Effect of activin and lithium on isolated *Xenopus* animal blastomeres and response alteration at the midblastula transition

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SUMMARY

Dorsoventral mesoderm patterning in the amphibian embryo involves a series of interactions mediated by several peptide growth factors. Animal blastomeres isolated at the 8-cell stage are useful for studying mesoderm patterning, since they contain the prospective (uninduced) mesoderm region and allow examination of the default state of animal cells. When activin is applied to these dorsal and ventral animal half explants, a competence prepattern for responding to activin is observed. In order to investigate the characteristics of prepatterning, we treated animal blastomeres with the embryo dorsalizing agent LiCl. Treatment with lithium alone did not induce normal trunk mesoderm in either blastomere. Lithium did, however, alter the competence of animal blastomeres to activin. Dorsal mesoderm was formed in the ventral blastomeres, as well as in the dorsal blastomeres. This result reveals that the early dorsoventral polarity in the animal hemisphere is not fixed.

Using *goosecoid (gsc)* and *Xwnt-8* genes as dorsal and ventral mesoderm markers, it was verified that lithium modifies the competence to activin. Unexpectedly, lithium treatment on its own resulted in *gsc* expression in the animal half explants. This suggests that embryo *goosecoid* expression may be induced by the effect of dorsal determination activity, but not by mesoderm induction. However, lithium induced also the expression of *brachyury (Xbra)* gene at very low levels. This would indicate the formation of dorsal-anterior mesoderm, which was not identified by the tissue observations.

Expression of *Xwnt-8*, a ventral mesoderm marker usually induced in blastula animal caps by activin, was hardly induced in the blastomere explants. We isolated whole animal half explants at the 8-cell stage and exposed to activin at different stages. It was found that the same concentration of activin induces *gsc* before the midblastula stage, and induces *Xwnt-8* at later stages. This suggests that the response of animal blastomeres alters depending on the stage of activin signaling.

Key words: *Xenopus laevis*, mesoderm induction, activin, lithium, dorsoventral axis formation, MBT

INTRODUCTION

Recent years have brought important advances in understanding the early events that guide axis formation in amphibian embryos. In *Xenopus*, the earliest step in dorsoventral axis formation involves the rotation of cortical cytoplasm in the fertilized egg. This process is postulated to activate cytoplasmic dorsal determinants in the future dorsal side (Gerhart et al., 1991). A number of studies have suggested that the vegetal and equatorial dorsal blastomeres of early cleavage stages contain a dorsal determinant that causes secondary axis formation when injected into ventral vegetal cells (Gimlich, 1986; Takasaki and Konishi, 1989; Kageura, 1990; Yuge et al., 1990; Fujisue et al., 1993). The dorsal region of the early blastula is referred to as the ‘Nieuwkoop center’ and is postulated to induce animal cells to become dorsal mesoderm.

In contrast to vegetal cells, animal cells have been considered to represent a relatively uniform group and usually used for the animal cap assay. Animal cap cells differentiate into mesoderm when treated with mesoderm-inducing factors (MIFs) such as members of fibroblast growth factor (FGF; Slack et al., 1987; Kimelman et al., 1988) and TGF-β including activin (Asashima et al., 1990; Smith et al., 1990; Green and Smith, 1990). It is reported that animal cap cells respond to increasing concentration of mesoderm-inducing factors by progressively forming dorsal structures (Green and Smith, 1990; Ariizumi et al., 1991). The existence of such factors in the egg and their importance in signaling pathways has been confirmed (Kimelman et al., 1988; Thomsen et al., 1990; Asashima et al., 1991; Amaya et al., 1991; Hemmati-Brivanlou and Melton, 1992; Dohrmann et al., 1993; Rebagliati and Dawid, 1993; Fukui et al., 1993, 1994). As yet, the observed spatial distribution of dorsal and ventral mesoderm cannot be fully explained, since the specificity and localization of those factors and/or their receptors have not been analyzed (reviewed by Smith, 1993). Yet Sokol and Melton (1991) have shown that the prospective dorsal and ventral regions of the blastula animal cap respond differently to the same concentration of
activin. That observation revealed that a competence prepattern to respond to activin exists in the animal cap cells. To investigate the process of prepatternning, we assayed the responsiveness of dorsal and ventral blastomeres isolated at the 8-cell stage to activin (animal-half assay), and showed that the competence prepattern is established by this stage (Kinoshita et al., 1993). However, a number of studies suggest that the polarity is not essential for creating normal body pattern. For example, Kageura and Yamana (1986) have shown that replacement of the two dorsal animal blastomeres with ventral animal cells at the 8-cell stage yields a normally patterned larva. The polarity in the animal hemisphere may be canceled under the influence of vegetal hemisphere.

In this paper, we examine the process of mesoderm patternning by treating animal-half explants with activin and lithium. The results show that the competence prepattern can be modified by lithium, that goosecoid expression can be induced by lithium alone, and that response to activin changes depending on the stage of blastomeres. We hypothesize that mesoderm patternning depends, in part, on the timing of induction and embryonic gsc expression is induced by the early dorsal determination signals.

MATERIALS AND METHODS

Embryos

Xenopus laevis embryos were obtained by artificial fertilization (Asashima et al., 1990). They were chemically dejellied with 2% cysteine hydrochloride (pH 8.0) at the 2- or 4-cell stage, washed with Steinberg’s solution (SS; 60.00 mM NaCl, 0.67 mM KCl, 0.34 mM Ca(NO₃)₂, 0.83 mM MgSO₄·7H₂O, 10.00 mM Hepes, pH 7.4), and selected for a reliable distinction between dorsal and ventral midline blastomeres. For the blastomere isolation, embryos were kept in CMF-PBS (Dulbecco’s formula, Takara Co., Kyoto, Japan) at 20°C. At the end of the 8-cell stage (stage 4.5), embryos with a strictly horizontal third cleavage plane were selected for the experiments. Care was taken so that animal dorsal blastomeres were always globular and smaller than ventral blastomeres. Staging was done according to Nieuwkoop and Faber (1967).

Explants

The 8-cell-stage blastomeres for animal half assay were isolated as described previously (Kinoshita et al., 1993). Only blastomere explants completely intact at 64-cell stage were used for the following assays, since small injuries in the surface often caused inhibition of cell cleavage and collapse of the whole explant. For the activin treatment on animal half explants at later stages, whole animal halves (composed of both dorsal and ventral animal blastomeres), isolated at the 8-cell stage, were allowed to develop in SS until blastula stages, then cleaved and opened with fine forceps to expose inner cells to activin. Explants were cultured in SS containing 0.1% BSA and 0.1 g/L kanamycin sulfate, in the presence or absence of activin A at 20°C. Human recombinant activin A was kindly provided by Dr. Eto at the Central Research Laboratories of Ajinomoto Co., Inc. (Kawasaki, Japan). The concentration of activin A was 50 ng/ml or about 500 units/ml. 1 unit/ml of activin is defined as the minimum concentration required to induce mesoderm in an animal cap explant (Cooke, 1987), and most animal cap explants form notochord at 500 units/ml (Ariizumi et al., 1991). Blastula animal caps were dissected from embryos between stages 7.5 and 9.5 embryos and treated in the same way.

Lithium treatment

For treatment with lithium, blastomeres isolated at the 8-cell stage were cultured for 1 hour (until the 32-cell stage) in SS, then incubated in SS containing 0.3 M LiCl at appropriate periods (see text) at 20°C. Animal caps dissected at stage 9 were exposed to 0.3 M LiCl-SS for 7 minutes. After lithium treatment, all explants were washed twice in SS and separately cultured in SS with or without activin A.

RNAse protection assays

At selected stages (see text), RNA was isolated from embryos as described previously (Kinoshita et al., 1993). Samples containing RNA from explants equivalent to 5 (for muscle actin) or 15 (for goosecoid, Xwnt-8 and Xbra) embryos in a final volume of 30 µl were hybridized with an excess of probe. T1 nuclease (Sigma) was used at 700 units/ml in the digestion reaction and samples were analyzed on a standard thin sequencing gel. The probes used were the muscle-specific α-actin probe SPAC9 (Sargent et al., 1986), subcloned into Bluescript II SK (Strategene), with a probe length of 600 nt and protected fragment length of 300 nt (muscle actin) and 170 nt (cytoskeletal actin); goosecoid (gsc), an organizer-specific homeobox gene probe (Blumberg et al., 1991; Cho et al., 1991), cloned into Bluescript II KS, with a probe length of 550 nt and a protected fragment length of 376 nt; Xwnt-8, a ventrolateral mesoderm marker (Christian et al., 1992), subcloned into Bluescript II SK, with a probe length of 305 nt and a protected fragment length of 251 nt; Xenopus Brachyury (Xbra), a mesoderm marker expressed throughout the marginal zone (Smith et al., 1991), cloned into pSP73, with a probe length of 297 nt and a protected fragment length of 216 nt; and the EF1-α probe, a loading control expressed in all embryonic cells (Krieg et al., 1989), cloned into Bluescript II SK, with a probe length of 152 nt and a protected fragment length of 93 nt. Plasmids were linearized and then transcribed with T7 or T3 RNA polymerases (Takara).

Histological examination

Explants and embryos cultured until stage 40 were fixed in Bouin’s fixative for 3 hours. They were dehydrated through ethanol series, embedded in Paraplast (Sherwood Medical), and sectioned at 6 μm. The sections were stained with hematoxylin-eosin.

RESULTS

Activin induces dorsal mesoderm in lithium-treated animal-ventral blastomeres

We have previously (Kinoshita et al., 1993) reported that neither animal dorsal (AD) nor animal ventral (AV) blastomeres isolated at the late 8-cell stage differentiated mesoderm when cultured alone, whereas 50 ng/ml (500 units/ml) activin A treatment induced mesoderm. As the isolated blastomeres were less sensitive to activin than blastula animal caps, it was necessary to treat them with this high concentration. The pattern of mesoderm differentiation in explants was different in AD and AV blastomeres. As seen in Fig. 1A, the AD explants cultured in activin (b) elongated at stage 20, exhibiting dorsal mesoderm formation, whereas AV explants (f) showed spherical forms similar to control explants (a,e). In order to examine the characteristics of this competence prepattern to activin, the animal blastomeres were exposed to lithium. It has been reported that lithium causes a sensitization of animal cells to mesoderm induction, while lithium itself does not act directly as a mesoderm inducer (Slack et al., 1988; Cooke et al., 1989; Kao and Elision, 1989). The blastomeres isolated at the 8-cell stage were allowed to develop until the 32-cell stage and exposed to 0.3 M LiCl for 10 minutes at 20°C. The dorsoanterior index (DAL, Kao and Elision, 1988) of the whole embryos treated simultaneously were between 7 and 8 (reduced trunks). In either explant (c and g), lithium by
itself did not cause morphological changes. However, it enhanced the effect of activin. Extreme elongation was observed in AD explants (d) and AV explants (h).

The above morphological observations were quantitatively examined by RNAase protection assays using mesoderm marker genes. Fig. 1B shows the results of an experiment probed by muscle-specific \( \alpha \)-actin gene, as a late marker for dorsolateral mesoderm (Gurdon et al., 1985). In the whole embryos treated with lithium, no significant difference to the control was detected (lanes 1 and 2). For explants (lanes 3 to 10), the results directly correlated with the morphology, showing that the AV explants treated with lithium express muscle-actin transcript in response to activin (lane 6). The amount of muscle-actin mRNA in these explants was almost equal to that of AD explants treated with activin alone (lane 8). These results reveal that lithium can modify the response of animal blastomeres to activin, so that ventral blastomeres acquire dorsal characters.

**gsc expression can be induced by lithium alone**

We also examined the responses to activin and/or lithium using several early gene markers. Homeobox gene \textit{goosecoid (gsc)} is a dorsal mesoderm marker (Cho et al., 1991) and \textit{Xwnt-8} is a ventral mesoderm marker (Christian and Moon, 1993) expressed at late blastula stages. Expressions of both genes are induced in animal cap explants by activin (Cho et al., 1991; Christian et al., 1991), and they are thought to play roles in dorsoventral patterning of the embryonic mesoderm. In a normal embryo, zygotic \textit{gsc} is expressed from stage 8.5 onwards in Spemann’s organizer field (Cho et al., 1991), and \textit{Xwnt-8} is expressed from stage 9 in all regions of the marginal zone except that of the organizer field (Christian and Moon, 1993). Expression patterns of the two markers in embryos and explants are presented in Fig. 2A. Also here the dorsoventral competence prepattern to activin is revealed, showing that \textit{gsc} signal in the AV blastomeres was weaker than that of the AD blastomeres (lanes 9 to 12). We took care to select ideal embryos with horizontal third cleavage planes, since animal blastomeres from diagonally cleaved embryos often expressed weak marker signals autonomously (data not shown). It is noteworthy that \textit{Xwnt-8} expressions in the activin-treated animal half explants were hardly induced (lanes 11 and 12). \textit{Xwnt-8} transcript in normal embryo (lane 4) was far stronger than \textit{gsc}.

Using \textit{gsc} and \textit{Xwnt-8} probes, lithium treatment was repeated as in Fig. 1B (Fig. 2B). In the stage 10.5 embryos treated with lithium, significant differences in the amounts of the two markers were detected (lanes 1 and 2). Lithium induced an increase of \textit{gsc} expression and a decrease of \textit{Xwnt-8}. The increase of \textit{gsc} in the embryo ventral region agrees with the result of the reported in situ hybridization experiment (Cho et al., 1991). For explants, treatment with both lithium and activin led to an increase of \textit{gsc} in both explants (lanes 6 and 10). This agrees with the results in Fig. 1A,B. Contrary to our expectations, \textit{gsc} expression was induced by treatment with lithium alone (lanes 5 and 9), which induced neither explant elongation nor muscle-actin gene expression. For stage 9 blastula animal

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**Fig. 1.** Modification of the competence to activin by lithium.

(A) Morphology of the explants after 15 hours of culture. Dorsal (a-d) and ventral (e-h) blastomeres were incubated in control medium (a-b, e-f) or treated with 0.3 M LiCl at the 32-cell stage for 10 minutes (c-d, g-h), and then cultured in the absence (a, c, e, g) or presence (b, d, f, h) of 50 ng/ml activin A for 10 hours. Ventral explants treated with lithium and activin (h) differ from ventral explants treated solely with activin (f), and resemble activin-treated dorsal explants (b). Bar in a, 0.5 mm. (B) Muscle actin gene expression in the embryos and explants at stage 27. RNA extracts at stage 27 from control (–), activin-treated (A), lithium-treated (Li), or lithium- and activin-treated (LiA) explants were assayed by RNAase protections with \(^{32}\)P-labeled muscle-specific \( \alpha \)-actin antisense RNA. Cytoskeletal \( \beta \)-actin RNA is also assayed by the same probe and serves as a control for RNA recovery. RNAs from embryos treated with (Li) or without (–) lithium at the 32-cell stage are also examined. RNA from 10 blastomeres and one embryo is loaded in each lane.
cap explants, gsc expression was not induced by lithium (lane 13). This shows that induction of gsc expression by lithium is effective only in the early animal blastomeres, not in the blastula animal caps.

To confirm the gsc-inducing effect of lithium, we isolated whole animal halves from the 8-cell-stage embryos and exposed them to lithium at different treatment times (Fig. 3A). In this experiment, we used Xbra, a mesoderm marker expressed throughout the marginal zone (Smith et al., 1991), to compare with the gsc expression. When the stage 10.5 embryos were treated with lithium, dramatic changes in gsc and Xwnt-8 (lanes 1 and 2) expressions were detected, but no significant difference in Xbra was shown. When the whole animal half explants were treated with lithium (lanes 3-6), expression of gsc increased in proportion to lithium-treatment time, whereas no expression of Xwnt-8 was detected at all. Although a minimal Xbra was also induced by lithium, no relation with the treatment-time was found. In Fig. 3B, the tissue sections of the explants are shown. A characteristic of the animal blastomere explants is that the control explants (a) contain a cluster of eosinophobic large cells, which are not found in the blastula animal cap explants (e.g. Ariizumi et al., 1991; Asashima et al., 1991). These bright cells are presumably derived from the prospective mesoderm region contained in the blastomere explants. Although this cluster often extended in the lithium-treated explants (b,d), no trunk mesoderm formation was observed. In contrast, the activin-treated explant (c) formed mesoderm and contained no bright cells. These results suggested that gsc gene, which has been called ‘dorsal mesoderm marker’, may not be induced by mesoderm induction, but induced by the effect of the dorsal determination event.

Activin treatment on the 8-cell-stage animal half explants induced mostly gsc (Fig. 2A, lanes 11 and 12) and that on the stage 9 blastula animal caps induced mostly Xwnt-8 (Fig. 2B, lane 12). One reason for this difference would be the type of cell population constituting the explants. The 8-cell-stage animal half explants contain prospective mesoderm which is lacking in blastula animal caps. Since gsc is expressed only in the embryo dorsal marginal zone (Cho et al., 1991), it is plausible that prospective mesodermal cells are more sensitive to activin than the ectoderm cells that constitute blastula animal caps. Another reason for this difference may be the timing of activin treatment. As Green et al.(1990) reported by a histological analysis, animal cap cells gradually lose their competence to respond to XTC-MIF (Xenopus activin) during late blastula to early gastrula stages.

**Alteration of response to activin at the MBT stage**

There is a good possibility that the cell type and/or timing of activin treatment influence the type of induced mesoderm. To test this idea, we isolated whole animal halves from the 8-cell-stage embryo, cultured in SS, then treated with 50 ng/ml activin for 1 hour at different stages. Here the explants were cleaved by two fine forceps to expose the cells of non-pigmented inner layer. In the previous study, we had confirmed that pigmented outer layer cells of animal caps do not react with mesoderm induction signals (Asashima and Grunz, 1983). The explants once opened soon shrunk and wound healed to recover original sphere forms within 1 hour. An example of the experiments is shown in morphology at stage 20 (Fig. 4A). Animal half explants (a-c) treated with activin at stage 7.5 elongated forming a large stalk of notochord, whereas those treated at stage 9.5 showed no significant extension, although they differed significantly from controls. The similar pattern of morphological change is seen in blastula animal cap explants (d-f). Examples of the tissue sections at stage 40 (g-i) are also shown. The animal half explants treated with activin at stage 7.5 (h) differentiated dorsal tissues. They contained large clusters of notochord, neural tissues and ventral mesoderm (mesenchyme, coelomic epithelium) tissues. In contrast, the explants treated at stage 9.5 formed transparent spheres including ventral tissues. The explant shown here (i) contains also a large cluster of eosinophobic large cells. These explants occasionally contained small amounts of muscle and notochord.

In Fig. 4B, the result of RNAase protection assay is shown. As seen in the expression pattern of Xbra gene, the competence
of animal cells to respond to activin is first low (stage 4.5), increases during early cleavage stages (stage 7.5-8.5), then decreases during the late blastula stages (stage 9.5-10). The loss of competence during the late blastula stages agrees well with the results of animal cap assay experiments (Green et al., 1990). In addition, the onset of competence is shown here. As for gsc and Xwnt-8, the animal half explants treated with activin at early stages (stages 4.5 and 7.5) expressed mostly gsc, but those treated at later stages (stages 8.5 and 9.5) expressed high amounts of Xwnt-8 and gsc. This suggests that an alteration of competence occurred at the midblastula transition (MBT) stage, when the cell division rate slows and zygotic gene transcriptions commence (Newport and Kirschner, 1982a,b). It is noteworthy that this phenomenon is different from the simple loss of competence. If a high competence to activin induces dorsal mesoderm and a low competence induces ventral mesoderm, treatment at stage 4.5 should induce Xwnt-8 as well as stage 9.5. Besides, gsc should preferentially decrease during the late blastula stages. However, the results indicate that activin treatment at stage 4.5 induced gsc, but not Xwnt-8. When the animal cells lose their competence to activin (stage 9.5-10), the three markers decreased synchronously. gsc did not decrease significantly earlier than the others. Similar results were obtained in the assay using differently staged blastula animal caps (data not shown).

Although the above descriptions concerned the effect of timing, the type of cell also contributes to the difference between the animal-half and animal-cap explants. When the proportion of gsc and Wnt-8 expression in the activin-treated animal cells were compared at the same stage, it is shown that a significantly larger amount of gsc is induced in the animal half explants (Fig. 4, lanes 4,5) than in the blastula animal caps (Fig. 2B, lane 12). This indicates that the cells of prospective mesoderm in the animal half explants preferentially express gsc in response to activin, even after MBT. These results suggest that early activin treatment can determine the dorsal part of marginal zone, and later, induce ventral mesoderm in the field where gsc does not exist.

**DISCUSSION**

**Dorsoventral prepattern in the animal hemisphere**

In *Xenopus laevis*, studies have established that specific members of the fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) are involved in mesoderm induction. The mechanism of dorsoventral mesoderm patterning, however, cannot be readily explained. The localizations of these factors or their receptors are still unclear (reviewed by Kimelman et al., 1992; Smith, 1993). Recent reports suggest that competence to respond to induction signals plays a sig-

![Fig. 3. Treatment-time-dependent induction of gsc by lithium without mesoderm tissue differentiation. (A) Expression of gsc, Xwnt-8 and Xbra in embryos and animal half explants treated with lithium at different treatment-time. Lanes 1 and 2 are RNA from embryos treated with (Li) or without (–) lithium for 15 minutes. Lanes 3–6 are RNA from whole animal half explants isolated at the 8-cell stage and treated with lithium for different periods. Lane 7 is RNA from animal half explants treated with activin. RNA from two embryos and 15 animal halves is loaded in each lane. (B) Histological sections through the control (a), lithium-treated (10 minutes) (b) and activin-treated (c) whole animal half explants. (d) A higher magnification of the section through a lithium-treated explant. Arrows show the position of the large-bright cells. epi, normal epidermis; not, notochord; mus, muscle. Scale bar= 100 μm for a,b,c; 40 μm for d.]
Fig. 4. Alteration of mesoderm types depending on the stage of activin treatment. Whole animal halves were isolated at the 8-cell stage, kept in normal SS, then cleaved and treated with 50 ng/ml activin at indicated stages. Control animal halves were kept throughout in SS. Animal caps were dissected from embryos at appropriate stages and readily transferred to medium containing activin. Control animal caps were dissected from stage 9 blastula. (A) Morphology of animal half (a-c) and animal cap (d-f) explants at stage 20 and histological sections of animal half explants (g-i) at stage 40. (a,d,g) Control; (b,e,h) activin treated at stage 7.5; (c,f,i) activin treated at stage 9.5. Arrows show the position of the large-bright cells. epi, normal epidermis; neu, neural tissue; not, notochord; mes, mesenchyme; coe, coelomic epidermis. Scale bar= 0.5 mm for a-f; 100 μm for g-i. (B) Expression of gsc, Xwnt-8 and Xbra at stage 10.5. RNA was isolated from ten animal half explants and two embryos.
significant role in dorsoventral mesoderm patterning. A number of studies have shown that prospective dorsal and ventral regions of *Xenopus* animal caps respond differently to the same concentration of activin. Explants from prospective dorsal ectoderm differentiate into dorsal structures whereas ventral or UV-irradiated explants do not (Sokol and Melton, 1991, 1992; Bolce et al., 1992; Christian and Moon, 1993). In the previous report, we assayed the response of animal blastomeres isolated at the 8-cell stage to activin A (animal-half assay) and showed that the competence prepattern is established at this stage (Kinoshita et al., 1993). As candidate molecules that create the prepattern, we suggest several groups of peptides. These factors modify the responsiveness of cells to mesoderm-inducing factors, but do not possess mesoderm-inducing activity by themselves (reviewed by Moon, 1993; Sive, 1993; Smith, 1993). The first candidate group that may be related to the dorsal determinant are the members of Wnt family and a different peptide noggin. These factors activate animal caps to form dorsal tissue in response to other factors, and mimic the effect of Nieuwkoop center when their mRNAs are injected into ventral blastomeres (Christian et al., 1992; Sokol and Melton, 1992; Steinbeisser et al., 1993; Ku and Melton, 1993; Smith and Harland, 1991, 1992; Smith et al., 1993). Another group of modifying factors is bone morphogenetic proteins (BMPs), which is a member of the TGF-β family. Transcripts of the *Xenopus* homologue of BMP-4 are present in the fertilized eggs and, in contrast to Wnts, modulate the effects of activin to form ventral tissue (Dale et al., 1992; Jones et al., 1992; Graff et al., 1994; Suzuki et al., 1994; Maeno et al., 1994b). The prepattern will be adequately explained if Wnts or noggin peptides were localized in the dorsal blastomeres and BMP-4 was localized in the ventral blastomeres.

The major differences between blastula animal-cap assay and the present 8-cell-stage animal-half assay are the stages of isolation and the cell types that constitute the explants. Blastula animal cap is composed exclusively of presumptive ectoderm cells, and the animal half explant includes part of the future marginal zone (Maslanski et al., 1992). The animal half explant enables us to keep culturing the presumptive mesoderm cells without mesoderm induction and to investigate the initial difference between dorsal and ventral animal cells. It is necessary in this assay to select embryos whose third cleavage plane is horizontal and close to animal pole. If not, the explant will contain endogenous mesoderm-inducing factor around the marginal zone, and form mesoderm. This may be the point that differs from the assay of Gallagher et al. (1991). They have shown that animal blastomeres from the 16-cell stage autonomously form dorsal mesoderm.

**Modification of the competence prepattern to respond to activin**

Lithium is known to dorsalize embryo pattern (Kao and Elinson, 1988), modifying normal mesoderm induction to produce dorsoanterior mesoderm, although lithium does not directly act as a mesoderm inducer (Cooke et al., 1989; Kao and Elinson, 1989). The effect of lithium seems closely related to the signaling pathways of Wnts and noggin, because lithium acts similarly to their mRNAs when injected into ventral side of an embryo (Kao and Elinson, 1989; Sokol and Melton, 1992). The action of lithium on mesoderm induction is not yet understood, but a relation to the inositol triphosphate-protein kinase C message transduction metabolism pathway (Busa and Gimlich, 1989) is suggested. The pathway has been shown to function in mesoderm induction and embryo axis determination (Maslanski et al., 1992).

Our present results showed that treatment of ventral animal cells with lithium modifies their response to activin, under conditions that preclude the influence of the vegetal cells. The competence prepattern in the animal hemisphere is likely not definitive to embryo axis determination. If the vegetal cells emit an active dorsal determining signal at later stages, the prepattern will be canceled. We suppose that dorsal animal cells inherit a small amount of dorsal determinant, but the Nieuwkoop center in the vegetal cells inherits a larger amount of dorsal determinant and mesoderm-inducing factors. The final dorsoventral mesoderm patterning may be established by a combination of maternal dorsal determinant and mesoderm-inducing signals passed from vegetal cells.

**Induction of gsc without mesoderm formation**

An additional finding obtained from the lithium treatment is that *gsc*, which is usually called dorsal mesoderm marker, was induced solely by lithium. The fact that trunk mesoderm was not formed suggests that induction of *gsc* by lithium may be independent of mesoderm induction. However, we cannot rule out the possibility that lithium induces *gsc* along with dorsal mesoderm, because *Xbra* is induced at very low levels. If the induced mesoderm were of a very dorsal-anterior type, such as head mesoderm, it would not cause explant elongation and would be expected to express low levels of *Xbra* at stage 10.5. We have to show the absence of other dorsal mesodermal markers to convincingly demonstrate that dorsal mesoderm is not formed.

If *gsc* were induced by a dorsalization signal but not by mesoderm induction, *gsc* induction by activin may reflect its dorsal determination effect. This finding may be related to the recent intriguing studies reported simultaneously (Cornell and Kimelman, 1994; LaBonne and Whitman, 1994), which showed that there are two types of activin-inducible mesodermal markers whose expressions were inhibited or not inhibited by the disruption of FGF signaling pathways. It is of interest in that explant elongation and muscle actin gene expression were inhibited and *gsc* inhibition was less effective.

**Temporal alteration of mesoderm patterning by activin**

It is well known that animal cap cells lose their competence to respond to mesoderm induction around early gastrula stage. For example, Jones and Woodland (1987) showed that animal cells treated with vegetal cells before stage 7 differentiate notochord at high frequency and those treated at later stages differentiate muscle. Green et al. (1990) have shown histologically a shift during blastula stages from dorsal to ventral types of response to activin. Also Lamb et al. (1993) showed that, in the activin-treated animal caps, N-CAM expression (a neural marker) diminishes and muscle actin expression increases after stage 8. In the present study, we showed that when we treated the animal blastomeres with activin before MBT they differentiated dorsal cells but when treated after MBT they differentiate ventral cells. The merit of our assay is that we can examine the onset of competence, and that we can use the same cell population throughout the experiment. In the report of
Xwnt-8 RNA injection experiment, Sokol (1993) demonstrates that the stage and size of the excised animal caps are critical parameters in determining the inductive response. Our present interpretation for these phenomena is not the simple loss of competence, but a positive dorsal determination effect on early animal blastomeres that may continue until MBT. There are several reports that may support this idea: embryo dorsalization effect by lithium is lost after MBT (Yamaguchi and Shinagawa, 1989), the ventral marker Xwnt-8 RNA acts as dorsal determinant before MBT (Christian et al., 1991; Smith and Harland, 1991) and erythroid differentiation induction by ventral mesoderm does not occur in stage 7 animal caps, but occurs in the stage 8-10 animal caps (Maeno et al., 1994a).

In recent models (Dawid, 1992; Kimelman, 1992; Sive, 1993), doroventral mesodermal patterning is explained primarily by two components: type or concentration of mesoderm-inducing factors, and the competence prepattern to respond to the induction signals. Our present results suggested that timing of induction may play a role in mesoderm patterning. In the earlier step, activin will induce dorsal structures with the help of dorsal determinants and, in the later step, it will induce ventral mesoderm. In embryos, the existence of maternal activin (Asashima et al., 1991; Dohrmann et al., 1993; Rebagliati and Dawid, 1993; Fukui et al., 1993, 1994) or Vg-1 product (a maternal TGF-β family protein that is a potential dorsal determinant: Dale et al., 1993; Thomsen and Melton, 1993) will induce the former action and zygotic activin B molecules synthesized after MBT (Thomsen et al., 1990; Dohrmann et al., 1993) will induce the latter action.

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REFERENCES


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