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REVIEW

Control of the Embryonic Body Plan by Activin during Amphibian Development

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ABSTRACT—Embryonic induction plays an important role in establishing the fundamental body plan during early amphibian development. The factors mediating this embryonic induction have, however, only recently been discovered. In the mid-1980's, certain peptide growth factors belonging to the FGF and TGF-β families were found to have a mesoderm-inducing effect on isolated *Xenopus* blastula ectoderm. The study of embryonic induction subsequently expanded rapidly and knowledge at the molecular level has gradually accumulated. One of these peptide growth factors, activin, a member of the TGF-β superfamily, is present maternally in the *Xenopus* early embryo and induces various mesodermal and endodermal tissues in isolated presumptive ectoderm. After exposure of presumptive ectoderm to activin, many genes are expressed in the same manner as in normal embryogenesis. Ectoderm treated with activin can induce a complete secondary embryo, the same as the organizer does in transplantation experiments. These findings suggest that activin is one of the first induction signals responsible for establishing the embryonic body plan in early amphibian development. In this article we shall review to what extent we can control the embryonic body plan *in vitro*, referring to some significant findings in this field.

INTRODUCTION

About seventy years ago, Spemann and Mangold [85] discovered the phenomenon of primary embryonic induction using a heteroplastic transplantation technique in newt embryos. Because the ability to induce a secondary embryo was restricted to the dorsal lip, this region was named "the organizer" in the sense of a morphogenetic center. Following the discovery of the organizer in amphibian embryos, many investigators tried to isolate factors which could act as the organizer, and to elucidate the source of the organizer, and to reveal the molecular mechanisms of the embryonic induction. As a result of these attempts, it was found that the mesodermal anlagen, including the organizer, develop from equatorial (marginal zone) cells under the influence of signals from vegetal hemisphere cells at an earlier stage of development [8, 59, 61, 65]. This is the first inductive phenomenon to be observed during amphibian development and is referred to as "mesoderm induction" (Fig. 1, A). During gastrulation, the invaginating cells of the dorsal marginal zone (the organizer) induce central nervous system anlagen by secreting certain factors to the overlying layer of ectodermal cells. This inductive phenomenon is called "neural induction" (Fig. 1, B). The fundamental body plan of the embryo is established as a result of these two major inductive interactions during the process of amphibian embryonic development.

During the past decade, several molecules which are responsible for mesoderm induction have been identified. These include certain peptide growth factors belonging to the bFGF (basic fibroblast growth factor) and TGF-β (transforming growth factor-β) families [9, 25, 88]. One of these molecules, activin, has a very potent mesoderm-inducing activity on *Xenopus* presumptive ectoderm [11, 26, 79] and induces almost all mesodermal tissues in a dose-dependent manner [6, 7, 31, 32]. The tissues induced by activin are exactly the same at the histological and molecular levels as those found in normal embryos. Genes normally expressed during development are also expressed sequentially after exposure of presumptive ectoderm to activin. The most characteristic property of activin is induction of organizer activity in presumptive ectoderm [4, 5, 21, 23, 72]. The isolated ectoderm of the late blastula or early gastrula itself forms atypical epidermis in the absence of activin. After treatment of isolated ectoderm with activin, however, the same region induces a secondary embryo, as the organizer does when implanted into the blastocoele of another embryo [21, 72]. These findings suggest that activin can not only induce various mesodermal tissues but elicit the organizer as a morphogenetic center. Thus, at present, activin is currently considered as a strong candidate for the first molecular signal to establish body plan during early amphibian development.

Using activin (as the inducer) and presumptive ectoderm (as the reacting tissue), it is possible in theory to reproduce embryonic induction and design a fundamental embryonic form *in vitro*. In this article, we have focused on activin as the first molecular signal in the chain of inductive events, and review the extent to which we can control cell differentiation

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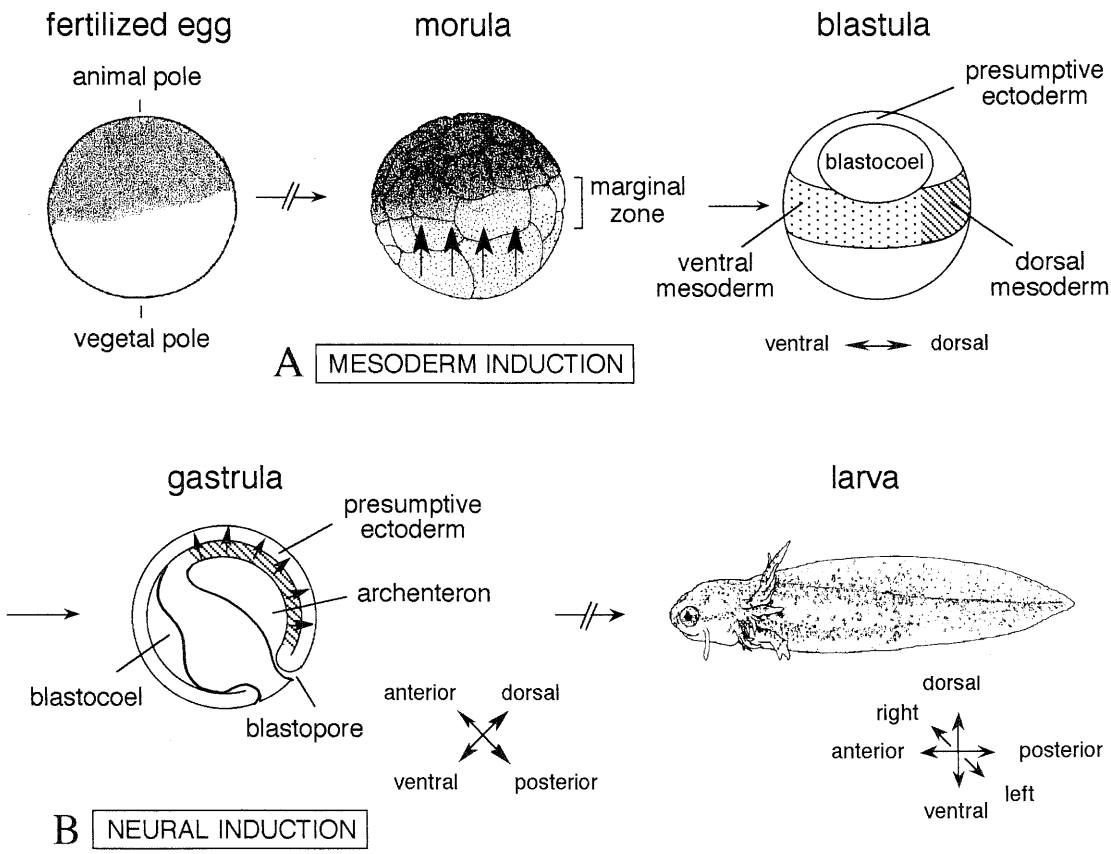

FIG. 1. Model of embryonic induction and early amphibian development. Mesoderm induction is the first embryonic induction, in which mesoderm anlagen including the Spemann organizer are induced by vegetal hemisphere cells (A). Dorsal mesoderm (the organizer) induces central nervous system anlagen in the overlying presumptive ectoderm during gastrulation (B).

and morphogenesis can be controlled *in vitro* with activin.

SURGICAL MANIPULATION OF EMBRYOS AND BIOASSAY OF INDUCERS

Amphibians provide excellent material for experiments to study the mechanisms of embryonic induction. Urodela, such as *Triturus, Cynops* and *Ambystoma*, have several advantages over other amphibians. Their eggs are relatively large (about 2 mm in diameter), and the embryos develop relatively slowly, so that it is very easy to perform surgical manipulation. Consequently, urodele embryos have proved the most useful material in traditional experimental embryology. Urodela are currently on the decrease throughout the world, and the most available material used today is the anuran, *Xenopus laevis*. *Xenopus* is easily maintained in the laboratory and produces large numbers of eggs at one spawning (about 1500–2000 eggs) in response to hormone injections every three months. Their embryos develop relatively rapidly, which is an advantage in biochemical and molecular biological studies.

Methods of testing induction activity using pluripotent presumptive ectoderm

Up to the early gastrula stage, presumptive ectoderm has pluripotency and can be induced to form neural tissue or mesodermal and endodermal tissue by addition of inducers. Taking an advantage of the pluripotency of presumptive ectoderm, three main methods of testing the inducing activity of tissues and substances have been devised. In the "implantation method" or "Einsteck method" [52], a piece of tissue or coagulated inducing substance is pushed into the blastocoele of a late blastula or early gastrula through a slit at the animal pole of the host embryo (Fig. 2, A). As gastrulation proceeds, the implant becomes pressed against the ventral ectoderm of the host embryo some hours after implantation. If the organizer (the dorsal lip of the blastopore) is implanted, a secondary embryo is eventually formed on the ventral side of the host embryo. To eliminate any possible influence by the host embryo, two explantation ways have been improved. In the "sandwich method", conceived by Holtfreter [40], the solid inducer is placed between two sheets of the presumptive ectoderm and interacts with them from the inside of a vesicle (Fig. 2, B). When the inducer is soluble in saline, the "piece culture method" or "animal cap assay" is generally used to test its inducing activity (Fig. 2, C) [17, 74, 98]. A piece of presumptive ectoderm (animal cap) is excised from a late blastula or early gastrula and dipped in the saline containing the inducing substance for a certain period. Bovine serum albumin is added to the saline to

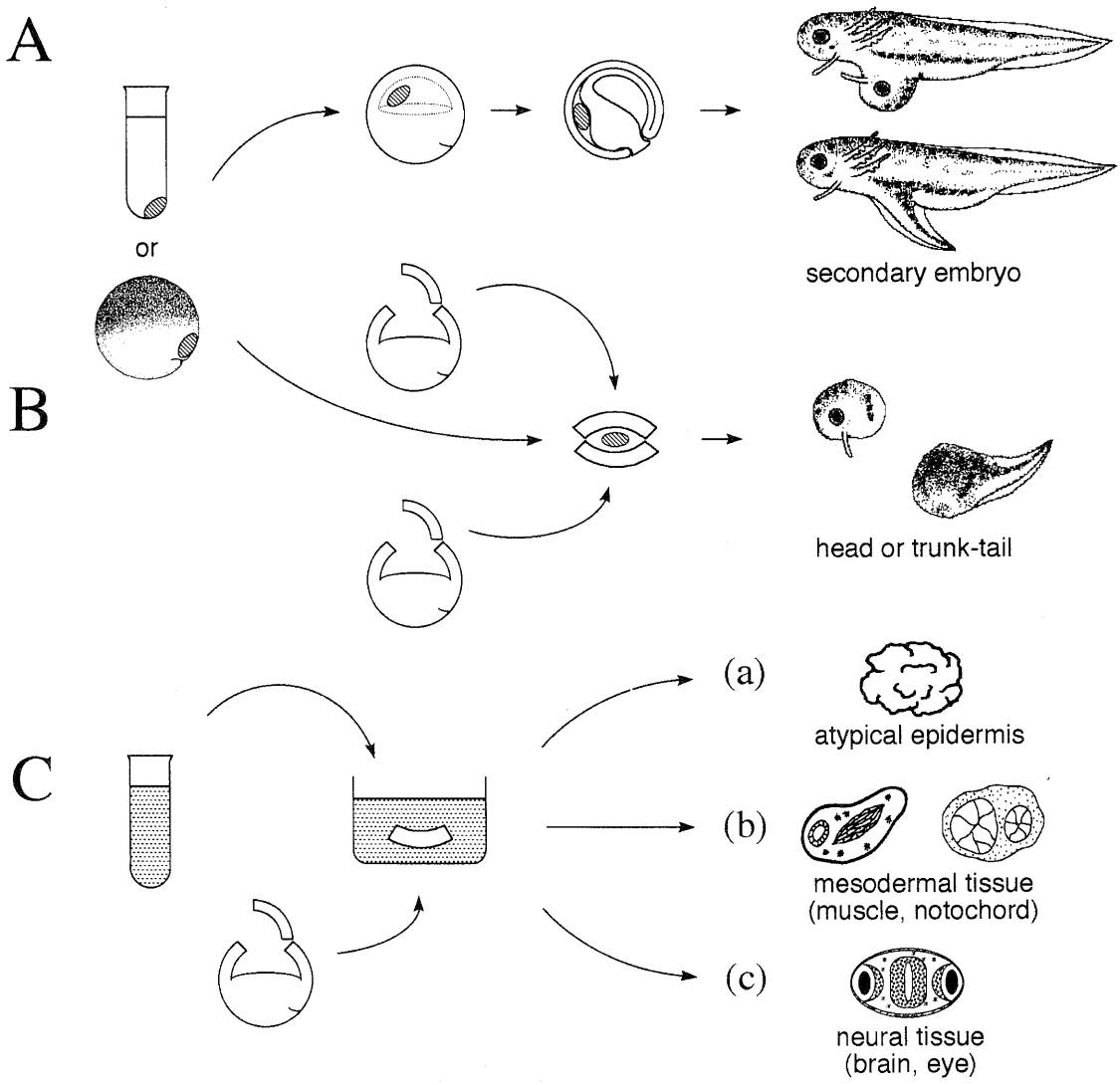

FIG. 2. Schematic diagrams of three methods of testing the inducing activity of tissues and substances. A, Implantation method. A piece of tissue (e.g., the organizer) or coagulated substance inserted into the blastocoele induces a secondary embryo on the ventral side of host embryo. B, Sandwich method. Inducer is placed between two pieces of presumptive ectoderm. C, Piece culture method. A piece of ectoderm is put in saline containing the inducing substance in solution. Ectoderm forms atypical epidermis in the absence of inducers (a). Differentiation of mesodermal tissues, such as notochord and muscle, indicates the presence of a mesoderm-inducing factor (b), whereas that of neural tissues, such as forebrain and eyes, indicates the presence of a neural inducing factor (c).

avoid adsorption of the inducing substance on the surface of the culture dish. The presumptive ectoderm is sometimes covered with a piece of nylon, silk, or filter paper to prevent it from curling up [17, 98]. This last method is easy to perform and has many advantages over other methods. Investigators can test large numbers of fractions of inducing substances in solution and estimate their inducing activity both qualitatively and quantitatively at the histological and molecular level. It is also quite easy to determine the induction properties of the substance and the competence of the reacting tissues when presumptive ectoderm at different stages or of different sizes is treated with various concentrations of the inducer for a fixed period.

In the piece culture method, the presumptive ectoderm forms irregular-shaped epidermis, referred to as "atypical epidermis", in the absence of inducers (Fig. 2, C, a), but can be induced to form neural tissue, or mesodermal and endodermal tissue by adding inducers. Differentiation of notochord and muscle in an explant indicates the presence of a mesoderm-inducing factor (Fig. 2, C, b). If the saline contains a certain neural inducer, such archencephalic structures as forebrain and eyes are induced in the explants (Fig. 2, C, c). The greatest care must be taken in identifying a neural inducer because presumptive ectoderm is susceptible to artificial stimulation [35, 88]. Ectoderm cultured in a highly saline solution sometimes forms neural tissue without inducers. Use of this culture method has enabled remarkable advances in the identification of mesoderm-inducing factors (MIFs) in recent years.

IDENTIFICATION OF MESODERM-INDUCING FACTORS

In the early studies, extracts of various vertebrate tissues, such as guinea pig bone marrow [92], chick embryo [29] and carp swim bladder [10, 43], were found to have a mesoderm-inducing effect on presumptive ectoderm. Although these "heterogenous" inducers could mimic mesoderm induction, the molecule which induces mesoderm in normal amphibian embryos remains unknown. The first molecule reported to possess potent inducing activity was isolated from chicken embryos [19, 30]. This factor is called "vegetalizing factor" because of its mesoderm- and endoderm-inducing effect on the presumptive ectoderm [33, 34, 50, 56]. More recently, a factor capable of inducing dorsal mesoderm was found in the culture medium of a *Xenopus* tadpole cell line (XTC cell) and designated XTC-MIF [78]. XTC-MIF is believed to be a natural mesoderm-inducing factor because of its origin.

Peptide growth factors as mesoderm-inducing substances

The most remarkable achievement in MIF research has been the identification of several peptide growth factors (PGFs) belonging to the FGF and TGF-β families as MIF candidates. In 1987, Slack *et al.* [77] reported that mammalian basic fibroblast growth factor (bFGF) has mesoderm-inducing activity. Presumptive ectoderm treated with bFGF differentiated solely into ventral mesoderm such as blood cells and coelomic epithelium, and no dorsal mesoderm with notochord was detected. These results suggest that other factors are required for complete mesoderm formation [36]. In the same study, they showed that heparin could block FGF-inducing activity, and suggested that an FGF-like substance which binds to heparin may be a natural inducer. Knöchel *et al.* [48, 49] and Rosa *et al.* [71] found that TGF-β1 and TGF-β2 also have mesoderm-inducing activity.

We ourselves have long been attempting to isolate MIFs from various sources, such as carp swim bladder, calf kidney, and mammalian cell lines. Eventually, we succeeded in isolating several peptides having mesoderm-inducing activity from the conditioned medium of the human K-562 cell line [64]. During their characterization, we found first that these peptides are closely related to activin A or erythroid differentiation factor (EDF) [11]. Activin A belongs to the TGF-β superfamily and was originally identified as a gonadal hormone that promotes the release of follicle stimulating hormone (FSH) from the anterior pituitary gland [51, 97]. There are three isotypes of activin: activin A is a homodimer consisting of two inhibin β_A chains, activin AB is a heterodimer of inhibin β_A and β_B chains, and activin B is a homodimer of two inhibin β_B chains (Fig. 3). Certain mesoderm-inducing factors derived from different sources have been shown to be identical to activin A or to be activin homologues: XTC-MIF from XTC cell conditioned medium [26, 79], WEHI-MIF from murine leukemia cells [1], vegetalizing factor from calf kidney and chicken embryo [12, 13], and PIF from mouse macrophage cell line P388D1 [83, 87]. From a historical perspective, it is very important to focus on one substance, such as activin or activin homologue, because many investigators have been afraid that there are numerous mesoderm-inducing substances, depending on the different starting materials such as animals or tissues.

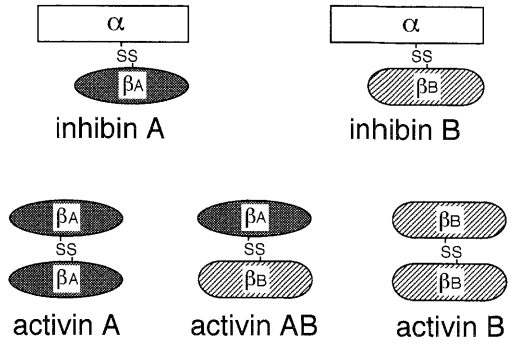

FIG. 3. Structure of activin and inhibin proteins. Three types of dimeric activin consist of inhibin β_A and β_B subunits.

Presence of activin in early Xenopus embryos

Activin A can induce a variety of mesodermal tissues in the *Xenopus* presumptive ectoderm *in vitro*, and thus it is the best candidate for the natural mesoderm-inducing factor. The next question was whether activin or a homologue actually exists in early embryos. To answer this question, we attempted to extract native activin protein directly from the early *Xenopus* embryos by reverse-phase HPLC [15]. After HPLC fractionation using thousands of unfertilized eggs or blastulae, only certain fractions exhibited the same retention times as activin A and showed potent mesoderm-inducing activity. The peptide(s) in these fractions was identified as an activin homologue because its mesoderm-inducing activity was inhibited by follistatin, an activin-specific binding protein. These fractions also possessed erythroid differentiation factor (EDF) activity. Estimates of the EDF activity in these fractions revealed that about 1 pg (0.5 ng/ml) of the activin homologue is present at least in a *Xenopus* egg. We further succeeded in extracting three types of activin (A, AB, B) and follistatin from early *Xenopus* embryos (st. 1–5) [28]. The cDNAs of both the activin β_A and β_B chains have been cloned by Thomsen *et al.* [87]. They tested the expression of activin mRNA and examined the inducing activity of *Xenopus* recombinant activins. The results showed that β_A chain mRNA is first expressed in late gastrulae and that β_B chain mRNA first appears in the blastula. Both of these gene expressions are too late to cause mesoderm induction during normal development. Their translation products seem to have a role in gastrulation or other types of cell differentiation, but not mesoderm induction.

We investigated the localization of activin and follistatin proteins in early *Xenopus* oocytes (st. 6) by electron microscopic immunolabeling with gold colloidal particles [95]. The

protein molecules were found to be localized uniformly in oocyte yolk platelets, but not in other cytoplasmic organelles. This suggests that a novel role of yolk platelets as a reservoir for inductive signals transported by vitellogenin, which is synthesized in the liver. These findings can be interpreted to mean that activin connects with vitellogenin forming the yolk platelets and the polarity during oogenesis and that this activin has an important role in producing the embryonic body plan by influencing the mesoderm induction.

INDUCTION PROPERTIES OF ACTIVIN ON PRESUMPTIVE ECTODERM

In the early 1950's, many investigators grappled with the analysis of embryonic induction using vertebrate tissues as "heterogenous" inducers. As a result of these attempts, hypotheses based on the gradients of inducers were proposed [39, 60]. The liver of guinea pig induces only archencephalic structures, such as forebrain, eye and nose, whereas bone marrow induces mesodermal tissues [90–92]. On the basis of experiments using these tissues, Toivonen and Saxén [93, 94] proposed the "two-gradient hypothesis" (Fig. 4). The gradient concept is very important in understanding cell differentiation and morphogenesis during early amphibian development. To examine whether cell and tissue differentiation can be controlled by a gradient of a single factor, we treated the presumptive ectoderm of *Xenopus laevis* and *Cynops pyrrhogaster* with various concentrations of activin A.

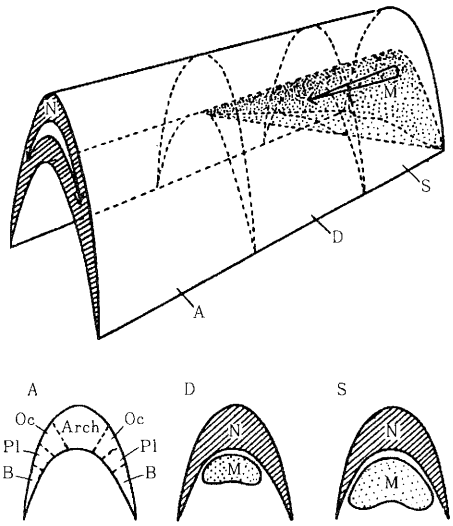

FIG. 4. The two-gradient hypothesis. The gradient of the neuralizing agent (N) is highest at the dorsal midline and declines toward the lateral and ventral parts of the embryo. That of the mesodermalizing agent (M) is highest at the posterior dorsal end and declines toward the anterior and lateral parts of the embryo. Each region of the embryo has a different concentration of the neuralizing and mesodermalizing agents. A, archencephalic region; D, deuterencephalic region; S, spinocaudal region; Arch, archencephalon; Oc, optic cup; Pl, placode; B, balancer. (From Toivonen and Saxén [94])

Dose and time-dependent mesoderm-inducing activity of activin A on Xenopus presumptive ectoderm

The concentration of activin A required to induce mesodermal tissues is inversely proportional to the duration of exposure of the presumptive ectoderm [7]. If the ectoderm is treated with activin A briefly, a high concentration is needed. Conversely, if treated for a long time, the concentration required is lower (Fig. 5, A).

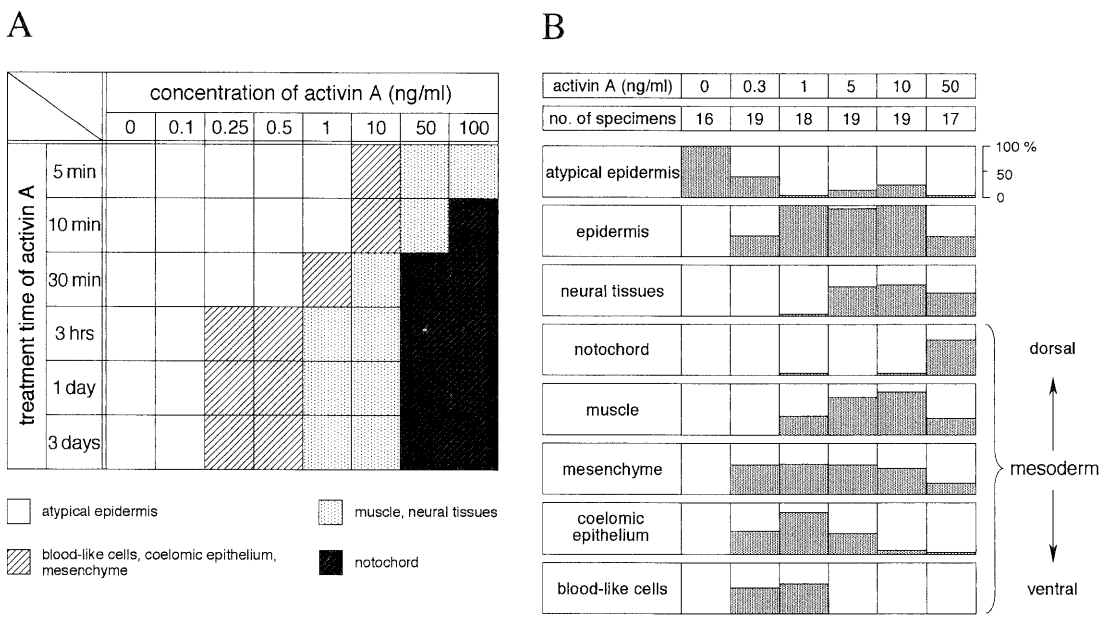

FIG. 5. A, Correlation between concentration and duration of activin A treatment on presumptive ectoderm. The concentration of activin A required to induce mesodermal tissues is inversely proportional to the duration of exposure of presumptive ectoderm. B, Dose-dependent mesoderm induction of activin A on presumptive ectoderm. Activin A induces mesodermal tissues from ventral to dorsal in character in a dose-dependent manner.

Depending upon the concentration of activin A, several different mesodermal tissues from ventral to dorsal in character are induced in ectodermal explants at clear dose thresholds (Fig. 5, B) [6, 7]. At low concentrations, ventral mesoderm, such as blood-like cells, coelomic epithelium and mesenchyme, are induced in the explants. At intermediate concentrations, muscle and neural tissue are induced as well. At high concentrations, notochord, the most dorsal mesoderm is induced. Similar results have also been reported by Grunz [34] using vegetalizing factor, and Green *et al.* [31, 32] using XTC-MIF. Moreover, no clear difference in induction properties was observed among the three types of activin (A, AB, B). They all induced several different mesodermal tissues in a gradient fashion, as described above [27, 63].

Follistatin, an activin-specific binding protein [62], inhibits the mesoderm-inducing activity of activin in a dose-dependent manner [14]. Follistatin was originally isolated from porcine follicle fluid and identified as an inhibitor of FSH secretion by the pituitary [96]. At high activin concentrations dorsal mesoderm, such as muscle and notochord, is induced in presumptive ectoderm. When follistatin is added to activin solution, the mesoderm-inducing activity of activin is weakened and the type of tissue induced shifts from dorsal mesoderm to ventral mesoderm. These findings indicate that follistatin binds to free activin, forming a complex that controls the concentration of activin and inhibits the mesoderm-inducing activity of activin.

The results of *in vitro* studies indicate that the type of mesodermal tissue may be determined by a concentration gradient of activin. Follistatin may also contribute to forming this gradient by its regulatory effect on the inducing activity of activin. Although activin protein is present in the early *Xenopus* embryos [15, 28], this putative concentration gradient, however, has not been confirmed. Another potent mesoderm-inducing factor, bFGF, preferentially induces ventral mesoderm *in vitro* [77]. bFGF exists in early *Xenopus* embryos in the form of both mRNA and protein [44, 76]. bFGF protein has been identified from oogenesis to the blastula stage, and is localized in the presumptive marginal zone [75]. Moreover, evidence that FGF plays a very important role in *Xenopus* embryogenesis has been accumulated during the past few years [2, 3, 41]. Thus, it may be impossible that all types of mesodermal tissues are induced by the activin gradient during normal development. Further study will be needed to determine how many factors are required for complete mesoderm induction.

Vegetalizing activity of activin A on the Cynops presumptive ectoderm

The presumptive ectoderm of the *Cynops* late blastula and early gastrula is made up of a single cell layer and is more homogeneous than that of *Xenopus*. The induction properties of activin A on *Cynops* ectoderm and *Xenopus* ectoderm are different. The concentration effect of activin A shown on *Xenopus* ectoderm is not clearly observed in *Cynops* ectoderm, and the frequency of mesoderm differentiation is also relatively low [57]. On the other hand, ectoderm treated with high concentrations of activin A differentiates solely into yolk-rich tissues [5]. These tissues are considered endoderm because they often exhibit a columnar appearance, a characteristic of endodermal epithelium in the alimentary canal. Although the frequency is low, a pulsating heart is also induced at high concentrations of activin A [5, 57]. Differentiation of the heart anlage during normal development is known to depend on the influence of endoderm [54, 69]. These findings suggest that activin A has a vegetalizing effect on *Cynops* ectoderm, the same as the "vegetalizing factor" [33, 34, 50, 56]. The "vegetalizing factor" has now been identified as activin A based on its chemical properties and biological activities [12–14, 89]. In *Cynops*, it is very likely that ectoderm determined to form endoderm induces mesodermal tissues from adjacent non-induced ectoderm as a secondary interaction [33, 56]. This possibility is also supported by the results of a sandwich experiment in which the inner activin-treated ectoderm formed endodermal tissues alone, and most of the mesodermal tissues differentiated from the surrounding untreated ectoderm [5]. Jones *et al.* [42] have identified endodermal tissues in *Xenopus* ectoderm treated with XTC-MIF using a monoclonal endodermal marker. This suggests that both endodermal tissues and mesodermal tissues are induced also in *Xenopus* ectoderm by activin A. Although we were unable to confirm the existence of endodermal tissues, such as intestine or liver, in *Xenopus* activin-treated ectoderm by histological examination, activin A may have a vegetalizing effect on *Xenopus* ectoderm. Further study will be needed to confirm whether mesodermal tissues are directly induced by activin A or formed as a result of secondary interactions between endodermalized and non-induced ectoderm.

SEQUENTIAL GENE EXPRESSION INDUCED BY ACTIVIN

A number of genes induced by activin on the *Xenopus* presumptive ectoderm have recently been reported (Table 1). These genes, called early response genes, do not necessarily require new protein synthesis, and are expressed within a few hours.

Mix. 1, a *Xenopus* homeobox gene, is expressed within 30 min after treatment with XTC-MIF (*Xenopus* activin) on the presumptive ectoderm [70]. *Mix. 1* seems to be one of the early response genes directly induced by activin, because its expression is not inhibited in the presence of cycloheximide, a specific inhibitor of new protein synthesis. In normal development, *Mix. 1* mRNA is transiently expressed during gastrulation, and localized in the presumptive endoderm and mesoderm region in the vegetal hemisphere. Although the function of this gene is not yet known, it seems to be closely related with the vegetalizing effect of activin as shown in *Cynops* ectoderm culture.

TABLE 1. Genes expressed in the presumptive ectoderm after activin treatment

gene	start of expression	characteristics	references
Mix. 1	30 min	homeobox gene, relative of *Drosophila paired*	Rosa (1989)
goosecoid	30 min	homeobox gene, relative of *Drosophila gooseberry* and *bicoid*, and *Xenopus Mix. 1*	Cho *et al.* (1991)
Xlim-1	2 hr	homeobox gene with LIM domain	Taira *et al.* (1992)
XFKH1		homeobox gene, relative of *Drosophila fork head*	Dirksen and Jamrich (1992)
Xbra	90-250 min	homologue of mouse *Brachuyry (T)*	Smith *et al.* (1991)
Xhox3	6 hr*	homeobox gene, relative of *Drosophila even-skipped*	*Ruiz i Altaba et al.* (1989)
XlHbox1	11 hr*	homeobox gene, relative of *Drosophila antennapedia*, homologue of mouse *Hox3.3*	Cho and De Robertis (1990)
XlHbox6	13 hr*	homeobox gene, relative of *Drosophila abdominal-B*, homologue of mouse *Hox2.5*	Cho and De Robertis (1990)
Xwnt-8	overnight	relative of oncogene *int-1*	Christian *et al.* (1991)
XMyoD	overnight	homologue of mouse *MyoD*	Hopwood and Gurdon (1989)
α-actin	19 hr	cardiac and skeletal muscle actin gene	Kinoshita and Asashima (1994)
N-CAM	19 hr	neural cell adhesion molecule gene	Kinoshita and Asashima (1994)

* time deduced by the author from the experimental conditions

Another homeobox gene, *goosecoid*, was cloned from the cDNA library of the organizer of *Xenopus* early gastrula [18, 20, 22]. Activin can directly induce the transcription of this gene within 30 min without protein synthesis. In normal development, the expression of *goosecoid* transcripts is limited to the dorsal lip of blastopore at the gastrula stage. Injection of the full-length *goosecoid* mRNA into either of the two ventral blastomeres of the 4-cell embryo results in the formation of a secondary axis. These results suggest that the *goosecoid* gene is closely related with the function of the organizer during embryonic induction.

Like *goosecoid, Xlim* is inducible by activin, and is expressed specifically in the dorsal lip of gastrulae [25, 86]. In the ectoderm culture, this gene is directly induced by activin, and also by retinoic acid (RA) [20]. A synergistic effect of the combination of activin and RA has been recognized in the expression of genes. Although RA itself has no mesoderm-inducing activity, RA is a candidate for controlling homeobox gene expression. The ectoderm co-incubated with activin and RA can induce expression of the *Xlim* gene, and these factors seem to be related with the formation of the organizer or the function of the organizer during early development.

Xbra, homologue of mouse *Brachyury (T)*, is expressed in the presumptive mesodermal cells around the blastopore, and then in the notochord during normal development [80]. This gene is immediately induced by both activin and FGF action on the presumptive ectoderm. The ectoderm isolated from *Xbra* mRNA-injected embryo differentiated into mesoderm [24]. These results suggest that *Xbra* is a gene required for the onset of differentiation after mesoderm induction.

In addition to the early response genes induced by activin, other homeobox genes are expressed regionally in response to growth factor treatment. *XlHbox 1* is a homeobox gene that is expressed in the diencephalic region after the neurula stage, and it is expressed by activin on the presumptive ectoderm [20]. Conversely, *XlHbox 6*, activated by FGF, is expressed in the posterior region of the embryo [20]. *Xhox 3* is the homeobox gene which is expressed in the posterior area after gastrulation. This gene is induced by FGF, but not by activin A [73]. These results suggest that activin can induce the expression of several homeobox genes which are located at anterior region, and that FGF can induce the homeobox genes which are located in the posterior region. Although these factors can control region-specific gene expression and the order of the gene expression, regional differentiation such as head, trunk and tail seems to occur following development. In addition to above genes, *XFKH 1, Xwnt-8, XMyoD, N-CAM* and others are expressed in the presumptive ectoderm after activin treatment. The synergistic effects of the mesoderm-inducing factors on gene expression and the interrelationships between the various genes are also important in understanding the cell differentiation.

IN VITRO CONTROL OF THE FUNDAMENTAL BODY PLAN BY ACTIVIN

As mentioned above, activin is currently considered as a strong candidate for the natural mesoderm-inducing factor (and the endoderm-inducing factor in *Cynops*) which acts as the first molecular signal in the chain of inductive events in amphibian embryogenesis. However, tissues induced in ectodermal explants *in vitro* are poorly organized, and explants never display a clear embryonic axis and form. Moreover, it has been shown that PIF, an activin A homologue derived from a mouse macrophage cell line, induces miniature embryos called "embryoids" from *Xenopus* blastula ectoderm [83, 87]. They are equipped with a rudimentary anterior-posterior axis, brain and eyes. However, embryoids are never formed when a small piece of ectoderm,

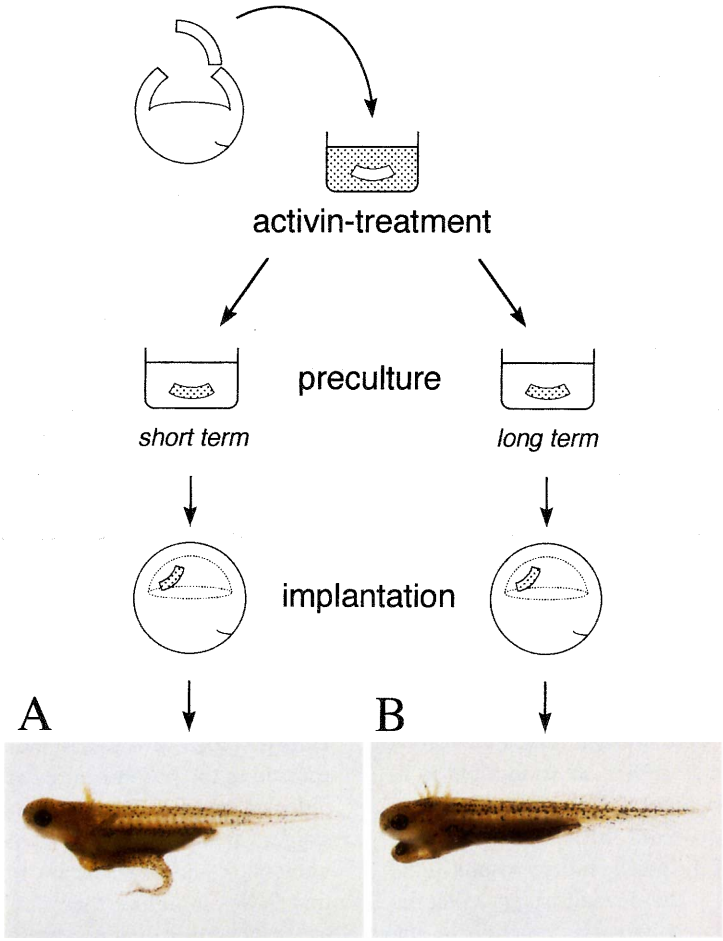

FIG. 6. Secondary embryos induced by activin-treated ectoderm. Activin-treated ectoderm precultured in saline for a short term induced a secondary trunk-tail (A), whereas that precultured for a long term induced a secondary head (B).

excluding cells close to the marginal zone (presumptive mesoderm), are used as a reacting tissue [6, 25]. Ectoderm treated with activin, on the other hand, induces a well-organized secondary embryo when transplanted into the blastocoele or ventral marginal zone of early gastrula (Fig. 6) [21, 23, 72]. As is well known, a similar embryo is also obtained by transplanting the organizer (the dorsal lip of the blastopore) [85]. Thus, activin induces organizer activity in the ectoderm. These findings suggest that activin can not only induce various meso- and endodermal tissues but can also facilitate formation of the organizer as a morphogenetic center.

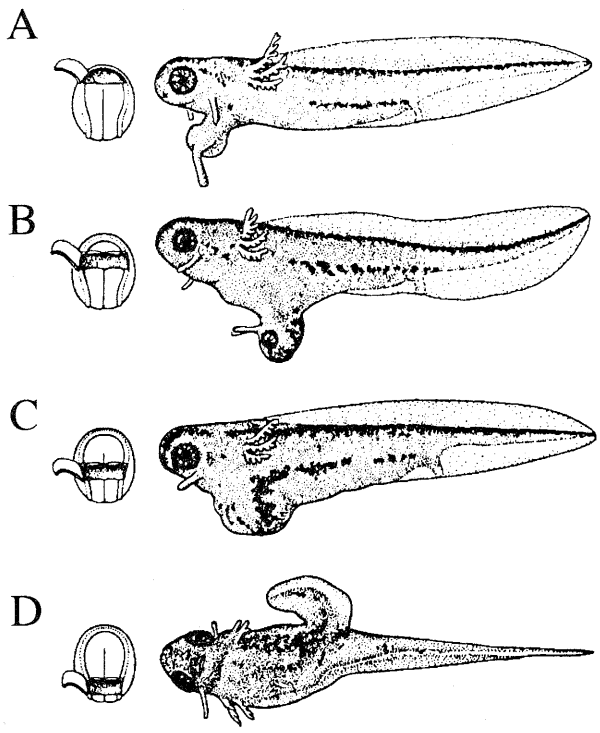

FIG. 7. Regional induction-specificity of the organizer (archenteron roof). Four quarters of the organizer after invagination were implanted into the blastocoele of early *Triturus gastrula*. A, The anterior-most region of archenteron roof induced head with balancers. B, The second quarter induced head with balancers, eyes and forebrain. C, The third quarter induced posterior part of head with hindbrain, spinal cord and somites. D, The posterior-most region induced trunk-tail with spinal cord, somites, and pronephros. (After Mangold [53]; from Nakamura and Toivonen [60])

Regional induction-specificity of the organizer

Mangold and Spemann [55] demonstrated by the implantation method that the dorsal lip of the early salamander gastrula induces a secondary head, while that of the *late* gastrula induces a trunk and tail structures. Spemann [84] defined the former as the "head organizer" and the latter as the "trunk organizer". Mangold [53] further examined the inducing abilities of the dorsal lip after invagination. The anterior region of the archenteron roof, corresponding to the invaginated head organizer, induced a head, and the posterior region, the trunk organizer, induced a trunk and tail (Fig. 7). These results indicate that at least two distinct regionalities are present in the organizer.

The dorsal lip of the early gastrula (head organizer), however, induces trunk-tail structures in sandwich culture or affixed transplantation. The same region induces the formation of heads when it is precultured in saline for a definite period [37, 66–68]. This means that the regional induction-specificity of the organizer changes autonomously during gastrulation. In the experiment performed by Mangold and Spemann [55], the dorsal lip of the early gastrula was implanted into the blastocoele. As gastrulation proceeds, its

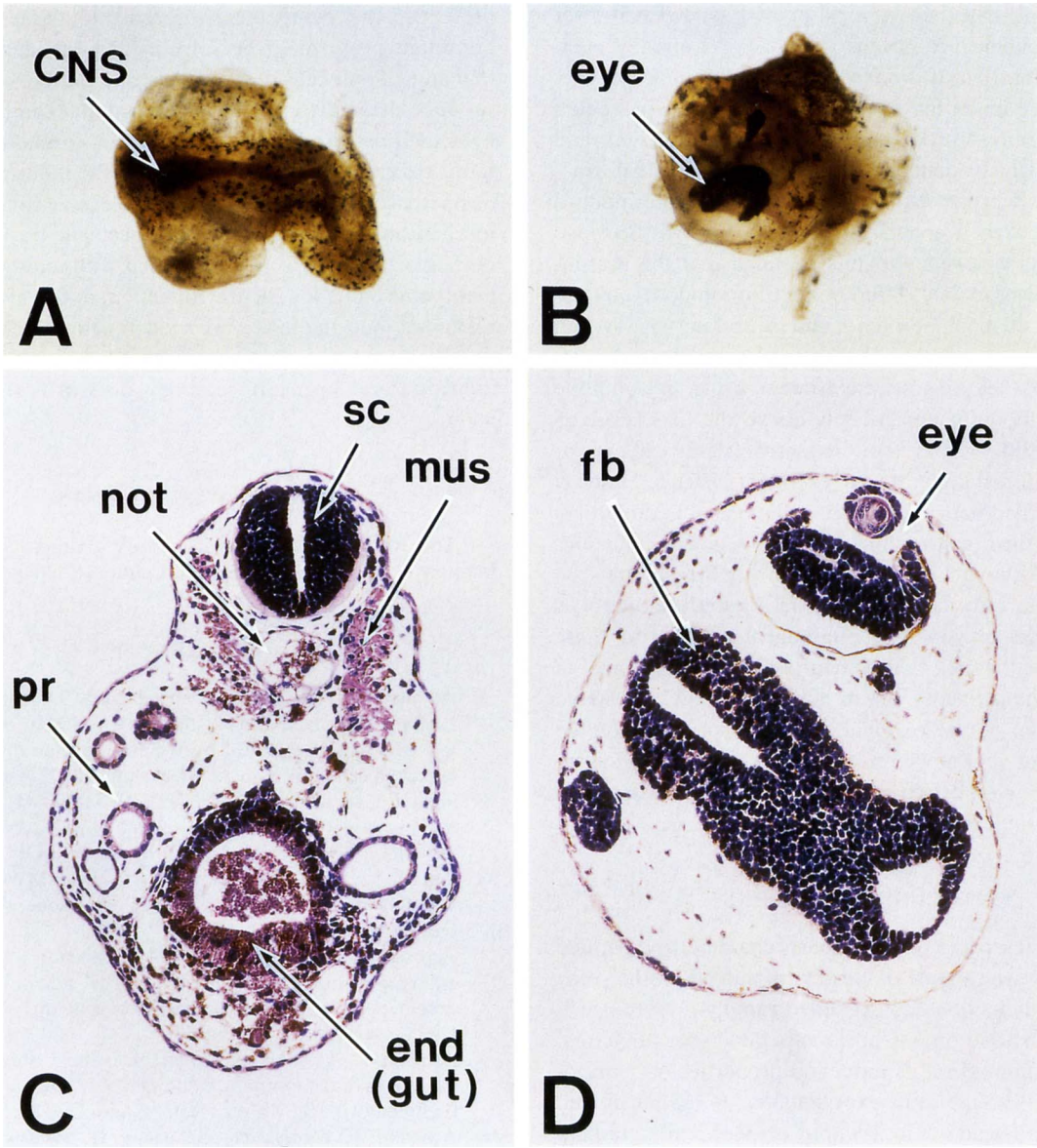

FIG. 8. Regional induction-specificity of activin-treated ectoderm. Presumptive ectoderm of early *Cynops* gastrula was treated with 100 ng/ml of activin A for 1 hr. After precultured in saline for various periods (0–24 hr), its induction-specificity was examined by the sandwich method. A, Explant with trunk-tail structures induced by the activin-treated ectoderm without preculture. B, Explant with head structures induced by the activin-treated ectoderm that was precultured for 12 hr in saline. C, Histological section of A. Spinal cord, axial mesoderm and gut differentiate in the same arrangement as in normal larvae. D, Histological section of B. Forebrain is accompanied by a well-differentiated eye. CNS, central nervous system; end, yolk-rich endodermal cells; fb, forebrain; mus, muscle; not, notochord; pr, pronephros; sc, spinal cord.

induction properties may transform from trunk-tail to head and eventually induce a secondary head on the ventral ectoderm of the host embryo. Although these findings seem to be very important in establishing the fundamental body plan, further investigation has long been awaited.

Regional induction-specificity of activin-treated ectoderm

If activin induces organizer-activity in ectoderm, it may well be that activin-treated ectoderm also exhibits regional induction-specificity, as shown in the classical organizer experiments. We have confirmed this possibility by employing the sandwich method on activin-treated *Cynops* ectoderm [5]. In this experiment, the presumptive ectoderm was treated with 100 ng/ml of activin A for 1 hr and sandwiched after preculture in saline for various periods. As already mentioned, ectoderm itself preferentially forms yolk-rich endodermal tissue under these conditions. The activin-treated ectoderm precultured for a short period induced trunk and tails characterized by deuterencephalic and spinocaudal structures, whereas when precultured for a long period it induced a head with archencephalic structures (Fig. 8). Lineage analysis of the sandwich explants revealed that the activin-treated ectoderm mainly differentiated into endodermal tissues and induced axial mesoderm and central nervous system in the untreated ectoderm. These results are in agreement with those of the previous experiment using presumptive pharyngeal endoderm immediately above the blastopore as the inducer [38]. From their extensive sandwich experiments on the dorsal lip of the early *Cynops* gastrula, Hama *et al.* [38] concluded that presumptive pharyngeal endoderm is an initiator of the organizer and that it has regional induction-specificity. Although it has not been confirmed that the yolk-rich tissue induced by activin in *Cynops* ectoderm is identical to the presumptive pharyngeal endoderm, these results support the idea that activin acts as the first signal in the chain of inductive events in amphibian embryogenesis. The mechanism of the regional induction-specificity of the organizer is not yet known on the molecular level. However, the above *in vitro* experiments will serve as a test system to analyze this problem.

CONCLUDING REMARKS

After the discovery that previously characterized peptide growth factors are capable of mesoderm induction, the study of embryonic induction has expanded rapidly. In this article, we have focused on one of these peptide growth factors, activin, and summarized its induction properties on pluripotent ectoderm through some experiments. Activin can control mesoderm formation in *Xenopus* ectoderm in a gradient fashion, which agrees with classical gradient theories to some extent. Further, ectoderm treated with activin acts as the organizer and exhibits clear regional induction-specificity. The regional induction-specificity of the organizer is closely related to the establishment of fundamental body plan in normal development. Although findings observed *in vitro* can not completely explain the role of activin *in vivo*, they will help and serve as an excellent model system for further analysis of morphogenesis, histological differentiation, and organogenesis at the molecular level. There are many problems still to be solved at the molecular level to understand the establishment of embryonic body plan more precisely. We should elucidate the molecular mechanism of signal transduction pathway involved in embryonic induction. Activin and FGF signals are transmitted to the target cells through the action of specific receptors: FGF receptor has tyrosine kinase in its intracellular domain [58], while activin receptors possess a serine/threonine kinase domain [16]. However, the pathway leads to gene activation of target cells is not clear at present. The competence of reacting tissues is also an important problem to be solved. Recent studies revealed that animal half cells of early *Xenopus* embryos have already set up a latent pattern of competence that can be developed by activin *in vitro* [45–47, 81, 82]. In normal development there are close relationships between the inducing signal and competence of target cells. The molecular components and mechanisms involved in competence will be important to elucidate. We were not concerned with another important embryonic induction, neural induction, in this article. Natural neural inducing factor involved in neural induction should also be identified to understand the mechanism of embryonic induction and formation of body plan in normal development.

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