The homeobox-containing gene XANF-1 may control development of the Spemann organizer

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SUMMARY

At the beginning of gastrulation the homeobox-containing gene, XANF-1, is expressed at a low level throughout the animal hemisphere of Xenopus laevis embryos, with a local maximum of expression in the region of the dorsal blastopore lip. By the end of gastrulation expression ceases everywhere except in the most anterior part of the neuroectoderm. We have investigated the functions of this gene by microinjecting XANF-1 mRNA in the blastomeres of the 32-cell stage embryo and have observed the following effects. First, microinjections of the mRNA in the animal blastomeres and the blastomeres of the marginal zone elicited massive migration of cells to the interior of the embryo at the early gastrula stage. Second, overexpression of XANF-1 in the ventral marginal zone (VMZ) resulted in the appearance of an additional centre of gastrulation movements and the formation of a secondary axis. In addition we showed that synthetic XANF-1 mRNA was able to cause dorsal-type differentiation in VMZ explants extirpated from the microinjected embryos at the beginning of gastrulation. These results suggest that XANF-1 may control the main functions of cells of the Spemann organizer.

Key words: homeobox, XANF-1, Spemann organizer, Xenopus laevis, axis formation, blastomere

INTRODUCTION

Much accumulated evidence suggests that a conserved system consisting of a set of homeobox-containing genes plays a critical role in establishing positional information along the anterior-posterior (AP) embryonic axis both in invertebrates and vertebrates (reviewed by McGinnis and Krumlauf, 1992). Protein products of these genes act as transcription factors and interaction between them and the genes that they regulate leads to the subdivision of the AP axis into different regions (reviewed by Levine and Hoey, 1988; Scott et al., 1989).

In this way, homeobox genes of the HOM complex in Drosophila and their homologues of the Hox complex in vertebrates provide positional information that is responsible for establishing the identity of the body segments along the AP axis in the trunk and posterior head regions (Lewis, 1978; Gehring, 1987; Akam, 1989; McGinnis and Krumlauf, 1992).

Less is known about the function of homeobox genes that might control the development of anterior-most structures. Only for the Drosophila maternal gene, bicoid, has it been firmly ascertained that its protein gradient, highest at the anterior pole of the egg, is sufficient to determine the polarity and pattern of the anterior half of embryo (Driever and Nusslein-Volhard, 1988; Driever et al., 1990). Several other homeobox genes with anterior-specific expression have been cloned recently from Drosophila (Dalton et al., 1989; Finkelstein et al., 1990), Xenopus (Papalopulu and Kintner, 1993) and mouse (Price et al., 1991; Price et al., 1992; Simeone et al., 1992). In Drosophila it was shown that loss-of-function mutations in three of them, normally expressed in the cephalic region of the embryo, orthodenticle (otd), empty spiracles (emsp) and buttonhead (btd), lead to the deletion of groups of adjacent head segments (Cohen and Jurgens, 1990; Finkelstein and Perrimon, 1990). Some evidence also suggests that the homologue of the Otx-2 gene may control development of the anterior-most structures (cement gland) in Xenopus embryos (Pannese et al., 1995).

In the present work, we have investigated the functions of the homeobox-containing gene of Xenopus laevis, XANF-1, which we cloned recently (Zaraisky et al., 1992). Mouse (Thomas and Rathjen, 1992), chicken (D. C. Beebe personal communication) and zebrasfish (Zaraisky et al., EMBL and GenBank accession numbers L38673 and Z47204 respectively) homologues of XANF-1 were subsequently cloned. The absence of extended homology between these and other homeobox genes suggests that XANF-1 and its homologues represent a novel class of homeobox genes.

In Xenopus, XANF-1 expression begins at the late blastula...
stage in the whole animal hemisphere, including the marginal zone. At the beginning of gastrulation XANF-1 shows highest expression levels in the deep cells of the dorsal blastopore lip, in cells that will give rise to the anterior (head) mesoderm. During gastrulation, mesodermal expression ceases and expression begins in the presumptive neurectoderm, eventually becoming intense at the anterior end of the presumptive neural plate. This pattern suggests that XANF-1 could participate in controlling the development of anterior structures.

To define more precisely the role of XANF-1, we have examined the effects of ectopic expression of this gene in the early embryo. The results obtained suggest that XANF-1 is able to control positional identities of cells along the AP embryonic axis, switching on a differentiation programme of a more anterior character, both in mesoderm and ectoderm.

**RESULTS**

**Analysis of XANF-1 expression by whole-mount in situ hybridisation**

Our previous analysis of the pattern of XANF-1 expression by the RT-PCR technique, provided us with only a crude picture (Zaraisky et al., 1992). Therefore, in the present study we have performed a detailed analysis of XANF-1 expression, using whole-mount in situ hybridisation. Expression of XANF-1 can be visualized for the first time at the late blastula stage (stages 9; Nieuwkoop and Faber, 1967). In confirmation of our earlier RT-PCR analysis, in situ hybridization revealed that XANF-1 is expressed at this stage at low levels in most cells of the animal half of the embryo (not shown).

By the beginning of gastrulation the expression of XANF-1 decreased everywhere except for a crescent-shaped region in the dorsal blastopore lip where, in contrast, the expression became more intense (Fig. 1A). Analysis of dissected embryos revealed that it was the cells of the deep layers of the lip, corresponding to the presumptive anterior mesoderm, that expressed XANF-1 transcripts most abundantly at the early gastrula stage (Fig. 1B). This tissue makes up the Spemann organizer (Spemann, 1938; Stewart and Gerhart, 1990), a region responsible for the formation of the embryonic axis.

Slightly later, at stage 10.5-11, intensive expression of XANF-1 could be seen in the presumptive neurectodermal cells, located just above the anterior mesoderm which still expresses this gene (Fig. 1C).

By the midgastrula (stages 11.5-12), expression was decreased everywhere except in the presumptive anterior neurectoderm, where it increased very sharply (Fig. 1D). Approximately the same expression pattern was detected in the late gastrula-early neurula embryos (stages 12.5-13.5). Dissection of embryos revealed that, in contrast with the early gastrula stage, label was now localised exclusively in cells of both neurectodermal layers, but was absent from cells of the underlying anterior mesoderm (Fig. 1E).

By the midneurula stage (stage 15), the zone of expression was restricted to a crescent-shaped area on the surface of the anterior neural ridge (Fig. 1F). Two stripes of more intense labelling within this spot bordered the medial anterior ridge from the anterior and posterior sides. According to the fate map of the Xenopus neural plate (Eagleson and Harris, 1990), cells from the medial part of the expression area should contribute mainly to the future telencephalon and anterior pituitary. The more weakly labelled part of the spot probably corresponds to some diencephalic structures.

It also seems very probable that at least some cells from the anterior-most part of the labelled region give rise to buccal structures, as well as the cement gland.

At later stages, the hybridization signal gradually receded into the posterior region of the initial crescent-shaped zone (Fig. 1G), eventually becoming restricted to the position of the prospective anterior pituitary (Fig. 1H) at the end of neurulation (stages 20-22).

**Injection of XANF-1 mRNA into the ventral blastomeres elicits formation of a secondary axis**

The results of in situ hybridization indicated that the XANF-1 gene is predominantly transcribed in two zones: the dorsal...
blastopore lip at the beginning of gastrulation and, subse-
sequently, the anterior part of the neural anlage. This expression
pattern is compatible with the idea that \textit{XANF-1} may take part
in controlling development both of the early dorsal blastopore
lip (Spemann organizer) and of the anterior part of the forebrain.

We therefore tested the ability of synthetic \textit{XANF-1} mRNA to induce
ectopic Spemann organizer activity in cells of the ventral marginal zone
(VMZ). It is well known that if these cells are grafted in the VMZ they can
cricket host cells to form a secondary embryonic axis and, moreover, to
induce them to differentiate with dorsal characteristics (Spemann, 1938;
Stewart and Gerhart, 1990).

In this series of experiments, we injected two marginal ventral blas-
tomeres, C4, at the 32-cell stage (Fig. 2A), with synthetic \textit{XANF-1} RNA at a
concentration of 50 pg/blastomere. These blastomeres normally contribute
to the ventral mesoderm, caudal somite mesoderm, epidermis and endoderm
(Dale and Slack, 1987; Moody, 1987), whereas at the early gastrula stage,
endogenous \textit{XANF-1} is expressed pre-
dominantly in the progeny of blas-
tomere C1 giving rise to the notochord,
inside mesoderm and endoderm.

The first visible changes after
injection were at the early gastrula
stage. About 30% of the injected
embryos exhibited a premature invagi-
nation furrow in the area of future
ventral blastopore lip (Fig. 2B, bottom
row, triangles). As a result, such
embryos had two independent centres
of invagination, the normal one
(dorsal) and an additional one
(ventral). This ventral centre usually
appeared somewhat later than the
dorsal one. By the end of neurulation,
all such embryos had an additional axis
on their ventral or lateral sides (Fig.
2C; Table 1). These secondary axes
were invariably incomplete: typically
they contained muscle, neural crest
derivatives (melanocytes) and neural
structures, resembling trunk and
caudal regions of normal CNS, but
lacked notochord and head structures
(15 embryos with secondary axes were
analysed by routine histology).

Secondary axes were generally not
seen (except for one embryo) in the
control embryos injected with the
truncated mRNA (Table 1).

To trace the fate of cells containing
synthetic \textit{XANF-1} mRNA, we

Fig. 1. Whole-mount in situ hybridization with \textit{XANF-1} anti-sense RNA labelled with
digoxigenin. (A) At the beginning of gastrulation (stage 10.25) the strongest expression of
\textit{XANF-1} is localized on the dorsal side of the embryo, in the Spemann organizer region (arrow).
Vegetal view, the dorsal side is to the top. (B) The same embryo, as in A, but cut in half. Within
the Spemann organizer region, \textit{XANF-1} transcripts are present predominantly in the deep zone
(closed), corresponding to the prospective head mesoderm and endoderm, and are much less
abundant in the surface cells (sc). Lateral view, dorsal side is to the right, animal pole is to the
top. Arrow indicates the position of the dorsal blastopore lip. The blastocoel roof (animal cap)
becomes destroyed during the whole-mount procedure. (C) At stage 10.5, \textit{XANF-1} begins to be
expressed intensely in the presumptive neurectoderm (pn), located just above the anterior
mesoderm (am), which still expresses this gene. Lateral view, dorsal side is to the right, animal
pole is to the top. Arrow indicates the position of the dorsal blastopore lip. The blastocoel roof
(br) was flattened during the whole-mount procedure. (D) By the middle-late gastrula stage
(stage 12), the expression of \textit{XANF-1} has increased and is restricted toward the anterior region of
the presumptive neurectoderm (pn). The embryo is shown from the anterior, dorsal side up.
(E) At the middle-late gastrula stage, the expression of \textit{XANF-1} is localised exclusively in cells
of both neurectodermal (ne) layers, but is absent from cells of the underlying anterior mesoderm
(am). The embryo, cut in half, is shown from the left side. The dorsal side is to the top, anterior
end is to the left. (F) At the mid-neurula (stage 15), the hybridization signal is horse-shoe-
shaped, localised on the surface of the anterior neural ridge, with two stripes of higher intensity
bordering the medial anterior ridge from the anterior and posterior sides. Orientation of the
embryo is the same as in D. (G) At the end of neurulation (stage 20), the only labelled spot is seen in a region corresponding to the location of the prospective anterior
pituitary. Orientation of the embryo is the same as in D. Scale bar, 200 µm.
**XlHbox6** and **goosecoid** (Niehrs and DeRobertis, 1991; Niehrs et al., 1993).

We found that almost all cells containing exogenous **XANF-1** mRNA became concentrated in the anterior end of the secondary axes, forming the anterior-most mesenchyme (Fig. 2D). In control embryos co-injected into the equivalent blastomeres with CG and truncated **XANF-1** RNA, relatively few labelled cells were seen in more posterior regions. Labelling

### Table 1. Microinjections of **XANF-1** mRNA

<table>
<thead>
<tr>
<th>Injected blastomere</th>
<th>Full-length mRNA <strong>XANF-1</strong></th>
<th>Truncated mRNA <strong>XANF-1</strong></th>
</tr>
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<tr>
<td></td>
<td>Secondary axis</td>
<td>Fate of injected cells*</td>
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<tr>
<td></td>
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<tr>
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</tr>
<tr>
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<td>53</td>
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<td>24</td>
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</tr>
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</table>

Embryos were injected in pairs of blastomeres at the 32-cell stage with 50 pg synthetic full-length or truncated **XANF-1** mRNA. In some experiments colloidal gold was co-injected with mRNA for lineage tracing.

*Colloidal gold labelling.
†Embryos with secondary axis.
‡Explants demonstrating signs of dorsal differentiation.
was particularly low in the myotomes, where labelled cells often had typical myotube morphology, and was not detected in the ectodermal derivatives in most injected embryos. In contrast, labelled cells in control embryos were widely distributed throughout both the ectoderm and mesoderm of the ventroposterior region of the body and never demonstrated any preferential anterior localisation (Fig. 2E).

**XANF-1 can induce dorsal-type differentiation in VMZ explants**

The effect obtained indicated that XANF-1 mRNA injected into marginal ventral blastomeres is able to mimic the central property of the Spemann organizer, namely the induction of a secondary axis. It was still uncertain, however, whether this gene caused differentiation of organizer cell types directly, or whether it merely induced the formation of an additional centre of gastrulation movements, which in turn modeled a secondary axis from the cells of more dorsal sectors of the marginal zone, i.e., from the cells already specified for axial differentiation. To distinguish between these two possibilities, we tested the effect of XANF-1 mRNA microinjection on the differentiation of ventral marginal zone explants, from the early gastrulae (Fig. 3A). It has long been shown that no dorsal structures develop from such explants if they are removed from normal embryos (Slack and Forman, 1980).

After 1 day in culture, about 40% of the explants (Table 1) taken from embryos injected with XANF-1 mRNA in the marginal ventral blastomeres C4 developed an elongated shape, characteristic of axial differentiation. By the second day numerous melanocytes were seen on their surfaces (Fig. 3B). Histological examination of such explants revealed the same set of dorsal structures as were observed in secondary axes: myotomes and neural derivatives, including melanocytes (Fig. 3C). By co-injecting the CG lineage tracer, we revealed that embryonic axes developing in such explants were composed, in their posterior parts (including somite tissue also), mostly of XANF-1-negative (non-labelled) cells. In contrast, the anterior-most regions of these axes were full of XANF-1-positive (labelled) cells. Thus, one may conclude that exogenous XANF-1 is able to cause anterior-dorsal-type differentiation in VMZ explants.

All the explants from the control embryos injected with the truncated XANF-1 mRNA remained rounded or slightly elongated (Fig. 3D; Table 1). Only epidermal, mesenchymal and blood tissues were seen in these explants (Fig. 3E).

**XANF-1 overexpression in cells of the dorsal marginal zone leads to an acceleration of their involution and incorporation into the anterior mesoderm and endoderm**

The data just presented show that the ability of XANF-1 mRNA to elicit the development of an additional axis is strikingly similar to the effect elicited by mRNA of another *Xenopus* homeobox gene – *goosecoid* (Blumberg et al., 1991; Niehrs et al., 1993). In addition, Blumberg and Niehrs reported that overexpression of *goosecoid* mRNA in the progeny of the marginal dorsal blastomeres, which normally gives rise to most of the Spemann organizer cells, accelerated the involution of these cells. As a result, even those cells that should normally remain on the surface of the embryo migrated inward and became primarily localised within the head mesoderm and endoderm.

To test whether XANF-1 mRNA was able to elicit a similar effect, we injected blastomere B1 at the 32-cell stage with a

Fig. 3. XANF-1 not only promotes formation of an additional centre of gastrulation movements, but elicits differentiation of more dorsal cell types. (A) A diagram of the experiment. Explants from the ventral marginal zone were removed at the early gastrula (stage 10.25) from embryos injected into the ventral blastomeres with full-length XANF-1 mRNA or control truncated XANF-1 mRNA, and incubated in saline for 2 days. (B) Differentiated explants from embryos injected with full-length XANF-1 mRNA have an elongated shape and contain numerous melanocytes. (C) Histological 7 μm section of one of the explants, shown in B, revealing a row of well developed myotomes (mt) surrounded by an ectodermal sac. (D) Analogous explants from embryos injected with control truncated XANF-1 mRNA are round and have no melanocytes. (E) Only mesenchymal (mes) and blood (b) cells can be seen in the explants of embryos injected with control truncated XANF-1 mRNA. Scale bars: B,D 100 μm; C,E 30 μm.
mixture of the mRNA and CG. According to Dale and Slack (1987), this blastomere contributes approximately equally to cells of the Spemann organizer and to cells of the neural anlage.

No visible morphological changes were noted until the late blastula-early gastrula stages in the injected embryos. The first typical effect seen at this time was the appearance at the surface of the dorsal marginal zone (DMZ) of groups of spindle-shaped cells elongated tangentially in different directions. The number of these elongated cells increased during the following 30-40 minutes, and then they started to contract their apical surfaces and plunge into the embryo. In many cases, this process was accompanied by the appearance of deep furrows, sometimes very similar to the natural blastopore lip furrow, but positioned animaly to it (Fig. 4A). If the amount of the injected XANF-1 RNA exceeded 100 pg/blastomere, such migration of cells from the DMZ was usually so intense that it resulted in a total erosion of the dorsal blastopore lip and retardation or even blocking of closure of the blastopore ring. Surprisingly, lower doses of RNA (40-50 pg/blastomere), which also stimulated the appearance of the surface furrows and immigration of cells, did not lead to any visible defects in the developing tadpoles. This result was even more surprising, given that co-injection of the CG lineage tracer revealed considerable changes in the course of gastrulation and modifications of the fate map. Two of the embryos illustrating this effect most strikingly (all of the embryos analysed displayed such an effect to different extents) are shown in Fig. 4C-E. Note that whereas by the late gastrula stage in the control embryo (in which the B1 blastomere had been injected with truncated XANF-1 mRNA), many of the cells derived from this blastomere remained within the neur ectoderm (Fig. 4C right, E), their counterparts in the embryo

Fig. 4. XANF-1 overexpression in the DMZ and animal cap leads to enhanced cell migration to the interior of embryo and, in the case of the migration from the DMZ, to the movement of these cells to an anterior position. (A) The embryo was injected with XANF-1 mRNA, at the 32-cell stage, into blastomere B1. At the beginning of gastrulation, cells that have plunged to the interior of the embryo form a furrow (arrowheads) on the surface of the presumptive neurectoderm. Dorsal view. Animal pole is to the top, natural invagination of the blastopore is to the bottom, beyond the field of view. (B) Cells starting to migrate from the blastocoel roof (animal cap) into the blastocoel (bl) of the embryo injected with XANF-1 mRNA into A1-A4 blastomeres have a bottle-shaped form (bc). Their neighbours are elongated along the blastocoel roof, which suggests that the bottle cells generate mechanical tensions by active contraction of their apices. At the same time, cells that have already left the blastocoel roof are spherical in shape and fail to form tight contacts either with each other or with other cells. Semithin 2 μm sagittal section of embryos fixed at the early gastrula stage (stage 10.5). Animal pole is to the top. (C) The embryo was injected into blastomere B1 with colloidal gold (CG) and synthetic XANF-1 mRNA (left). By the late gastrula stage, the majority of cells containing this mixture have migrated into the interior of embryo. In the control embryo injected with CG mixed with the control truncated XANF-1 mRNA, many of the labelled cells remain on the surface, in the presumptive neurectoderm (right). Both embryos are shown from the dorsal side, anterior end is to the top, posterior to the bottom. Position of blastopore (bp) is indicated in each embryo. (D) Histological 7 μm medial sagittal section of the left-hand embryo shown in C reveals that the majority of cells containing CG and synthetic XANF-1 mRNA have become incorporated into the axial mesoderm (axm) and have migrated within it toward the prospective head mesoderm and endoderm. At the same time the presumptive neurectoderm (pne) is almost totally lacking labelled cells. Dorsal is to the top, anterior to the left. The dorsal blastopore lip (dbl) and ventral blastopore lip (vbl) are indicated. (E) An analogous section of the control embryo (C, right) demonstrates that the majority of cells containing both CG and control truncated XANF-1 mRNA remain in the presumptive neurectoderm. Only a minor proportion of the labelled cells involutes through the dorsal blastopore lip with the posterior mesoderm. Scale bars: A, 20 μm; B, 10 μm; C-F, 200 μm.
injected with full-length XANF-1 mRNA migrated to the interior and were recruited into the mesoderm and endoderm (Fig. 4C left, D). Interestingly, marked cells within the mesoderm were obviously attracted to an anterior location, moving from the posterior regions toward the head mesoderm. This was also confirmed by the observation that cells containing exogenous XANF-1 RNA were found at later stages predominantly within head mesoderm, head and trunk somites, notochord and the anterior part of the CNS. At the same time, the number of such cells in the caudal region of the embryo was significantly lower in the experimental embryos than in the controls.

Microinjections of XANF-1 mRNA in the most animal or vegetal blastomeres are able to cause cell migration but cannot induce secondary axes

The results described above clearly demonstrate the ability of XANF-1 mRNA to elicit the properties of the Spemann organizer in prospective mesodermal cells. Thus the question arises as to whether the same effect would be exerted on the descendants of the most animal and vegetal blastomeres, which are not specified to the mesodermal fate in normal development. To test this, we injected one of the animal-most pair of blastomeres, A4, with XANF-1 mRNA at the 32-cell stage. These blastomeres normally give rise almost entirely to ectodermal derivatives (Dale and Slack, 1987; Moody, 1987).

The first abnormalities seen around the beginning of gastrulation looked very similar to the initial steps of cell migration that were observed in the case of the B1 injections (see above). As can be seen (Fig. 4B), cells just starting to migrate from the blastocoel roof to the blastocoel cavity had a characteristic bottle shape. At the same time, the cells that had already left the blastocoel roof were round and failed to form tight contacts, either with each other or with the bottom of the blastocoel. This process resulted in a dramatic thinning of the ectodermal sheet and, if more than 100 pg RNA was injected per blastomere, it usually led to a severe destruction of the blastocoel roof by the midgastropl stage. However, these defects were not seen when control truncated XANF-1 mRNA was injected in the same blastomeres, even at higher concentration (150 pg/blastomere).

Despite the clear ability of exogenous XANF-1 to elicit migratory behaviour of the A4 descendants, no additional axial structures were observed. Similarly, synthetic XANF-1 RNA failed to induce development of axial structures in animal cap explants removed from the embryos injected with mRNA in A1-A4 blastomeres. This indicates that some additional factor(s), present in marginal zone cells but lacking in animal cap, must act with XANF-1 to cause the formation of axial structures.

We also found that XANF-1 mRNA was unable to induce secondary axes when injected into the most vegetal ventral pair of blastomeres, D4 (Table 1). The results of this series of experiments are very important because they demonstrate that XANF-1 cannot induce formation of an additional centre of dorsalizing activity, the so-called Nieuwkoop centre. The latter is formed normally in the vegetal dorsal region of the embryo by the mid-blastula stage, and subsequently acts as an inducer of the Spemann organiser (reviewed by Gerhart et al., 1991). Cells derived from the Nieuwkoop centre do not populate the axial structures, but only release a signal which induces the Spemann organiser in DMZ. This behaviour, very similar to that of cells of the natural Nieuwkoop centre, was demonstrated recently for the descendants of blastomere D4 microinjected with mRNA of the genes Xwnt-8 and noggin (Smith and Harland, 1991, 1992).

Thus we concluded that XANF-1 directly induced Spemann’s organiser properties in cells of ventral mesoderm when its mRNA was injected in C4 blastomeres, but did not act via the production of a secondary Nieuwkoop centre. This is the same conclusion, as was made recently for goosecoid (Niehrs et al. 1993). However, there is a clear difference between the action of XANF-1 and goosecoid. In contrast to goosecoid, microinjections of XANF-1 mRNA in D4 blastomeres led to the formation of an additional axis which was not accompanied by an incorporation of D4 progeny into the axis.

DISCUSSION

XANF-1 can influence identity of cells in the deep zone of the Spemann organiser

To investigate the possible role of XANF-1, we have compared the behaviour of cells overexpressing this gene with that of XANF-1-expressing cells in normal development.

At the beginning of gastrulation, endogenous transcripts of XANF-1 are most abundant in deep zone cells of the dorsal blastopore lip, or Spemann organiser. During gastrulation, these cells crawl along the inner surface of the blastocoel roof, forming the leading edge of the dorsal mesoderm. Finally, they occupy the most anterior region of the AP body axis, giving rise to the head mesenchyme and pharyngeal endoderm. At the same time, presumptive mesodermal cells from the outer zone of the dorsal blastopore lip display another type of movement: involuting through the lip, they move by mediolateral intercalation, which leads to the dorsal convergence and elongation of the trunk part of the body axis. These intercalating cells create somites and notochord (Shih and Keller, 1992).

The following results suggest that XANF-1 is able to control at least the migratory behaviour of deep cells of the Spemann organiser. First, cells containing synthetic XANF-1 mRNA start to migrate into the embryo at the beginning of gastrulation, i.e., at the same time as do deep cells of the Spemann organiser. Second, in the dorsal blastopore lip, cells overexpressing XANF-1 migrate to the interior of the embryo much more quickly than their counterparts in control embryos that also involute through the lip but give rise to the trunk mesoderm. Finally, both types of cells migrate to an anterior position, where they occupy an area of the anterior-most head mesenchyme and endoderm.

XANF-1 may also control another important property of cells of the presumptive anterior mesoderm, namely to function as cells of the Spemann organiser. Indeed, in VMZ explants cells overexpressing XANF-1 not only recruit other cells in their movement, but they also cause dorsal-type (somite) differentiation of these cells. In this regard, however, one might ask why, if these cells do indeed acquire the identity of presumptive anterior mesoderm, they never induce forebrain structures in the secondary axis and never cause enlargement or other malformations of such structures in the primary axis. To explain this, it could be suggested that XANF-1 alone cannot
induce the full set of properties characteristic of the presumptive anterior mesoderm, but only subset of them. Alternatively, it seems reasonable to suppose that the number of organizer cells induced in our experiments was insufficient. It is known that if the total amount of organizer tissue is less than half of normal, the axis that subsequently develops lacks anterior regions (Stewart and Gerhart, 1990). In contrast, the organizer can be increased to at least twice its normal size without any visible consequences upon the developing axis (Cooke, 1972).

It is still unknown if the two observed effects (induction of cell movement and dorsal differentiation) of exogenous XANF-1 have some causal relationship with each other. For example, it is possible that stimulation of MZ cell movement per se could be a factor sufficient for the induction, in them of a programme of dorsal-type differentiation, or vice versa. Nevertheless, this is clearly not the case as far as animal cap cells are concerned. Indeed, we could not reveal any development of dorsal structures in animal cap explants, despite the clear ability of exogenous XANF-1 to elicit migratory behaviour in cells of such explants.

Interestingly, functions very similar to those suggested for XANF-1 have been attributed to another homeobox gene, goosecoid (Blumberg, 1991; Niehrs, 1993). This gene, like XANF-1, is expressed at the late blastula and early gastrula stages in cells of the deep zone of the dorsal blastopore lip, but, in contrast to XANF-1, goosecoid expression is restricted to this site during early embryogenesis. As for XANF-1, microinjection of goosecoid mRNA into marginal ventral blastomeres resulted in the formation of secondary axes, which were generally incomplete. In contrast with two other genes displaying secondary axis-inducing activity, noggin and XWnt-8 (Smith and Harland, 1991, 1992), neither goosecoid nor XANF-1 act via the creation of a novel Nieuwkoop centre. Rather, the latter two genes appear to directly convert marginal zone cells into Spemann organizer tissue. Another very similar effect produced by injection of both goosecoid and XANF-1 is the acceleration of DMZ cell involution and induction in them of an attraction to the anterior region of the embryo. Unfortunately we do not know whether goosecoid, like XANF-1, is able to induce migratory behaviour not only in MZ cells but in animal cap cells. However, it is known that goosecoid alone, like XANF-1, is unable to induce mesodermal differentiation in animal caps (Niehrs, 1993).

Such a striking similarity between the effects elicited by goosecoid and XANF-1 allows us to suppose that both homeobox genes might be involved in the same regulatory pathway at the beginning of gastrulation, controlling at least some of the properties of cells in the deep zone of the Spemann organizer.

Abnormal migration of cells might account for the apparent lack of abnormalities within the CNS anlage as a result of XANF-1 mRNA microinjection into the dorsal blastomeres

Because the expression of XANF-1 is localised to the neuroectoderm by the late gastrula stage, the CNS anlage seemed to be the most probable region in which misexpression of this gene might result in an abnormal phenotype. However, when we injected XANF-1 mRNA into A1, A2 or B2 blastomeres which normally give rise to the neuroectoderm (Dale and Slack, 1987; Moody, 1987), no abnormalities were observed except massive migration of cells to the interior of embryo.

As we have shown, such migration led in many cases to the complete depletition of cells containing XANF-1 in the anterior part of the neural anlage, i.e., just in the area where endogenous XANF-1 is normally expressed. Thus, one may conclude that such a ‘disappearance’ of cells, containing exogenous XANF-1 mRNA in high concentration and representing a potential source of CNS malformations, was the reason for the lack of other abnormalities. However, low amounts of synthetic mRNA, which do not provoke such an intensive migration of cells at the beginning of gastrulation, might be insufficient to provide an abnormally high concentration of the protein in the neural anlage cells during neurulation.

To circumvent these obstacles, it might be possible, for example, to microinject XANF-1 in the form of a DNA vector under the control of a promoter which would allow one, firstly, and in contrast with synthetic mRNA, to avoid intense expression of mRNA at the early gastrula stage, and secondly, to obtain a sufficient level of the RNA within the neuroectoderm at the late gastrula stage, i.e., around the time when natural XANF-1 is expressed in the anterior neuroectoderm. We are currently undertaking such experiments using a vector expressing XANF-1 under the control of its own promoter.

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