Int. J. Dev. Biol. 38: 605-611 (1994)

Original Article

Expression of mesoderm markers in *Xenopus laevis* Keller explants

JEAN-PIERRE SAINT-JEANNET[1], ALEXANDER A. KARAVANOV and IGOR B. DAWID*

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland, USA

ABSTRACT In an attempt to document at the molecular level the behavior of mesodermal cells in Keller explant preparation, we have analyzed the time course of expression of four molecular markers of mesoderm *gsc, Xbra, Xnot* and XLIM-1. Our findings demonstrate that, (i) all mesodermal markers tested were expressed in the explants, but patterning of the mesoderm appeared incomplete; (ii) during convergence and extension of the explants, mesodermal cells did not invade the ectodermal tissue at any time tested, supporting the view that mesoderm establishes exclusively planar contacts with the ectoderm in this preparation; (iii) planar contacts were not sufficient to promote the neural expression of XLIM-1 protein in these explants.

KEY WORDS: *neural induction, regionalization, mesoderm, amphibia*

Introduction

Neural induction has been an area of intensive studies for several decades (Hamburger, 1988; Saxén, 1989, Gilbert and Saxén, 1993), and for a long time this aspect of vertebrate development was rather confusing. The recent identification in *Xenopus laevis* of two secreted proteins, noggin (Lamb *et al.*, 1993) and follistatin (Hemmati-Brivanlou *et al.*, 1994), that can trigger neuralization of the ectoderm without its mesodermalization, represents a major advance in the understanding of the molecular aspects of this process. In parallel with attempts to identify inducing factors, the general biological context of induction and the sources and routes of propagation of inducing signals have been studied intensely. Two experimental systems, the exogastrula (Holtfreter, 1933; Ruiz i Altaba, 1992) and the Keller explant (Keller and Danilchick, 1988; Doniach *et al.*, 1992), have proven to be practical approaches for addressing certain questions regarding the general properties of neural induction. From studies using these systems there emerged evidence that, in addition to the conventional vertical signals that travel from the involuting mesoderm to the overlying ectoderm, planar signals that spread from the dorsal mesoderm through the plane of the ectoderm can initiate neural development (Guthrie, 1991; Doniach, 1993; Ruiz i Altaba, 1993). In exogastrulae and Keller explants, where the mesoderm moves away from the ectoderm, vertical contacts are believed to be absent but induction of many neural marker genes occurred at normal level (Kintner and Melton, 1987; Dixon and Kintner, 1989; Ruiz i Altaba, 1990, 1992' Doniach *et al.*, 1992; Papalopulu and Kintner, 1993; Zimmerman *et al.*, 1993); however, several other neural genes failed to be induced in such preparations (Sharpe and Gurdon, 1990; Dirksen and Jamrich, 1992; Ruiz i Altaba and Jessell, 1992; Ruiz i Altaba *et al.*, 1994; Taira *et al.*, 1994).

In evaluating the results of neural differentiation in these preparations, the behavior of the prospective mesodermal cells in relation to ectodermal cells is critical. During exogastrulation, the mesoderm undergoes complex cell movements which remain incompletely understood so that transient vertical contacts between mesoderm and ectoderm cannot be excluded with certainty. The movement of different cell groups in Keller explants has been documented by lineage tracing (Keller *et al.*, 1992b), indicating that presumptive mesodermal cells do not invade the ectoderm. Nevertheless, early molecular markers have not been used to identify and trace mesodermal cells and their migration in such explants; the use of such markers has proven to be highly valuable in determining cell fate (e.g., Sokol and Melton, 1991; Bolce *et al.*, 1992) and localizing specific groups of cells during embryogenesis (e.g., Izpisua-Belmonte *et al.*, 1993). While there are no reasons to doubt the appropriate identification of cell types by Keller and his colleagues in characterizing mesoderm behavior in explants (Keller and Danilchick, 1988; Keller *et al.*, 1992a,b), we felt that the analysis of mesodermal marker gene expression in this system would be of interest.

In the present paper we describe the time course of expression of four specific molecular markers of mesoderm, *gsc, Xbra, Xnot* and XLIM-1, in Keller explants; XLIM-1 is also expressed in the neurectoderm from mid-gastrula stages onward. Our findings demonstrate that mesodermal cells, as identified by these markers, do not invade at any time the ectodermal tissue, supporting the

***Address for reprints:** Building 6B Room 413, National Institutes of Health, Bethesda, MD 20892, USA. FAX: 301-496.0243.

[1]Permanent address: Centre de Biologie du Développement, Centre National de la Recherche Scientifique-Unité Mixte de Recherche 9925, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex, France.

0214-6282/94/$03.00

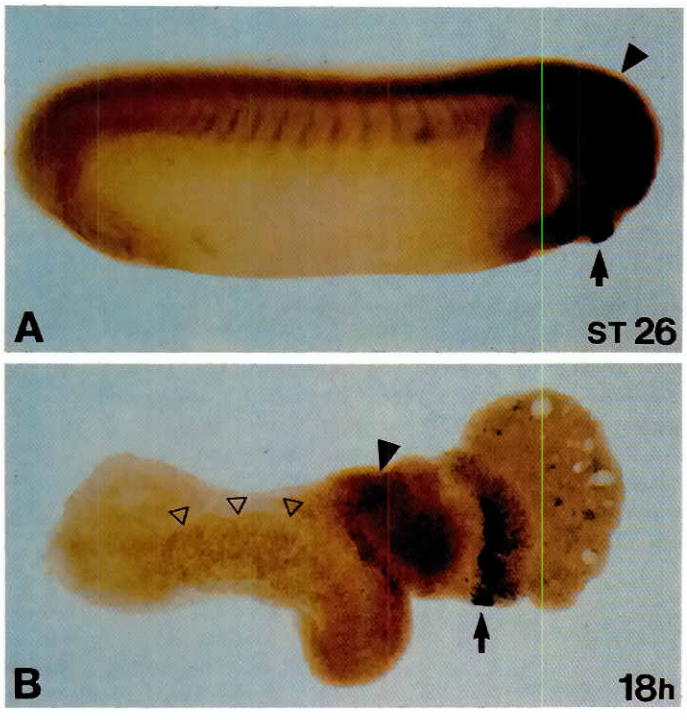

Fig. 1. Expression of neural and cement gland markers in normal embryo (A) and Keller explant (B). *Anti-NCAM antibody (filled triangle) outlines the CNS (brown staining). The black/blue staining specific to the cement gland is indicated by an arrow. The notochord in the mesodermal region of the explant (bottom) is indicated by open triangles.*

view that only planar contacts exist between mesoderm and ectoderm in Keller explants. In studying the expression of XLIM-1 in both germ layers, we noted that planar contacts are insufficient to promote the neural expression of this protein in the explants.

Results

A Keller explant consists of two sheets of dorsal mesoderm and ectoderm sandwiched together and cultured flat under a coverslip. This arrangement prevents involution of the mesoderm, but convergence and extension movements still proceed such that the anterior ends of the mesoderm and of the ectoderm elongate in opposite directions. Clean dissection, in particular the complete removal of the head mesoderm, is a prerequisite for correct establishment of these explants (Keller and Danilchick, 1988).

In order to test the accuracy of our dissections, Keller explants after 18 h of culture (equivalent stages 25-26) were processed for double whole-mount immunostaining with the pan-neural marker NCAM and a cement gland marker (Fig. 1A). In our hands, as in previous reports (Doniach *et al.*, 1992; Papalopulu and Kintner, 1993), NCAM is expressed in Keller explants in a zone that covers the proximal region of the ectoderm with sharp boundaries of expression (Fig. 1B). Interestingly, cement gland forms and the corresponding staining occurs anterior and outside the region that expresses NCAM. The notochord can be identified morphologically (Fig. 1B, open triangles) in the mesoderm as distinct from the NCAM-expressing region, as expected from earlier work (Keller and Danilchick, 1988; Keller *et al.*, 1992a,b).

Time course of expression of Xbra, Xnot and gsc in Keller explants

Keller explants at different times during convergence and extension (cf. Materials and Methods) were processed for whole-mount *in situ* hybridization using probes for *Xbra*, *gsc* and *Xnot*. In control embryos, *Xbra* is expressed in the entire mesoderm at the early gastrula stage, and during further development becomes restricted to the notochord and posterior mesoderm, forming a ring surrounding the closing blastopore (Smith *et al.*, 1991; Green *et al.*, 1992). *Xnot* is expressed most intensely in the chordal mesoderm, but also in different regions of all three germ layers in a dynamically changing pattern (von Dassow *et al.*, 1993). The *gsc* gene marks the dorsal marginal zone at late blastula stage and becomes associated with the extreme anterior edge of the dorsal mesoderm, the head mesoderm, during gastrulation (Cho *et al.*, 1991; Steinbeisser and De Robertis, 1993). Thus, the expression domains of these three transcription factors cover overlapping areas within the developing mesoderm.

In Keller explants after 30 min of culture, all three markers were found in a restricted area at the vegetal, i.e. mesodermal, edge of the explant (Fig. 2A,G). Staining for *gsc* is less intense than in control embryos since prospective head mesoderm has been removed from the explants (Keller and Danilchick, 1988); *gsc* expression that is detected at this stage in the explants corresponds to its appearance in the deep marginal zone. After 2 h of culture (equivalent stage 11), no *gsc* staining was detected in any of the explants (not shown). The areas of expression of the *Xbra* and the *Xnot* gene were maintained within a narrow band during the several hours of culture while the explants underwent elongation. The posterior limit of the expression region seems to correspond to the boundary between the ectoderm and the mesoderm (Fig. 2B,D,E). Staining for the two gene products is distinct. The *Xbra* pattern appears in two transverse bands (Fig. 2B), which may correspond to one band for each side of the original sandwich. Each of the bands probably represents a portion of the circumblastoporal ring that is known to express *Xbra* during gastrulation (Smith *et al.*, 1991). Since in a Keller explant the posterior of the mesoderm and ectoderm abut, the location of the *Xbra*-positive region agrees with its localization in posterior mesoderm. After 6 h of culture, *Xbra* is still predominantly expressed in the posterior mesoderm, but a weaker signal becomes apparent in an area that outlines the differentiating notochord (Fig. 2C). This pattern agrees broadly with that in the embryo but is quantitatively different: by stage 14, corresponding to 6 h incubation, *Xbra* is most intensely expressed in the notochord in the embryo (Smith *et al.*, 1991), but is quite weakly expressed in this tissue in the explant. This result suggests that the explant reproduces the early gastrula mesodermal expression patterns quite well, but not their subsequent elaboration. This suggestion is supported by the pattern of *Xnot* staining, which appears in two patches in explants after 2 and 4 h of incubation (Fig. 2D,E). After longer times, *Xnot* expression becomes more diffuse within the mesodermal part of the explants but does not outline a notochord-like structure (Fig. 2F). Thus, *Xnot* expression in the explants also reflects the early gastrula pattern where it is predominantly in dorsal mesoderm, but does not recapitulate changes in later gastrulation when *Xnot* is most abundant in the notochord (von Dassow *et al.*, 1993).

At all time points studied, cells expressing these three marker genes and, by extension, mesodermal cells in general, do not invade the ectodermal region of the explant, in accordance with the lineage analysis of Keller *et al.* (1992b). Therefore, vertical con-

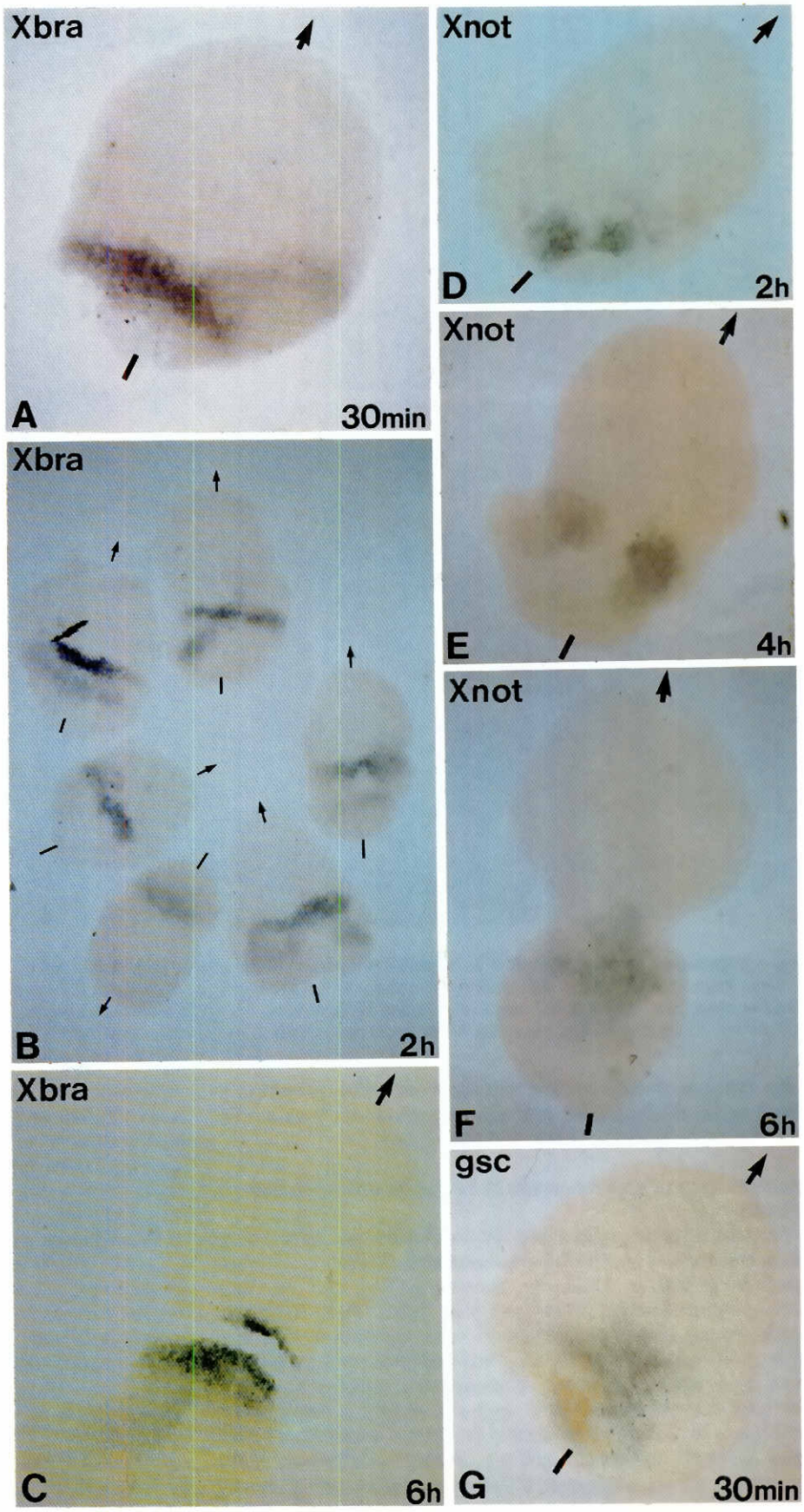

Fig. 2. Time course of expression of early mesoderm markers during convergence and extension of Keller explants, as visualized by whole-mount *in situ* hybridization. *The time of explant culture, and the probe are indicated in each panel. The arrow points towards the anterior portion of the ectoderm of each explant.*

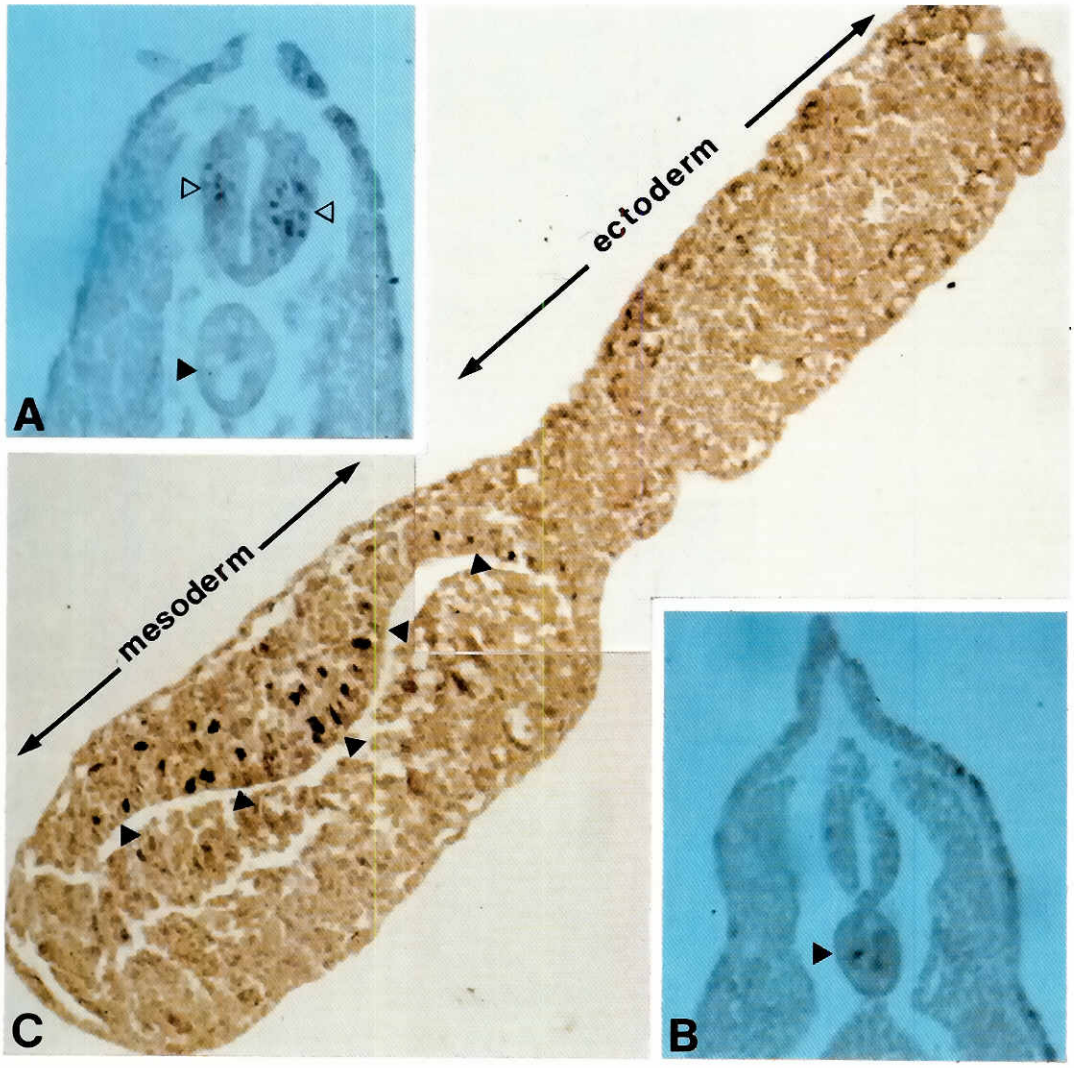

Fig. 3. Expression pattern of XLIM-1 in normal embryo and Keller explant, as visualized by immunocytochemistry. (A,B) *Transverse sections through stage 25-26 embryo in the anterior* **(A)** *and posterior* **(B)** *trunk level, showing XLIM-1-positive nuclei in the neural tube (open arrowheads in* **A***) and the notochord (closed arrowhead in B). Sagittal section through a Keller explant cultured for 18 h* **(C)***; XLIM-1-positive nuclei are confined to the notochord-like structure in the mesodermal region of the explant. No staining is detected in the ectodermal portion of the explant.*

tacts between mesoderm and ectoderm are unlikely to occur in these explants, supporting the view that planar contacts are the dominant type of interaction in this preparation.

Requirement of vertical contacts for the neural expression of XLIM-1

The *Xlim-1* gene is expressed in three cell lineages, (i) first in the dorsal mesoderm and the forming notochord, (ii) starting in the late gastrula, in certain cells of the nervous system, and (iii) in the pronephros and nephric duct (Taira *et al.*, 1992, 1994). To characterize the expression of this gene in Keller explants we analyzed specimens cultured for 18 h by immunocytochemistry with an antibody directed against the carboxyterminal region of XLIM-1. In the corresponding stage 25-26 embryos, XLIM-1 is expressed in two opposite and non-overlapping gradients: an anteroposterior gradient in the spinal cord, and a posteroanterior gradient in the notochord. As a consequence of this graded distribution, XLIM-1 is detected anteriorly in the spinal cord in a subset of cells localized in an intermediate position along the dorsoventral axis of the neural tube (Fig. 3A), and posteriorly in the notochord (Fig. 3B). The figure also illustrates the nuclear localization of the antigen, as expected for a homeodomain-containing protein. Sections of Keller explants at an equivalent stage display nuclear staining solely in the mesodermal area in a tissue with notochord-like morphology, while the ectodermal segment remained completely devoid of staining (Fig. 3C). Therefore, the notochord-specific expression of XLIM-1 arises in these explants in a similar way as in the embryo, but the neural expression is absent. This result suggests that planar contacts in this preparation are not sufficient to allow the neurectodermal expression of XLIM-1.

Discussion

The Keller explant preparation has been extensively used as a paradigm for the study of neural induction. It consists of two pieces of dorsal mesoderm and ectoderm cultured together

apposed at their inner surfaces under a coverslip preventing involution of the mesoderm, such that mesoderm and ectoderm elongate in opposite directions by convergence and extension movements (Keller and Danilchick, 1988; Doniach, 1993). As a consequence, the mesoderm is believed to be unable to establish vertical contact with the ectoderm, but neuralization of the ectoderm can occur and many neural marker genes are expressed (Dixon and Kintner, 1989; Doniach *et al.*, 1992; Papalopulu and Kintner, 1993; Zimmerman *et al.*, 1993). Experiments with Keller explants, in addition to those using exogastrulae, are the basis for the conclusion that signal transmission through planar contacts between mesoderm and ectoderm and further signal conductance through the plane of the ectoderm are possible during neural induction (reviewed in Doniach, 1993 and Ruiz i Altaba, 1993). The relative importance of planar as compared to vertical inductive signalling remains under discussion (Saint-Jeannet and Dawid, 1994).

The experimental evidence for the view that vertical contacts are completely excluded from the Keller explant preparation is based on lineage tracing of labeled marginal zone cells recombined with unlabeled ectoderm (Doniach *et al.*, 1992; Keller *et al.*, 1992b). Keller *et al.* (1992b) showed that in 50% of cases analyzed no cells from the labeled marginal zone were found in the ectoderm at the end of the experiment, while in the remaining cases very few cells were seen. Given the importance of the conclusions based on this preparation it appeared worthwhile to reinvestigate the behavior of mesodermal cells in Keller explants with the aid of specific markers. While multiple neural markers have been used to characterize the differentiation of such explants (Dixon and Kintner, 1989; Doniach *et al.*, 1992; Papalopulu and Kintner, 1993; Zimmerman *et al.*, 1993), this is the first study, to our knowledge, in which the expression of mesodermal markers has been tested.

A time course of expression of three mesodermal markers, *gsc*, *Xbra* and *Xnot*, during elongation of Keller explants led to two conclusions. (1) Mesodermal cells as identified by these three markers never invaded the ectoderm during convergence and extension movements. This result reinforced at a molecular level the conclusions based on direct observation and lineage tracing initially reported by Keller and his colleagues (Keller and Danilchick, 1988; Keller *et al.*, 1992b), and further supports the view that planar contacts are the predominant if not exclusive contacts between ectoderm and mesoderm in this preparation. (2) The patterning of the mesoderm in Keller explants is incomplete, as seen in the expression patterns of the three marker genes tested. While the low level and transient nature of *gsc* expression is simply explained by the removal of prechordal mesoderm where this gene is active through gastrulation, the situation is more complex with respect to the other two genes. A notochord forms in Keller explants, yet *Xbra* expression was low and *Xnot* expression was not observed in this structure after 6 h of incubation (Fig. 2C,F). *Xbra* expression was restricted to a ring of posterior mesoderm, presumably corresponding to its circumblastoporal pattern (Fig. 2A-C); *Xnot* staining was usually seen in two patches which became diffuse after longer incubation (Fig. 2D-F), and which cannot easily be related to any aspect of the *in vivo* pattern of this gene. Thus it appears that notochord development in the explants is either incomplete or delayed. The latter possibility is suggested by the observation that explants cultured for a longer period express the XLIM-1 antigen (Fig. 3) as well as the Tor 70 antigen (not shown; the Tor 70 monoclonal antibody is specific for the notochord as shown by Bolce *et al.*, 1992).

The use of Keller explants and exogastrulae in *X. laevis* has established that planar contacts can induce many but not all neural markers normally expressed in the embryo (Sharpe and Gurdon, 1990; Dirksen and Jamrich, 1992; Ruiz i Altaba and Jessell, 1993; Ruiz i Altaba *et al.*, 1994; Taira *et al.*, 1994). While a high degree of anteroposterior patterning of the central nervous system could be obtained in such preparations (Ruiz i Altaba, 1990, 1992; Doniach *et al.*, 1992; Papalopulu and Kintner, 1993; Zimmerman *et al.*, 1993), very little patterning along the dorsoventral axis was observed (Ruiz i Altaba, 1992; Taira *et al.*, 1994). The dorsoventral patterning of the neural tube could be partially rescued in exogastrulae by grafting of a notochord into the ectoderm (Ruiz i Altaba, 1992). Similarly, in the chick embryo, notochord ablation and grafting experiments have demonstrated that dorsoventral patterning of the neural tube depends on vertical signals from the underlying notochord (Yamada *et al.*, 1991). Together, these data suggest that vertical apposition of the dorsal mesoderm is required, at least for certain aspects of the regionalization of the neurectoderm (reviewed in Ruiz i Altaba, 1994).

Making use of an antiserum against the homeodomain-containing protein XLIM-1, we analyzed the expression of this marker in Keller explants. In addition to its presence in the notochord, XLIM-1 is normally expressed in a subset of neurons in an intermediate position along the dorsoventral axis of the neural tube (Taira *et al.*, 1994); thus, it is a suitable marker for the analysis of the regionalization of the neural tube. In Keller explants XLIM-1 was never found to be expressed in the ectodermal portion of the explants but was restricted to the notochord. This result indicates that planar contacts are not sufficient to induce the neural expression of XLIM-1, in good agreement with the observations on *Xlim-1* transcripts in exogastrulae (Taira *et al.*, 1994). The lack of expression of this marker serves to illustrate the importance of the vertical apposition of the dorsal mesoderm (notochord) in the patterning of the neural tube along its dorsoventral axis.

Materials and Methods

Embryos

Mature albino oocytes were stripped from adult *Xenopus laevis* injected with HCG (Sigma) and fertilized with a minced testis. Half an hour after fertilization eggs were dejellied in 2% cysteine, washed, reared in 0.1xNAM (Normal Amphibian Medium; Peng, 1991) and staged according to Nieuwkoop and Faber (1967).

Keller explants

Keller explants were prepared as initially described by Keller and Danilchik (1988). Two pieces of dorsal ectoderm and marginal zone devoid of endoderm were dissected from stage 10+ embryos and cultured face-to-face for 5 min in 1xNAM. After healing explants were transferred into 0.5xNAM and cultured under a coverslip for different times: 30 min (equivalent stage 10.5), 2 h (stage 11), 4 h (stage 12), 6 h (stage 14) and 18 h (stage 25-26).

Antibodies

4d (anti-N-CAM) hybridoma supernatant was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Biology, University of Iowa, under contract N01-HD-2-3144 from NICHD. The anti-cement gland anti-serum whose specificity will be described elsewhere was a gift of Peter Good. The anti-XLIM-1 polyclonal antibody was raised in rabbits against a fusion protein between the GST and the carboxy-terminal region of Xlim-1. The specificity of this antibody and the pattern of expression of XLIM-1 will be described elsewhere (Karavanov *et al.*, in preparation). Secondary antibodies conjugated to peroxidase and to alkaline phosphatase were from Sigma and Boehringer Mannheim, respectively.

Immunocytochemistry

Whole-mount immunostaining followed the procedure described by Hemmati-Brivanlou and Harland (1989). Embryos were fixed in MEMFA for 1 h and then washed in methanol. Embryos were then rehydrated, blocked in PBT plus 10% sheep serum, incubated overnight at 4°C with primary antibody, washed extensively in PBT and developed with secondary antibody conjugated to peroxidase overnight at 4°C. Enzymatic reaction was performed until development of appropriate staining and stopped in methanol. Embryos were then transferred and observed in Murray clearing solution (Benzyl alcohol/Benzyl benzoate). For double staining the two reactions were carried out consecutively.

Immunostaining on sections followed standard protocols. Briefly, embryos were fixed in MEMFA for 1 h, transferred successively into methanol and xylene, embedded in paraplast, and 10 μm sections were collected on glass slides. Sections were deparaffinized, rehydrated, and incubated in 2% Boehringer Mannheim blocking reagent for 1 h. Staining was performed by successive incubation with anti-XLIM-1 antibodies and anti-rabbit Ig conjugated to peroxidase. Enzymatic reaction was performed using Pierce reagent, and staining was enhanced with osmium tetroxide (0.4%). The reaction was stopped in water, sections were dehydrated and mounted in permount.

Whole-mount in situ hybridization

The procedure described by Harland (1991) was followed with minor modifications (Cho *et al.*, 1991; Ruiz i Altaba, 1992). Digoxigenin-labeled antisense RNAs were generated by *in vitro* transcription of cDNA clones using the digoxigenin labeling kit (Boehringer Mannheim) following the manufacturer's instructions.

Acknowledgments

We thank E. De Robertis, D. Kimelman and J. Smith for providing probes.

References

BOLCE, M.E., HEMMATI-BRIVANLOU, H., KUSHNER, P.D. and HARLAND, R.M. (1992). Ventral ectoderm of *Xenopus* forms neural tissue, including hind-brain, in response to activin. *Development 115:* 681-688.

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.

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.

DIXON, J.E. and KINTNER, C.R. (1989). Cellular contacts required for neural induction in *Xenopus* embryos: evidence for two signals. *Development 106:* 749-757.

DONIACH, T. (1993). Planar and vertical induction of anteroposterior pattern during development of the amphibian central nervous system. *J. Neurobiol. 24:* 1256-1275.

DONIACH, T., PHILLIPS, C.R. and GERHART, J.C. (1992). Planar induction of anteroposterior pattern in the developing central nervous system of *Xenopus laevis*. *Science 257:* 542-545.

GILBERT, S.F. and SAXÉN, L. (1993). Spemann's organizer: models and molecules. *Mech. Dev. 41:* 73-89.

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.

GUTHRIE, S. (1991). Horizontal and vertical pathways in neural induction. *Trends Neurosci. 14:* 123-126.

HAMBURGER, V.K. (1988). *The Heritage of Experimental Embryology. Hans Spemann and the Organizer.* Oxford University Press, New York.

HARLAND, R.M. (1991). *In situ* hybridization: an improved whole-mount method for *Xenopus* embryos. In *Methods in Cell Biology*, Vol. 36 (Eds. B.K. Kay and H.B. Peng). Academic Press, San Diego, pp. 675-685.

HEMMATI-BRIVANLOU, A. and HARLAND, R.M. (1989). Expression of an *engrailed*-related protein is induced in the anterior neural ectoderm of early *Xenopus* embryos. *Development 106:* 611-617.

HEMMATI-BRIVANLOU, A., KELLY, O.G. and MELTON, D.A. (1994). Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell 77:* 283-295.

HOLTFRETER, J. (1933). Die totale Exogastrulation, eine Selbstablosung des Ektoderms vom Entomesoderm. *W. Roux Arch. EntwMech. 129:* 669-793.

IZPISUA-BELMONTE, J.C., DE ROBERTIS, E.M., STOREY, K.G. and STERN, C.D. (1993). The homeobox gene *goosecoid* and the origin of organizer cells in the early chick blastoderm. *Cell 74:* 645-659.

KELLER, R.E. and DANILCHIK, M. (1988). Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. *Development 103:* 193-209.

KELLER, R.E., SHIH, J. and SATER, A.K. (1992a). The cellular basis of the convergence extension of the *Xenopus* neural plate. *Dev. Dynamics 193:* 199-217.

KELLER, R.E., SHIH, J., SATER, A.K. and MORENO, C. (1992b). Planar induction of convergence and extension of the neural plate by the organizer of *Xenopus*. *Dev. Dynamics 193:* 218-234.

KINTNER, C.R. and MELTON, D.A. (1987). Expression of *Xenopus* N-CAM RNA in ectoderm is an early response to neural induction. *Development 99:* 311-325.

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.

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

PAPALOPULU, N. and KINTNER, C.R. (1993). *Xenopus* distal-less related homeobox genes are expressed in the developing forebrain and are induced by planar signals. *Development 117:* 961-975.

PENG, H.B. (1991). Solutions and protocols. In *Methods in Cell Biology*, Vol. 36 (Eds. B.K. Kay and H.B. Peng). Academic Press, San Diego, pp. 657-662.

RUIZ I ALTABA, A. (1990). Neural expression of the *Xenopus* homeobox gene *Xhox3:* evidence for a patterning neural signal that spread through the ectoderm. *Development 108:* 595-604.

RUIZ I ALTABA, A. (1992). Planar and vertical signals in the induction and patterning of the *Xenopus* nervous system. *Development 115:* 67-80.

RUIZ I ALTABA, A. (1993). Induction and axial patterning of the neural plate: planar and vertical signals. *J. Neurobiol. 24:* 1276-1304.

RUIZ I ALTABA, A. (1994). Pattern formation in the vertebrate neural plate. *Trends Neurosci. 17:* 233-243.

RUIZ I ALTABA, A. and JESSELL, T.M. (1992). Pintallavis, a gene expressed in the organizer and midline cells of frog embryos: involvement in the development of the neural axis. *Development 116:* 81-93.

RUIZ I ALTABA, A., COX, C., JESSELL, T.M. and KLAR, A. (1994). Ectopic neural expression of a floor plate marker in frog embryos injected with midline transcription factor Pintallavis. *Proc. Natl. Acad. Sci. USA 90:* 8268-8272.

SAINT-JEANNET, J-P. and DAWID I.B. (1994). Vertical versus planar neural induction in *Rana pipiens* embryos. *Proc. Natl. Acad. Sci. USA 91:* 3049-3053.

SAXÉN, L. (1989). Neural induction. *Int. J. Dev. Biol. 33:* 21-48.

SHARPE, C.R. and GURDON, J.B. (1990) The induction of anterior and posterior neural genes in *Xenopus laevis*. *Development 109:* 765-774.

SMITH, J.C., PRICE, B.M.J., GREEN, J.B.A., WEIGEL, D. and HERRMANN, B.G. (1991). Expression of a *Xenopus* homolog of *brachyury* (T) is an immediate-early response to mesoderm induction. *Cell 67:* 753-766.

SOKOL, S. and MELTON, D.A. (1991). Pre-existent pattern in *Xenopus* animal pole cells revealed by induction with activin. *Nature 351:* 409-411.

STEINBEISSER, H. and DE ROBERTIS, E.M. (1993). *Xenopus* goosecoid: a gene expressed in the prechordal plate that has dorsalizing activity. *C.R. Acad. Sci. (Paris) 316:* 966-971.

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 embryos. *Genes Dev. 6:* 356-366.

TAIRA, M., OTANI, H., JAMRICH, M. and DAWID, I.B. (1994). Expression of the LIM class homeobox gene *Xlim-1* in pronephros and CNS cell lineages of *Xenopus* embryos is affected by retinoic acid and exogastrulation. *Development 120:* 1525-1536.

VON DASSOW, G., SCHMIDT, J.E. and KIMELMAN, D. (1993). Induction of the *Xenopus* organizer: expression and regulation of Xnot, a novel FGF and activin-regulated homeobox gene. *Genes Dev. 7:* 355366.

YAMADA, T., PLACZEK, M., TANAKA, H., DODD, J. and JESSELL, T.M. (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell 64:* 635-647.

ZIMMERMAN, K., SHIH, J., BARS, J., COLLAZO, A. and ANDERSON D.J. (1993). XASH-3, a novel *Xenopus* achaete-scute homolog, provides an early marker of planar neural induction and position along the mediolateral axis of the neural plate. *Development 119:* 221-232.

Accepted for publication: September 1994