamura *et al.*, 1992; Fukui *et al.*, 1993) (Fig. 3). The minimum concentration of activin A to induce mesodermal tissues was inversely proportional to its treatment time. The explants differentiated into different types of mesodermal tissues, from the ventral-type to the dorsal-type depending on the concentration of activin and its treatment time (Ariizumi *et al.*, 1991b). Activins may be the natural mesoderm inducer, and thus it may be responsible for establishing axial organization in the amphibian embryo.

Though the above described mesoderm induction by activin is remarked from the histological level, several biochemical and molecular biological approaches have been reported. Activins can induce in the explants several kinds of homeobox genes such as *Mix*.1, *goosecoid*, *Xlim-1*, *XFKH*, oncogene related genes such as *Xwnt*-8, and key differentiation genes such as MyoD, α-actin and myosin in muscle differentiation. Almost all of the activin-responsive genes are expressed in the dorsal region of the blastula stage embryo (Table 1). Activin can also act on explanted blastomeres to induce tissues ranging from posterolateral mesoderm to dorsoanterior organizer mesoderm. By contrast, FGF induces only posterolateral markers and does so over relatively broad dose ranges (Green *et al.*, 1992).

There are many reports about gene expression in explants after activin A treatment (Dawid *et al.*, 1992; Kinoshita and Asashima, 1994). The genes expressed in the explants (animal cap) by activin A are all observed in normal embryogenesis, and the order of these expressed genes is also the same during normal development. Thus gene expression induced by activin treatment of the animal cap seems to really mimic the cell differentiation and morphogenesis which occurs during normal development.

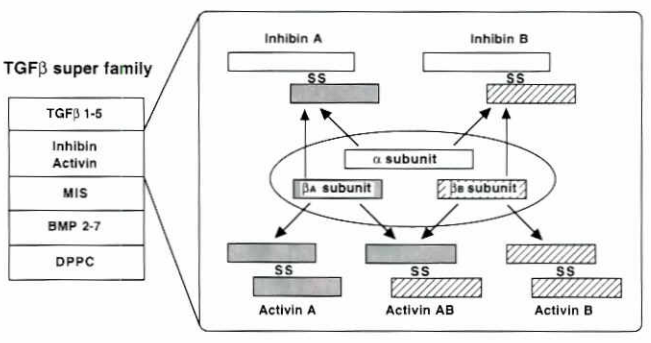

Fig. 2. Diagram of chemical structure of activins and inhibins which belong to TGFß superfamily proteins. *In TGFß superfamily we can observe the well conserved cysteine residues in their amino acid sequences. MIS: Müllerian inhibitory substance, BMP: bone morphogenetic protein, DPPC: decapentaplegic gene complex.*

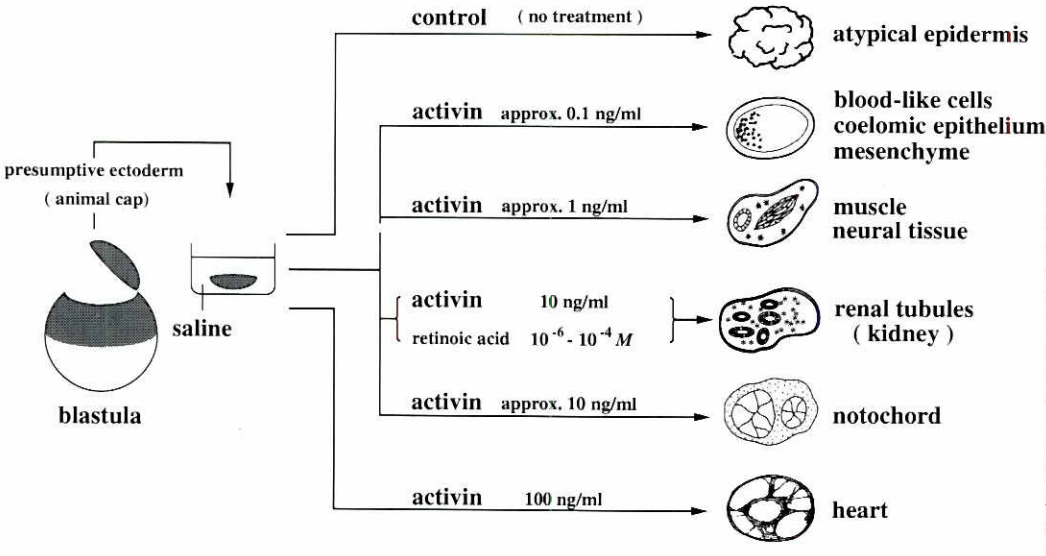

Fig. 3. Diagrammatic representation of the animal cap assay and explants after activin treatment. *Depending on the activin concentration, many mesodermal tissues from ventral type mesoderm (low concentration of activin treatment) to dorsal type mesoderm (middle or high concentration of activin treatment) are induced (Asashima et al., 1990; Ariizumi et al., 1991a,b; Nakamura et al., 1992; Fukui et al., 1993). High concentration of activin also induced the beating heart in the explant (Moriya and Asashima 1992) and the combination of activin and retinoic acid induced the renal tubules (Moriya et al., 1993).*

Receptors and signal transduction pathway of mesoderm-inducing factor

The onset of mesoderm induction occurs in the early blastula, prior to the MBT. The structure of activin receptors has been demonstrated by cDNA cloning from mammalian cell lines (Mathews and Vale, 1991) and has shed light on research into the signal transduction mechanism of activin. This receptor appears to have the predicted structure of a transmembrane ligand-activated protein serine/threonine kinase. These findings raise the possibility that a new class of receptor-coupled kinases may play a central role in signal transduction by members of the TGFß family.

The presence of activin receptor isoforms has also been reported (Attisano *et al.*, 1992) and the possibility that heterogeneity of the isoforms may underlie the dose- and cell-specific features of activin function has been discussed.

The sequence of the type II *Xenopus* activin receptor genes (XactRII) has also been established (Kondo *et al.*, 1991; Hemmati-Brivanlou *et al.*, 1992; Mathews *et al.*, 1992; Nishimatsu *et al.*, 1992). XAR7, one of the XactRII genes, is represented as a transcript in the embryo from the oocyte to the tailbud stage, and has 87% homology at the level of deduced amino acid sequence with the mouse activin receptor ActRII (Kondo *et al.*, 1991). In addition, when cloned XactRIIB mRNA was injected into one of the embryo's ventral blastomeres, a secondary body axis was induced (Mathews *et al.*, 1992). On the other hand, XAR1, which is highly

TABLE 1

EXPRESSION OF SEVERAL KINDS OF GENES IN *XENOPUS* EMBRYOGENESIS BEFORE GASTRULATION

		genes expressed from/ after MBT	activin responsive genes	FGF responsive genes	expression region at pre-gastrulation	reference
homeobox genes	goosecoid (sc)	+* (St. 9)	+	—	blastopore lip	Blumberg *et al.* (1991) Cho *et al.* (1991)
	Mix.1	+	+	—	vegetal half	Rosa (1989)
	Lim-1	+	+	—	dorsal mesoderm	Taira *et al.* (1992)
	Xhox3	+	+	+	mesoderm?	Ruiz i Altaba and Melton (1989)
	Xnot	+	+	+	blastopore lip	von Dassow *et al.* (1993)
	XFKH1	+	+	+	dorsal mesoderm	Dirksen and Jamrich (1992)
	Xtwi	+	N.D.	N.D.		Hopwood *et al.* (1989)
	Xlab	+ (St.10)	N.D.	N.D.		Sive and Cheng (1991)
Brachyury (T)	brachyuty (Xbra)	+	+	+	marginal zone-notochord	Smith *et al.* (1991)
secretory factors	Xwnt-8	+	+	N.D.	ventral marginal zone	Smith and Harland (1991) Sokol *et al.* (1991)
	noggin	+*	+	N.D.	dorsal marginal zone	Smith *et al.* (1992)

*genes which can be detected from cleavage stage but at a very low expression level; N.D., no data.

homologous to XactRIIB (Hemmati-Brivanlou *et al.*, 1992), is expressed continuously in the ovary, unfertilized egg and neurula stage embryo. The maternal mRNA is found uniformly in the early embryo, but analysis by *in situ* hybridization showed that the XactRIIB mRNA does not localize before blastula, and is restricted to the neural plate region at the neurula stage. These findings suggest that activins also play a role in both neural tube formation and mesoderm induction much earlier, at the early cleavage stage.

Moreover, a dominant negative activin receptor, a receptor molecule with its cytoplasmic domain truncated so that its capacity to respond to ligand is abolished, was constructed and overexpressed in the *Xenopus* embryo (Hemmati-Brivanlou and Melton, 1992). This dominant negative experiment yielded embryos that cannot form axial structures. This observation suggests that activin is required for both the induction of mesoderm and the patterning of the embryonic body plan *in vivo*.

It can easily be speculated, therefore, that activin, follistatin, and activin receptors participate in a regulatory circuit.

It is known that the FGF receptor has an extracellular ligand-binding domain containing a globin-like sequence, and cytoplasmic tyrosine kinase domain. Disruption of the FGF signaling pathway by expression of a dominant negative construct of the FGF receptor generally results in gastrulation defects that are later evident during formation of the trunk and tail, although head structures are formed nearly normally (Amaya *et al.*, 1991). This phenotype resembles the result from cultured explants of neural plate from which the archenteric roof was discarded. In those experiments it was reported that this dominant negative receptor inhibits the expression of *Xbra*. It does not, however, inhibit *goosecoid*, the dorsal lip marker (Amaya *et al.*, 1993). Those results suggest that the intracellular signal pathway of FGF are different from activin. Perhaps the two candidates for the natural mesoderm-inducing factor, activin and FGF, may act cooperatively during embryogenesis (Fig. 4).

Many intracellular signal-transducing molecules have been observed, and recent studies have shown that *ras* and *raf*-1 proto-oncogenes are involved in mesoderm signal transduction. The *ras* and *raf*-1 products (Ras and Raf-1) are indispensable sequential elements in the transduction of growth and differentiation signals initiated by receptor and non-receptor tyrosine kinases. Raf has been positioned downstream of Ras in numerous signal transduction pathways, and Ras interacts directly with the Raf (Vojtek *et al.*, 1993). Whitman and Melton (1992) demonstrated using microinjection of RNAs encoding $p21^{ras}$ variants and the piece culture method that dominant inhibitory ras affects the inhibition of FGF and activin signaling for mesoderm induction. Constitutively active *ras* injected explants show differentiation alone without mesoderm-inducing factors. These results do not show that the *ras* signaling pathway depends upon natural mesoderm induction, but suggests that a *ras* dependent signaling step exists in the mesoderm-inducing pathway. A more direct experiment was done using Raf-1 serine/threonine kinase (MacNicol *et al.*, 1993). Animal cap explants injected with dominant negative Raf-1 mutant (NAF) demonstrated a complete block to bFGF-stimulated mesoderm induction, but NAF had no effect on the activin-stimulated formation of mesoderm. Injection of NAF RNA into embryos blocked normal development, and the phenotype induced by NAF showed posterior truncations in the tadpole such as the injection of dominant negative mutants of FGF receptor in *Xenopus* embryos. The failure of NAF to inhibit activin-stimulated mesoderm induction suggests that Raf-1 is not an obligatory component of the activin receptor signaling pathway.

Recently, another type of activin receptor type I (ActRI) has been cloned (Attisano *et al.*, 1993). Though the relationship between ActRI and ActRII receptors is not clear, inducing signals seem to move into the nucleus through two types of activin receptors. Kinase activity related with these receptors needs to be examined more at the molecular level. In eggs signal transduction pathways seem to be not strict but are flexible in regulating the signals which control sequential gene expressions during development.

Sequential gene expression in development

Several genes that are expressed in the early *Xenopus* embryo have been cloned. Some of these genes have been classified

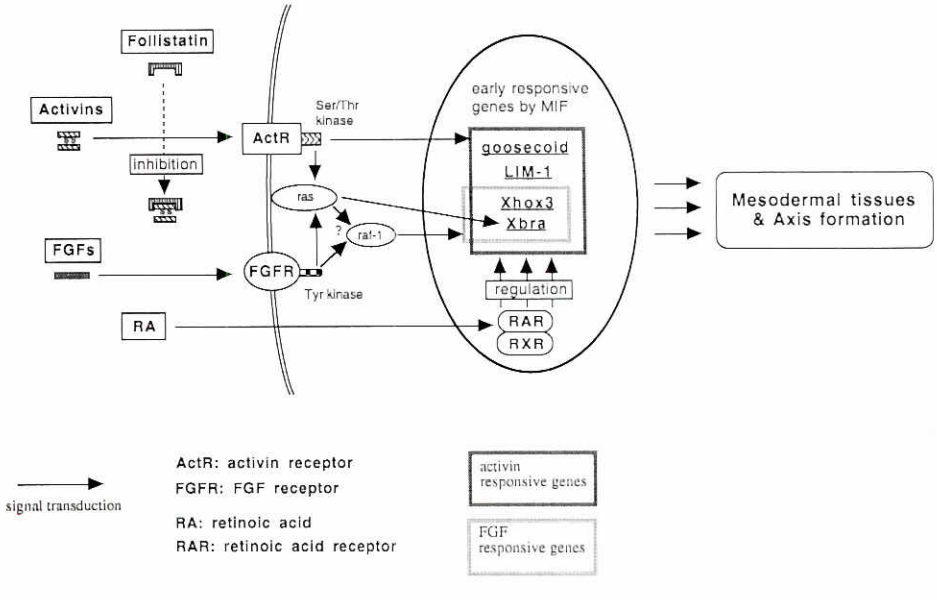

Fig. 4. Diagram of regulation of mesoderm-inducing factor (MIF) and signal transduction. *Activins bind with follistatin to make inactive form. MIFs activate their own receptors to transfer their signals into the cytoplasm and nucleus to express the early response genes and retinoic acid (RA) modulates the MIFs signals.*

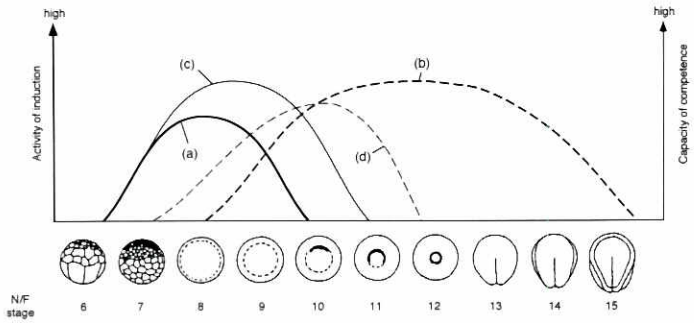

Fig. 5. Changes in inducing activity and capacity of competence following the development in *Xenopus laevis*. **(a)** *Mesoderm inducing activity from vegetal hemisphere to animal hemisphere.* **(b)** *Neural inducing activity from mesoderm layer to ectoderm layer.* **(c)** *Competence for mesoderm differentiation in ectoderm.* **(d)** *Competence for neural differentiation in ectoderm.*

either as coding DNA binding factors or secretory factors (Table 1). Distinctions between those various DNA binding factors — i.e., genes containing homeobox region or *brachyury* gene (Kispert and Herrmann, 1993) — include increasing levels of expression levels from the MBT onwards, response to FGF or activin, and localized activin responsive genes expression (generally in the dorsal marginal region of early gastrulae). It is known that some of those genes are expressed in the presence of cycloheximide, a protein synthesis inhibitor. This suggests the presence of an immediate, early response mechanism to a mesoderm-inducing factor in the early blastomeres of the embryo. Among those genes, *Xlim-1* is activated by retinoic acid alone.

Wnt family members and Noggin are secretory factors. That is, they possess a hydrophobic signal peptide sequence at the amino terminus (Christian *et al.*, 1991; Smith and Harland, 1991, 1992). The mRNAs for these factors rescue axis formation in ultraviolet-irradiated embryos when injected into the marginal zone (Sokol *et al.*, 1991; Smith and Harland, 1991, 1992). The expression pattern of *noggin* is especially interesting. It is not localized maternally, but becomes restricted to the dorsal mesoderm region after the MBT. The gene *noggin* was isolated from a *Xenopus* expression library and shown to be expressed in the dorsal midline of the mesoderm during gastrulation (Smith and Harland, 1992). When tested using the ventral mesoderm assay, *noggin* protein showed dorsalizing activity and neuralizing activity (Lamb *et al.*, 1993; Smith *et al.*, 1993). *noggin* protein also has an important role through association with Wnt family, activin, FGF in the process of early development.

Anyway, *noggin* and *Xwnt*-8 are expressed activin-treated piece cultures. Activin and FGF are most likely situated in an upstream position in a cascade of gene expression, whereas *noggin* and Xwnt family are downstream genes which are controlled as early response genes.

Retinoic acid as a modulator of development

Retinoids appear to play a major role in embryogenesis and differentiation. Retinoic acid (RA), a derivative of retinol, can modify formation of the chick limb bud and budding of the ascidian embryo. It is known that *Xenopus* embryos treated with RA exhibit defective head formation (Durston *et al.*, 1989). It has also been reported that expression levels of homeobox box genes such as *goosecoid*, *Xlim-1* and *X1hbox-6* (Cho and DeRobertis, 1990) change after RA treatment, using the piece culture method. Expression levels of *X1hbox-6* and *Xlim-1* are increased after RA treatment. *goosecoid* expression is lower after the treatment with activin (XTC-MIF) and RA than after the treatment with activin (XTC-MIF) alone.

RA modulates these activin effects. Presumptive ectoderm treated with activin and RA differentiates pronephric tubules (Moriya *et al.*, 1993). Activin at a concentration of 10 ng/ml induces notochord in presumptive ectoderm. In combination with 10^{-6} *M* RA, muscle is induced instead of notochord. In combination with 10^{-5} *M* RA, activin frequently induced pronephric tubules. RA appears to be the modular of the lateralization related to axis formation in embryogenesis.

Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are ligand-inducible trans regulators that modulate the transcription of target genes by interacting with cis-acting DNA response elements. RAR-RXR heterodimers form more efficiently than homodimers (Durand *et al.*, 1992). The presence of RA at 1.5×10^{-7} *M* in the *Xenopus* embryo after gastrulation has been reported by Durston *et al.* (1989). Two types of RAR genes, RARα and RARγ, and two types of RXR genes, RXRα and RXRγ, have been isolated from unfertilized *Xenopus* eggs (Blumberg *et al.*, 1992). RXRγ and RARα are synthesized during oogenesis and persist in the cleaving embryo at approximately constant levels until they are degraded just before gastrulation (stage 10), suggesting the regulation of gene expression by the retinoid.

Role of the determinant and axis formation

Components of a cell that commit it and its descendants to a particular pathway of differentiation are generally referred to as "determinants". During the first cell cycle after fertilization the cortex of the egg rotates about 30 degrees relative to the inner cytoplasm. This rotation is essential for the establishment of the dorsal-ventral axis and for the production of dorsoanterior structures in the embryo. UV irradiation of fertilized eggs blocks the cortical rotation and eliminates dorsal mesoderm and dorsoanterior structures in irradiated embryos. It is generally accepted that axis specification depends both on the presence of an axis-inducing determinant and on activation by sperm-mediated cortical rotation. It has been reported that a determinant in the vegetal pole of an uncleaved *Xenopus* egg is moved to the equatorial region by cortical rotation (Fujisue *et al.*, 1993). The molecular nature of the determinant is unknown but it has been demonstrated that cytoplasm of the presumptive dorsal cell of an embryo contains a determinant that is responsible for dorsoventral axis specification.

Candidate genes for that determinant exist. *Vg1* is a maternal mRNA that is localized in the vegetal hemisphere of the developing *Xenopus* egg (Weeks and Melton, 1987). *Vg1* mRNA is synthesized during early oogenesis and translocated to the vegetal cortex of fully grown oocytes (Melton, 1987), and consequently *Vg1* mRNA and protein become partitioned within cells of the vegetal hemisphere during early development. Thus *Vg1* mRNA and protein are localized to cells that induce embryonic mesoderm, and the protein is a member of a family of cell growth and differentiation factors, the TGFßs, other members of which have the ability to induce mesoderm. Moreover, a genetically engineered constructed chimeric mRNA containing a latent associated region of bone

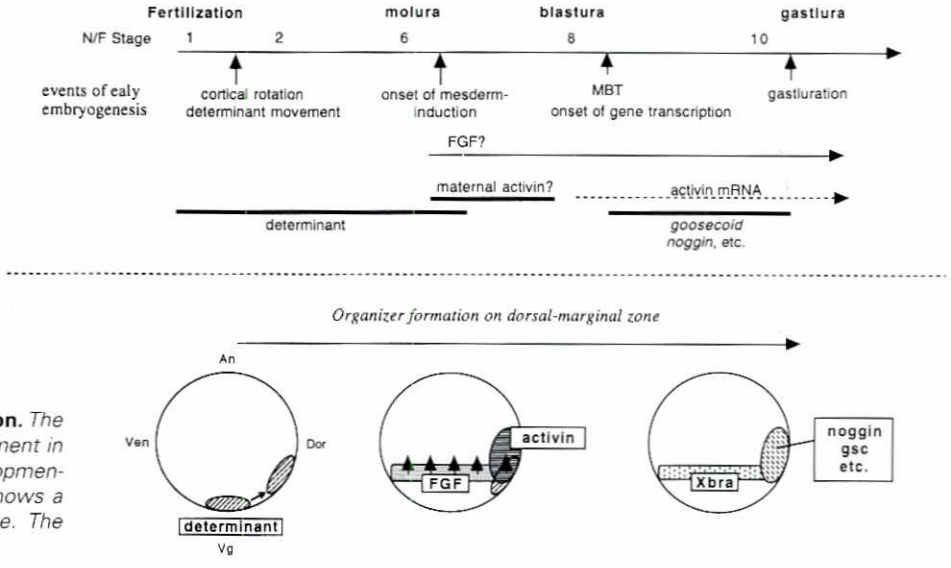

Fig. 6. Model of a process of organizer formation. *The upper figure shows an outline of early development in Xenopus and factors acting throughout the developmental stage based on the reported data. Lower shows a hypothetical model supporting the upper figure. The model is described more fully in the text.*

morphogenetic protein-4 (BMP4) and mature region of *Vg1* was injected (Thomsen and Melton, 1993). When this chimeric mRNA was injected into the ventral cell of *Xenopus* a secondary axis developed. Despite these suggestive observations, no function for Vg1 protein has yet been assigned.

Candidates for determinants in unfertilized eggs include factors such as *Vg1*, FGF, activin, noggin, retinoic acid, Xwnt family, BMP as well as structures and other cell organelles such as yolk platelets, mitochondria and ER. It will be important to establish the relationship or the interactions between these factors and cell organelles in cell differentiation and morphogenesis.

Acquisition and diminution of competence

During embryogenesis from a fertilized egg to a larva, the developmental programs are deployed with a relationship between the inducing factors and competent cells. The first regional differentiation is discernible as separations of presumptive ectoderm, mesoderm and endoderm cells. According to Holtfreter and Hamburger (1955), competence is "the physiological state of a tissue which permits it to react in a morphogenetically specific way to determinative stimuli".

Tissue or cell differentiation is generally due both to intrinsic factors in the competence cells and to extrinsic inducing factors such as activin. Concerning inducing factors, it is important to know what kind of inducing factors are involved and where parts of the embryo are activated in the process of development. So, it is also very important to know the state of the competent cell. The competence of cells during development changes (Fig. 5). It is known that animal-half cells change their responding ability (= competence) against mesoderm-inducing factor or neuralizing factor (Chuang, 1955; Kuusi, 1961). Gastrula ectoderm, isolated from *Xenopus laevis*, consists of two cell sheets, representing a superficial and a deep layer. An endodermal character of the deep layer can be ruled out by induction experiments with vegetalizing factor (= activin-like factor). Under the influence of vegetalizing factor the outer as well as the inner ectoderm layer differentiated into mesodermal tissues. The results of experiments with dorsal blastopore lip as inducer indicate that both inner and outer ectoderm layers are responsive to the neural stimulus (Asashima and Grunz, 1983).

When animal cap cells of *Xenopus* blastulae were exposed to activin A, low concentrations induced ventrolateral mesoderm, whereas high concentrations induced formation of dorsal mesoderm (Green and Smith, 1990; Ariizumi *et al.*, 1991b; Green *et al.*, 1992; Fukui *et al.*, 1993). These results suggested that the blastula animal cap may consist of multipotent cells that can form all states ranging from posterolateral mesoderm to dorsoanterior organizer mesoderm in response to activin. Animal-half blastomeres from the late 8-cell stage *Xenopus* embryo have been isolated, and these blastomeres have been examined for their response to activin A (Kinoshita *et al.*, 1993). Dorsal blastomeres from the 8-cell stage gave rise to trunk and tail structures containing dorsal mesoderm, whereas the ventral blastomere explants from 8-cell stage formed spheres containing solely ventral mesoderm. Both muscle actin transcription and *goosecoid* transcription were induced primarily in dorsal blastomeres. These results suggest that a competence prepattern of response to activin exists as early as the 8-cell stage. But at the moment we cannot find any difference in the molecular substance between dorsal cells and ventral cells. For the receptor of activin type II in the cell membrane, no difference has been found. The signal transduction or metabolism in the cytoplasm and nucleus in both cells are not clear at the present time. Though there are clearly responding cells and a prepattern in ventral and dorsal cells during development, the mechanism which generates competent cells at the molecular level is not clear.

Formation of the organizer

Differentiation of tissues and organized formation of organs can be understood as a system of serial inductive reactions originating from the organizer. Some exploratory experiments have demonstrated that only dorsal marginal zone can form the axial mesoderm with self-differentiation before gastrulation.

Muscle precursor cells from early, mid-, and late gastrula stages of *Xenopus* embryos were isolated and transplanted singly into the ventral region of late gastrula hosts (Kato and Gurdon, 1993). Single cells from late gastrulae differentiated into muscle when

surrounded by nonmuscle cells. Similar cells from early or mid-gastrula did not, unless they were transplanted as a group of adjacent cells taken from the same region of an embryo. These results suggest that the pre-gastrula embryo did not determine muscle differentiation in the presumptive somite region of fate map. Therefore, mesoderm induction of normal embryo represents "determination of Spemann's organizer".

Now, there is not sufficient evidence to construct a model for "formation of the organizer". However, we have attempted to adapt the results from recent studies to normal *Xenopus* development (see Fig. 6). The upper arrow indicates the actual time of normal development using Nieuwkoop and Faber's staging series (1967). Formation of the organizer can be understood as a cascade of competence and molecular pathways. At first, cortical rotation with rearrangement of cytoplasmic factors occurs and morphogenetic determinants shift from the vegetal pole to the dorsal region. Mesoderm induction does not occur at this stage. The potency of the determinant is lost at about the 128-cell stage (Fujisue *et al.*, 1993). Mesoderm induction may occur in the early blastula (Jones and Woodland, 1987). Competence of animal blastomeres to activin or natural inducing factors is obtained at this stage between the 32- and 64-cell stage (Kinoshita *et al.*, 1993; Bessho and Asashima, unpublished data). The onset of embryonal FGF synthesis and activation of maternal activins suppressed by follistatin may occur at this stage. It is suspected that activin acted on dorsal mesoderm as a presumptive organizer region and FGF acted on the whole marginal zone.

Then the regionally specific gene expression described previously occurred through the MBT period. Some genes exist maternally, but few mRNAs and their proteins have been recognized, so we must wait for further investigations before discussion. It is clear that secretory factors which may correlate with axis formation, such as *Xwnt* and *noggin*, increase gene expression levels at this stage. Many DNA binding factors responding to mesoderm-inducing factor such as activin or FGF also commence expression (see Table 1).

The inducing potency of the organizer can be expected to have a close connection with embryonic morphogenesis. This idea is supported by the fact that after invagination of the organizer region, remarkable morphogenetic movement may be observed in the course of archenteric roof formation, and this has a close relationship to the regional inductive effect of the organizer. In normogenesis the uninvaginated blastopore lip of the early gastrula develops into the frontal part of the archenteric roof and induces differentiation of the archencephalic region of the central nervous system. But when the blastopore lip is isolated before invagination and cultured with the ectodermal sheets, spinocaudal differentiation occurs, including formation of the notochord. This seems closely connected with the fact that when the blastopore lip is isolated before invagination, it undergoes a remarkable elongational movement in subsequent development. In contrast to the frontal region of the archenteric roof which induces the brain and is formed by proliferation, the rear region of the archenteric roof, which induces the spinal cord, is the tissue formed by the elongating movement (Hama, 1949, 1950).

Conclusion

Although at the present time many findings on mesoderm-inducing factors and receptors of amphibian embryos have been reported, the general problem of the mechanism of embryonic induction remains unsolved.

Is there any relationship between the elongation movements which occur in gastrulation and cell differentiation? What are the differences in the molecular signals and competence between head organizer and trunk-tail organizer? Concerning these issues, it is important to ask what factors are controlling the formation of the body plan, formation of the organizer, neural induction, determination of segmentation? In the process of embryonic development, many genes are connected or related to each other in a sequential chain of induction. Some genes play a major role and initiate cell differentiation and morphogenesis, but some genes appear in the shade, or behind, other genes which drive the process of development.

Many factors such as FGF, activins, RA, Xwnt, which are related with embryonic inductions, have been reported. These factors play a very important role in normal embryogenesis, but they also have important functions in the developmental process throughout the life cycle. For example, activin genes or proteins appear to play some roles throughout the development. It is very interesting that these substances appear in the key processes or stages such as oogenesis, mesoderm induction, gastrulation, limb bud formation and the adult changing function, while using the same genes. Indeed, activins successfully unite classical embryology and endocrinology and molecular developmental biology.

We need to understand the dynamic behavior of these key substances during development. We must also investigate new signaling genes or signaling systems which relate competence to embryonic development, and connect molecules and biological phenomena.

Acknowledgments

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

References

ALBANO, R.M., GODSAVE, S.F., HUYLEBROECK, D., VAN NIMMEN, K., ISAACS, H.V., SLACK, J.M.W. and SMITH, J.C. (1990). A mesoderm-inducing factor produced by WEHI-3 murine myelomonocytic leukemia cells is activin A. *Development 110:* 435-443.

AMAYA, E., MUSCI, T.J. and KIRSCHNER, M.W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. *Cell 66:* 257-270.

AMAYA, E., STEIN, P.A., MUSCI, T.J. and KIRSCHNER, M.W. (1993). FGF signaling in the early specification of mesoderm in *Xenopus*. *Development 118:* 477-487.

ARIIZUMI, T., MORIYA, N., UCHIYAMA, H. and ASASHIMA, M. (1991a). Concentration-dependent inducing activity of Activin A. *Roux Arch. Dev. Biol. 200:* 230-233.

ARIIZUMI, T., SAWAMURA, K., UCHIYAMA, H. and ASASHIMA, M. (1991b). Dose and time-dependent mesoderm induction and outgrowth formation by activin A in *Xenopus laevis*. *Int. J. Dev. Biol. 35:* 407-414.

ASASHIMA, M. (1975). Inducing effects of the presumptive endoderm of successive stages in *Triturus alpestris*. *Roux Arch. Dev. Biol. 177:* 301-308.

ASASHIMA, M. and GRUNZ, H. (1983). Effects of inducers on inner and outer gastrula ectoderm layers of *Xenopus laevis*. *Differentiation 23:* 206-212.

ASASHIMA, M., NAKANO, H., SHIMADA, K., KINOSHITA, K., ISHII, K., SHIBAI, H. and UENO, N. (1990a). Mesodermal induction in early amphibian embryos by activin A. *Roux Arch. Dev. Biol. 198:* 330-335.

ASASHIMA, M., NAKANO, H., UCHIYAMA, H., DAVIDS, M., PLESSOW, S., LOPPNOW-BLINDE, B., HOPPE, P., DAU, H. and TIEDEMANN, H. (1990b). The vegetalizing factor belongs to a family of mesoderm-inducing proteins related to erythroid differentiation factor. *Naturwissenschaften 77:* 389-391.

ASASHIMA, M., NAKANO, H., UCHIYAMA, H., SUGINO, H., NAKAMURA, T., ETO, Y., EJIMA, D., DAVIDS, M., PLESSOW, S., CICHOCKA, I. and KINOSHITA, K. (1991a). Follistatin inhibits the mesoderm-inducing activity of activin A and the vegetalizing factor from chicken embryo. *Roux Arch. Dev. Biol. 200:* 4-7.

ASASHIMA, M., NAKANO, H., UCHIYAMA, H., SUGINO, H., NAKAMURA, T., ETO, Y., EJIMA, D., NISHIMATSU, S., UENO, N. and KINOSHITA, K. (1991b). Presence of activin (erythroid differentiation factor) in unfertilized eggs and blastulae of *Xenopus laevis*. *Proc. Natl.Acad. Sci. USA 88:* 6511-6514.

ASASHIMA, M., SHIMADA, K., NAKANO, H., KINOSHITA, K. and UENO, N. (1989). Mesoderm induction by activin A (EDF) in *Xenopus* early embryo. *Cell Differ. Dev. (Suppl.) 27:* 53.

ASASHIMA, M., UCHIYAMA, H., NAKANO, H., ETO, Y., EJIMA, D., SUGINO, H., DAVIDS, M., PLESSOW, S., BORN, J., HOPPE, P., TIEDEMANN, H. and TIEDEMANN, H. (1991). The vegetalizing factor from chicken embryos: its EDF (activin A)-like activity. *Mech. Dev. 34:* 135-141.

ATTISANO, L., CÁRCAMO, J., VENTURA, F., WEIS, F.M.B., MASSAGUÉ, J. and WRANA, J.L. (1993). Identification of human activin and TGFß type I receptors that form heteromeric kinase complexes with type II receptors. *Cell 75:* 671-680.

ATTISANO, L., WRANA, J.L., CHEIFETZ, S. and MASSAGUÉ, J. (1992). Novel activin receptors: distinct genes and alternative mRNA splicing generate a repertoire of serine/threonine kinase receptors. *Cell 68:* 97-108.

BECKER, U. and TIEDEMANN, H. (1961). Zell- und Organdetermination in der Gewebekultur, ausgeführt am presumptiven Ektoderm der Amphibiengastrula. *Verhandl. Deut. Zool. Ges. 25:* 259-267.

BLUMBERG, B., MANGELSDORF, D.J., DYCK, J.A., BITTNER, D.A., EVANS, R.M. and DE ROBERTIS, E.M. (1992). Multiple retinoid-responsive receptors in a single cell: families of retinoid "X" receptors and retinoic acid receptors in the *Xenopus* egg. *Proc. Natl. Acad. Sci. USA 89:* 2321-2325.

BLUMBERG, B., WRIGHT, C.V.E., DE ROBERTIS, E.M. and CHO, K.W.Y. (1991). Organizer-specific homeobox genes in *Xenopus laevis* embryos. *Science 253:* 194-196.

CHO, K.W.Y. and DE ROBERTIS, E.M. (1990). Differential activation of *Xenopus* homeobox genes by mesoderm inducing growth factors and retinoic acid. *Genes Dev. 4*: 1910-1916.

CHO, K.W.Y., BLUMBERG, B., STEINBEISSER, H. and DE ROBERTIS, E.M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid*. *Cell 67:* 1111-1120.

CHRISTIAN, J.L., McMAHON, J.A., McMAHON, A.P. and MOON, R.T. (1991). Xwnt-8, a *Xenopus* Wnt-1/int-1 related gene responsive to mesoderm-inducing growth factors, may play a role in ventral patterning during embryogenesis. *Development 111:* 1045-1055.

CHUANG, H.H. (1955). Untersuchungen über die Reaktionsfähigkeit des Ektoderms mittels sublethaler Cytolyse. *J. Acad. Sinica 4:* 151-186. (In Chinese with German summary).

DAWID, I.B., TAIRA, M., GOOD, P.J. and REBAGLIATI, M.R. (1992). The role of growth factors in embryonic induction in *Xenopus laevis*. *Mol. Reprod. Dev. 32:* 136-144.

DIRKSEN, M.L. and JAMRICH, M. (1992). A novel, activin-inducible, blastopore lip-specific gene of *Xenopus laevis* contains a fork head DNA-binding domain. *Genes Dev. 6:* 599-608.

DOHRMANN, C.E., HEMMATI-BRIVANLOU, A., THOMSEN, G.H., FIELDS, A., WOOLF, T. and MELTON, D.A. (1993). Expression of activin mRNA during early development in *Xenopus laevis*. *Dev. Biol. 157:* 474-483.

DURAND, B., SAUNDERS, M., LEROY, P., LEID, M. and CHAMBON, P. (1992). All-trans and 9-cis retinoic acid induction of CRABPII transcription is mediated by RAR-RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell 71:* 73-85.

DURSTON, A.J., TIMMERMANS, J.P.M., HAGE, W.J., HENDRIKS, H.F.J., DEVRIES, N.J., HEIDEVELD, M. and NIEUWKOOP, P.D. (1989). Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature 340:* 140-144.

ETO, Y., TSUJI, T., TAKEZAWA, M., TAKANO, S., YOKOGAWA, Y. and SHIBAI, H. (1987). Purification and characterization of erythroid differentiation factor (EDF) isolated from human leukemia cell line THP-1. *Biochem. Biophys. Res. Commun. 142:* 1095-1103.

FUJISUE, M., KOBAYAKAWA, Y. and YAMANA, K. (1993). Occurrence 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.

FUKUI, A., NAKAMURA, T., SUGINO, K., TAKIO, K., UCHIYAMA, H., ASASHIMA, M. and SUGINO, H. (1993). Isolation and characterization of *Xenopus* follistatin and activin. *Dev. Biol. 158:* 131-139.

FUKUI, A., NAKAMURA, T., UCHIYAMA, H., SUGINO, K., SUGINO, H. and ASASHIMA, M. (1994). Identification of activins A, AB and B and follistatin proteins in the *Xenopus* embryos. *Dev. Biol. 163:* 279-281.

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 (Suppl.):* 37-51.

GREEN, J.B.A. and SMITH, J.C. (1990). Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature 347:* 391-394.

GREEN, J.B.A., HOWES, G., SYMES, K., COOKE, J. and SMITH, J.C. (1990). The biological effects of XTC-MIF: quantitative comparison with *Xenopus* bFGF. *Development 108:* 173-183.

GREEN, J.B.A., NEW, H.V. and SMITH, J.C. (1992). Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell 71:* 731-739.

GURDON, J.B. (1987). Embryonic induction — molecular prospects. *Development 99:* 285-306.

HAMA, T. (1949). Explantation of the urodelen organizer and the process of morphological differentiation attendant upon invagination. *Proc. Jpn. Acad. 25:* 4-11.

HAMA, T. (1950). Morphogenesis of vertebrate, II. Studies on the early development of the urodele. *Science (Japan) 19:* 539-545. (In Japanese).

HEMMATI-BRIVANLOU, A. and MELTON, D.A. (1992). A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos. *Nature 359:* 609-614.

HEMMATI-BRIVANLOU, A., WRIGHT, D.A. and MELTON, D.A. (1992). Embryonic expression and functional analysis of a *Xenopus* activin receptor. *Dev. Dynamics 194:* 1-11.

HOLTFRETER, J. (1933) Organisierungsstufen nach regional Kombination von Entomesoderm mit Ektoderm. *Biol. Zbl. 53:* 404-431.

HOLTFRETER, J. and HAMBURGER, V. (1955) Embryogenesis: progressive differentiation. Amphibians. In *Analysis of Development* (Eds. B.H. Willier, P.A. Weiss and V. Hamburger). Sanders, Philadelphia and London, pp.230-296.

HOPWOOD, N.D., PLUCK, A. and GURDON, J.B. (1989). A *Xenopus* mRNA related to *Drosophila* tracers. II. Experiments with $Na_2S^{35}O_4$ and methionine-^{35}S. *Arch. Soc. Zool.-bot. Fenn. Vanamo 14:* 4-28.

LAMB, T.M., KNECHT, A.K., SMITH, W.C., STACHEL, S.E., ECONOMIDES, A.N., STAHL, N., YANCOPOLOUS, G.D. and HARLAND, R.M. (1993). Neural induction by the secreted polypeptide Noggin. *Science 262:* 713-718.

LING, N., YING, S.Y., UENO, N., SHIMASAKI, S., ESCH, F., HOTTA, M. and GUILLEMIN, R. (1986). Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. *Nature 321:* 779-782.

MacNICOL, A.M., MUSLIN, A.J. and WILLIAMS, L.T. (1993). Raf-1 kinase is essential for early *Xenopus* development and mediates the induction of mesoderm by FGF. *Cell 73:* 571-583.

MANGOLD, O. (1923). Transplantationsversuche zur Frage der Spezifität und der Bildung der Keimblätter. *Arch. Mikrosk. Anat. EntwMech. 100:* 193-301.

MATHEWS, L.S. and VALE, W.W. (1991). Expression cloning of an activin receptor, a predicted transmembrane serine kinase. *Cell 65:* 973-982.

MATHEWS, L.S., VALE, W.W. and KINTNER, C.R. (1992). Cloning of a second type of activin receptor and functional characterization in *Xenopus* embryos. *Science 255:* 1702-1705.

MELTON, D.A. (1987). Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. *Nature 328:* 80-81.

MITRANI, E., ZIV, T., THOMSEN, G., SHIMONI, Y., MELTON, D.A. and BRIL, A. (1990). Activin can induce the formation of axial structures and is expressed in the hypoblast of the chick. *Cell 63:* 495-501.

MORIYA, N. and ASASHIMA, M. (1992). Mesoderm and neural inductions on newt ectoderm by activin A. *Dev. Growth Differ. 34:* 589-594.

MORIYA, N., UCHIYAMA, H. and ASASHIMA, M. (1993). Induction of pronephric tubules by activin and retinoic acid in presumptive ectoderm of *Xenopus laevis*. *Dev. Growth Differ. 35:* 123-128.

NAKAMURA, O., TAKASAKI, H. and ISHIHARA, M. (1971). Formation of the organizer from combinations of presumptive ectoderm. *Proc. Jpn. Acad. 47:* 313-318.

NAKAMURA, T., ASASHIMA, M., ETO, Y., TAKIO, K., UCHIYAMA, H., MORIYA, N., ARIIZUMI, T., YASHIRO, T., SUGINO, K., TITANI, K. and SUGINO, H. (1992). Isolation and characterization of Activin B: evidence for its potent *Xenopus* mesoderm-inducing activity. *J. Biol. Chem. 267:* 16385-16389.

NAKAMURA, T., TAKIO, K., ETO, Y., SHIBAI, H., TITANI, K. and SUGINO, H. (1990). Activin-binding protein from rat ovary is follistatin. *Science 247:* 836-838.

NAKANO, H., KINOSHITA, K., ISHII, K., SHIBAI, H. and ASASHIMA, M. (1990). Activities of mesoderm-inducing factors secreted by mammalian cells in culture. *Dev. Growth Differ. 32:* 165-170.

NEWPORT, J. and KIRSCHNER, M. (1982a). A major developmental transition in early *Xenopus* embryos. I. Characterization and timing of cellular changes at the midblastula stage. *Cell 30:* 675-686.

NEWPORT, J. and KIRSCHNER, M. (1982b). A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell 30:* 687-696.

NIEUWKOOP, P.D. (1969). The formation of the mesoderm in urodelean amphibians. I. Induction by the endoderm. *W. Roux Arch. EntwMech. Org. 162:* 341-373.

NIEUWKOOP, P.D. and FABER, J. (1967). *Normal Table of Xenopus laevis (Daudin)* 2nd. ed. North-Holland Publishing Company, Amsterdam.

NISHIMATSU, S., IWAO, M., NAGAI, T., ODA, S., SUZUKI, A, ASASHIMA, M., MURAKAMI, K. and UENO, N. (1992). A carboxyl-terminal truncated version of the activin receptor mediates activin signals in early *Xenopus* embryos. *FEBS Lett. 312:* 169-173.

ROSA, F.M. (1989). Mix.1, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of *Xenopus* embryos. *Cell 57:* 965-974.

RUIZ I ALTABA, A. and MELTON, D.A. (1989). Interaction between peptide growth factors and homoeobox genes in the establishment of antero-posterior polarity in frog embryos. *Nature 341:* 33-38.

SIVE, H.L. and CHENG, P.F. (1991). Retinoic acid perturbs the expression of Xhox.lab genes and alters mesodermal determination in *Xenopus laevis. Genes Dev. 5:* 1321-1332.

SLACK, J.M.W. and ISAACS, H.V. (1989). Presence of basic fibroblast growth factor in the early *Xenopus* embryo. *Development 105:* 147-153.

SLACK, J.M.W., DARLINGTON, B.G., HEATH, J.K. and GODSAVE, S.F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature 326:* 197-200.

SMITH, J.C. (1987). A mesoderm-inducing factor is produced by a *Xenopus* cell line. *Development 99:* 3-14.

SMITH, J.C., PRICE, B.M.J., GREEN, J., 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:* 753-765.

SMITH, J.C., PRICE, B.M.J., VAN NIMMEN, K. and HUYLEBROEK, D. (1990). Identification of a potent *Xenopus* mesoderm inducing factor as homologue of activin A. *Nature 345:* 729-731.

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

SMITH, W.C. and HARLAND, R.M. (1992). Expression cloning of *noggin*, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell 70:* 829-840.

SMITH, W.C., KNECHT, A.K., WU, M. and HARLAND, R.M. (1993). Secreted *noggin* protein mimics the Spemann organizer in dorsalizing *Xenopus* mesoderm. *Nature 361:* 547-549.

SOKOL, S., CHRISTIAN, J.L., MOON, R.T. and MELTON, D.A. (1991). Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. *Cell 67:* 741-752.

SPEMANN, H. and MANGOLD, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *Arch. Mikrosk. Anat. EntwMech. 100:* 599-638.

TAIRA, M., JAMRICH, M., GOOD, P.J. and DAWID, I.B. (1992). The LIM domain-containing homeo box gene Xlim-1 is expressed specifically in the organizer region of *Xenopus* gastrula embryo. *Genes Dev. 6:* 356-366.

THOMSEN, G.H. and MELTON, D.A. (1993). Processed Vg1 protein is an axial mesoderm inducer in *Xenopus. Cell 74:* 433-441.

THOMSEN, G.H., WOOLF, T., WHITMAN, M., SOKOL, S., VAUGHAN, J., VALE, W. and MELTON, D.A. (1990). Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell 67:* 485-493.

TIEDEMANN, H. (1990). Cellular and molecular aspects of embryonic induction. *Zool. Sci. 7:* 171-186.

UENO, N., LING, N., YING, S.Y., ESCH, F., SHIMASAKI, S. and GUILLEMIN, R. (1987). Isolation and partial characterization of follistatin: a single-chain Mr 35,000 monomeric protein that inhibits the release of follicle-stimulating hormone. *Proc. Natl. Acad. Sci. USA 84:* 8282-8286.

VALE, W., RIVIER, J., VAUGHAN, J., McCLINTOCK, R., CORRIGAN, A., WOO, W., KARR, D. and SPIESS, J. (1986). Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature 321:* 776-779.

VOJTEK, A.B., HOLLENBERG, S.M. and COOPER, J.A. (1993). Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell 74:* 205-214.

VON DASSOW, G., SCHMIDT, J. and KIMELMAN, D. (1993). Induction of the *Xenopus* organizer: Expression and regulation of Xnot, a novel FGF and activin-inducible homeobox gene. *Genes Dev. 7:* 355-366.

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-ß. *Cell 51:* 861-867.

WHITMAN, M. and MELTON, D.A. (1992). Involvement of $p21^{ras}$ in *Xenopus* mesoderm induction. *Nature 357:* 252-254.