

Inducing factors and the control of mesodermal pattern in *Xenopus laevis*

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Summary

The mesoderm of *Xenopus laevis* and other amphibia is formed through an inductive interaction during which cells of the vegetal hemisphere act on cells of the animal hemisphere. Two groups of factors mimic the effects of the vegetal hemisphere. One group consists of members of the fibroblast growth factor (FGF) family, while the other is related to transforming growth factor type β (TGF- β). In this paper we discuss the evidence that the FGF family represents 'ventral' mesoderm-inducing signals, and the TGF- β family 'dorsal' signals. The

evidence includes a discussion of the cell types formed in response to each type of factor, the fact that only XTC-MIF (a member of the TGF- β family) and not bFGF can induce animal pole ectoderm to become Spemann's organizer, and an analysis of the timing of the gastrulation movements induced by the factors.

Key words: mesoderm induction, mesoderm-inducing factors, bFGF, XTC-MIF, thresholds, gastrulation, amphibian embryo, *Xenopus laevis*, developmental timers.

Introduction

The mesoderm of *Xenopus laevis*, and of other amphibian embryos, is formed through an inductive interaction in which cells of the vegetal hemisphere of the embryo act on blastomeres of the overlying marginal zone (Nieuwkoop, 1969, 1973; Sudarwati and Nieuwkoop, 1971; reviewed by Smith, 1989; Fig. 1A). This interaction is usually demonstrated by juxtaposing tissue from the animal cap and the vegetal pole (Fig. 1B). Normally, the animal cap material is too far from vegetal blastomeres to receive an inductive signal and when cultured alone it forms 'atypical epidermis' – cells that stain with antibodies to keratin but which do not adopt normal epidermal morphology (see Smith *et al.* 1985). However, when animal cap cells are placed in contact with vegetal cells a significant proportion (about 40%) differentiate as mesodermal cell types, including notochord and muscle (Dale *et al.* 1985; Gurdon *et al.* 1985).

Recently, significant progress has been made towards identifying the 'mesoderm-inducing factors' (MIFs) produced by vegetal pole cells. The candidates fall into two classes. One consists of members of the TGF- β family and includes XTC-MIF (Smith, 1987; Smith *et al.* 1988; Rosa *et al.* 1988), TGF- β 2 (Rosa *et al.* 1988) and perhaps the protein encoded by the localized mRNA Vg1 (Rebagliati *et al.* 1985; Weeks and Melton, 1987). TGF- β 1 is unusual in that it has no mesoderm-inducing activity alone, but acts synergistically with members of the other class of MIFs, the FGF family (Kimelman and Kirschner, 1987). Active members of this family include aFGF, bFGF and embryonal carcinoma-derived growth

factor (ECDGF) as well as the protein products of the *kFGF* and *INT-2* oncogenes (Slack *et al.* 1987; Kimelman and Kirschner, 1987; Paterno *et al.* 1989; see preceding paper by Slack and his colleagues).

In this paper, we compare the effects of the two classes of MIF and discuss how they might act together to establish the correct spatial pattern of cellular differentiation in the mesoderm of *Xenopus*. We then review what problems remain in coming to understand how combinations of inducing factors might set up the remarkably constant mesodermal pattern of *Xenopus* (Cooke and Smith, 1987).

The three-signal model

A series of experimental embryological experiments has led to the 'three-signal' model for the formation of mesoderm in the early amphibian embryo (see Smith *et al.* 1985). This model is explained in detail in the preceding paper in this volume by Slack *et al.* and will only be described briefly here (Fig. 2). Thus during oogenesis, which takes several months in *Xenopus*, the egg becomes polarized such that yolk, pigment and, most importantly, informational macromolecules, become localized to particular regions along the animal–vegetal axis (see Gerhart, 1980; Wylie *et al.* 1985; Melton, 1987; Yisraeli and Melton, 1988; Yisraeli *et al.* this volume). Until fertilization, however, the egg is radially symmetrical around the animal–vegetal axis: every meridian has the potential to form dorsal axial structures.

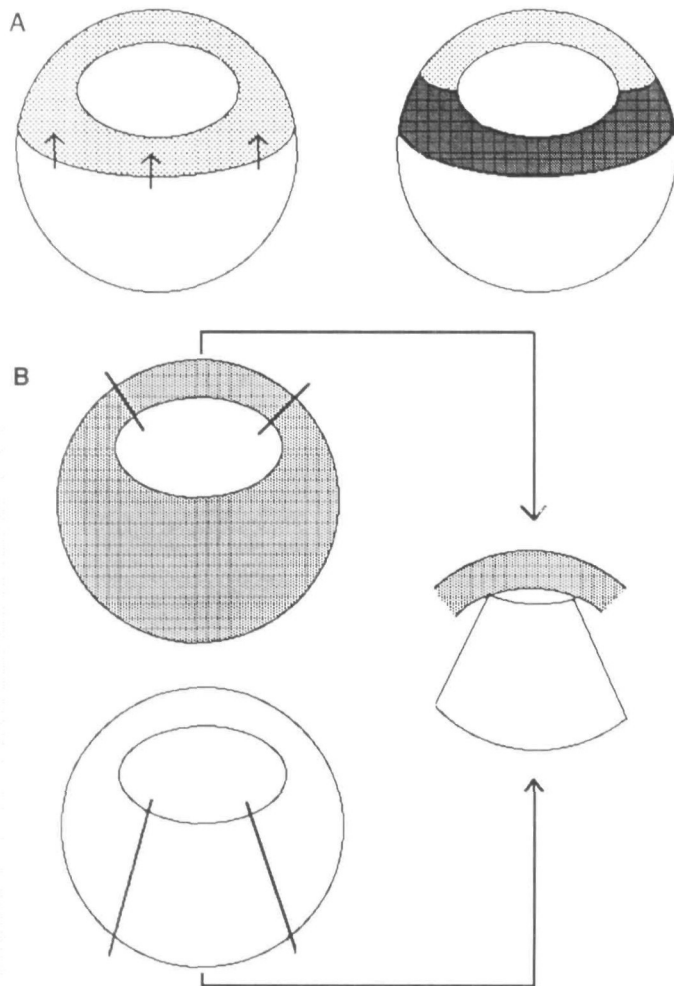


Fig. 1. Mesoderm induction. (A) At early blastula stages the *Xenopus* embryo can be considered to consist of two cell types: presumptive ectoderm in the animal hemisphere (light stippling) and presumptive endoderm in the vegetal hemisphere (no stippling) (Jones and Woodland, 1986). During mesoderm induction a signal from the vegetal hemisphere induces overlying equatorial cells to form mesoderm (heavy stippling). This view is somewhat simplified for it is not possible to draw an accurate line between vegetal inducing cells and animal pole responding cells. Indeed, it may be that some cells both produce and respond to the signal. (B) The classical demonstration of mesoderm induction. Blastomeres from the animal pole of a lineage-labelled blastula-staged embryo are placed in contact with cells from the vegetal pole of an unlabelled embryo.

This symmetry is broken at fertilization, when the sperm entry point defines the direction of rotation of the cortex of the egg with respect to the subcortical cytoplasm. The orientation of this rotation in turn accurately predicts the future dorsoventral axis of the embryo (see Gerhart *et al.* this volume). The mechanism by which this occurs is unknown, but it is clear that most of the dorsoventral patterning information thus created is in the vegetal region of the egg. This can be demonstrated using animal-vegetal combinations; when animal pole cells are combined with dorsal

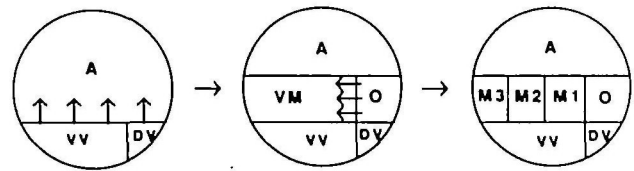


Fig. 2. The three-signal model. Two mesoderm induction signals are assumed to derive from the vegetal region of the early blastula. The dorsal-vegetal (DV) signal induces dorsal mesoderm, or 'organizer' tissue (O) while the ventral vegetal signal (VV) induces general ventral mesoderm (VM). The ventral mesoderm then receives a signal from the organizer, probably during gastrulation, which results in the formation of additional muscle (M1) and perhaps pronephros (M2); only the most remote tissue (M3) remains as ventral blood-forming mesoderm.

vegetal blastomeres they form notochord, a dorsal mesodermal cell type, while ventral vegetal blastomeres induce blood, mesenchyme and mesothelium which are usually regarded as ventral mesodermal cell types (Boterenbrood and Nieuwkoop, 1973; Dale *et al.* 1985; Dale and Slack, 1987b; see Smith, 1989). An analogous experiment was performed by Gimlich and Gerhart (1984), who showed that embryos made radially symmetrical by UV irradiation of the vegetal hemisphere of the egg shortly after fertilization could be 'rescued' by dorsal vegetal, but not ventral vegetal, blastomeres.

It should be noted that the terms 'dorsal' and 'ventral' are somewhat misleading when applied to the preneurula-stage embryo. As a result of the earlier and more vigorous gastrulation movements on the 'dorsal' side of the embryo, these cells form most of the head and anterior of the larva, including structures which in the final morphology are 'ventral', as well as the notochord and some somitic tissue of the trunk. Consequently, blastomeres on the 'ventral' side tend to form ventral structures in the trunk region of the embryo, but axial structures more posteriorly (Cooke and Webber, 1985; Keller, 1975, 1976; Smith and Slack, 1983; Cleine and Slack, 1985; Dale and Slack, 1987a).

Thus, at the blastula stage one can distinguish, by operational criteria, at least two types of mesoderm induction signal. One of these induces predominantly dorsal cell types and one ventral. But two signals, or even three or four, from the vegetal hemisphere are unlikely to be sufficient to establish directly a complex and reproducible pattern of mesodermal cell types. One indication that more signalling subsequently takes place is that the fate map of *Xenopus* shows that at least half of the muscle of the embryo arises from the ventral side of the embryo (Cooke and Webber, 1985; Keller, 1976; Dale and Slack, 1987a), whereas the ventral vegetal blastomeres induce very little muscle from responding animal pole tissue (Dale *et al.* 1985; Dale and Slack, 1987b). This paradox can be resolved by the third signal of the model, which is produced by dorsal mesoderm cells and which acts on adjacent ventral mesoderm to cause it to form muscle instead of ventral tissues. This interaction can be demonstrated by juxtaposing dorsal and ventral marginal zones *in vitro*. Normally, in

isolation, the ventral tissue forms blood, mesenchyme and mesothelium, but in response to the dorsal tissue it forms muscle instead. The dorsal tissue, meanwhile, forms notochord as usual (Slack and Forman, 1980; Smith *et al.* 1985; Dale and Slack, 1987b). An alternative demonstration of this phenomenon of dorsalization is to implant dorsal marginal zone tissue into the ventral marginal zone of a host early gastrula (Smith and Slack, 1983). This is the famous organizer graft of Spemann and Mangold (1924), and results in an embryo with mirror-image symmetry in the mesoderm which is also reflected in the induction of two neural tubes (Gimlich and Cooke, 1983; Smith and Slack, 1983; Jacobson, 1984).

The three-signal model is summarized in Fig. 2. It is important to note that the model does not address the acquisition of anteroposterior positional values. This is rather poorly understood although the current models are discussed in this volume by Gerhart and his colleagues. One suggestion these authors make, however, is that the completeness of the anteroposterior axis depends upon the size of the organizer, the region designated 'O' in Fig. 2. We present some results below which are consistent with this view.

Mesoderm-inducing factors

As outlined in the Introduction, mesoderm-inducing factors in *Xenopus* fall into two families: FGF and TGF- β . The members of these families are summarized in Table 1. At present, no member of either family satisfies all the criteria for being a true morphogen (see Slack and Isaacs, 1989; Wolpert, this volume). Thus for the TGF- β family the most active inducer is XTC-MIF (Smith *et al.* 1988; Green *et al.*, in preparation) but it is not yet known whether the molecule is present in the early embryo. One member of the TGF- β family which is present in the embryo is the Vg1 protein (Dale *et al.* 1989; Tannahill and Melton, 1989), but it is not yet known whether this has mesoderm-inducing activity.

The situation is slightly clearer with the FGF family, where it is known that bFGF is present in the embryo in sufficient quantity to act as a mesoderm inducer (Kimelman *et al.* 1988; Slack and Isaacs, 1989). It is not known, however, if the protein is localized to the vegetal region of the embryo, and indeed this seems unlikely because bFGF is synthesized throughout oogenesis and would be expected to be free to diffuse throughout the oocyte. In addition, *Xenopus* bFGF, like bovine bFGF, carries no secretion signal sequence (Abraham *et al.* 1986; Kimelman *et al.* 1988), so it is not clear how it might escape from vegetal blastomeres. To confirm that bFGF plays a role in mesoderm induction it is necessary, therefore, to ablate the protein from the embryo and show that mesoderm formation is disrupted.

Thus it is not clear whether the two *Xenopus*-derived inducing factors available in pure form – XTC-MIF and bFGF – play a role in normal development. Nevertheless, it has been possible to carry out important experiments with the molecules, and as we discuss below the

results are consistent with bFGF being the ventral vegetal inducing signal and XTC-MIF, or something like it, the dorsal signal.

XTC-MIF and bFGF: dorsal and ventral signals

We outline below three types of experiment that indicate that XTC-MIF and bFGF mimic, respectively, the dorsal and ventral mesoderm-inducing signals represented in Fig. 2. This conclusion is important in itself, but, as we show, the work should lead to improved understanding of thresholds in development, of Spemann's organizer, of the control of cell motility, and of timing in development.

Concentration-dependent effects of XTC-MIF and bFGF

The first series of experiments investigates the effects of exposing *Xenopus* animal pole regions to different concentrations of XTC-MIF or *Xenopus* bFGF (XbFGF). Animal caps were dissected from embryos at the midblastula stage and cultured in inducing factor until sibling embryos reached the swimming tadpole stage. In order that all the cell types present could be recognized, and to preserve the three-dimensional structure of the explants, the specimens were fixed, sectioned and stained by conventional histological procedures, before being examined under the microscope.

Two conclusions could be drawn from this experiment. First, only XTC-MIF, and not XbFGF, induced notochord from animal pole tissue (Smith *et al.* 1988; Green *et al.* in preparation; see Godsave *et al.* 1988 and Slack *et al.* 1988 for results with bovine bFGF). This strongly suggests that XTC-MIF more resembles the dorsal mesoderm-inducing signal and XbFGF the ventral. The second conclusion is that the type of induction observed depends upon the concentration of inducing factor. Thus for XbFGF, 1 ng ml⁻¹ causes all explants to become induced, but the cell types formed only rarely include muscle; most of the induced tissue is best described as mesenchyme and mesothelium (Fig. 3E). These cell types are often regarded as 'ventral mesoderm' but it is worth emphasizing that without molecular markers this classification is tentative at best, and may result from a regrettable tendency to classify any tissue formed in response to a 'mesoderm-inducing factor' as 'mesoderm'. The best justification for the view is that the cells resemble mesoderm in their social behaviour and adhesive preferences (Cooke and Smith, 1989) and that they resemble the cell types formed by animal pole tissue after juxtaposition with ventral vegetal pole tissue (Dale *et al.* 1985; see Smith, 1989). Higher concentrations of XbFGF begin to induce muscle, but 25–50 ng ml⁻¹ is required for the formation of significant amounts (Fig. 3F) (Green *et al.*, in preparation; see Godsave *et al.* 1988 and Slack *et al.* 1988 for results with bovine bFGF). As stated above, notochord is never observed.

A similar transformation in cell types is seen with XTC-MIF. Low concentrations (less than 1 ng ml⁻¹)

tend to induce mesenchyme and mesothelium (Fig. 3B) while higher concentrations result in muscle formation (Fig. 3C). However, with XTC-MIF muscle formation is maximal at about 5 ng ml^{-1} and in some experiments muscle differentiation falls off beyond this point to be replaced by notochord and neural tissue (Fig. 3D) (Smith *et al.* 1988). The transformation from muscle differentiation to notochord formation is, however, somewhat variable (Green *et al.* in preparation) and this is currently under investigation.

Rather than considering XbFGF as a weaker meso-

derm inducer than XTC-MIF, it may be more accurate to regard the two factors as producing qualitatively different types of mesoderm rather than different 'grades' of the same type. Some cell-biological evidence for this point of view is provided by recent studies on MIF-induced gastrulation movements (below) but additional molecular evidence comes from recent work by Rosa (1989) which shows that the gene *Mix.1* is strongly activated by XTC-MIF but not by XbFGF, at concentrations where both induce mesoderm.

The observation that different concentrations of

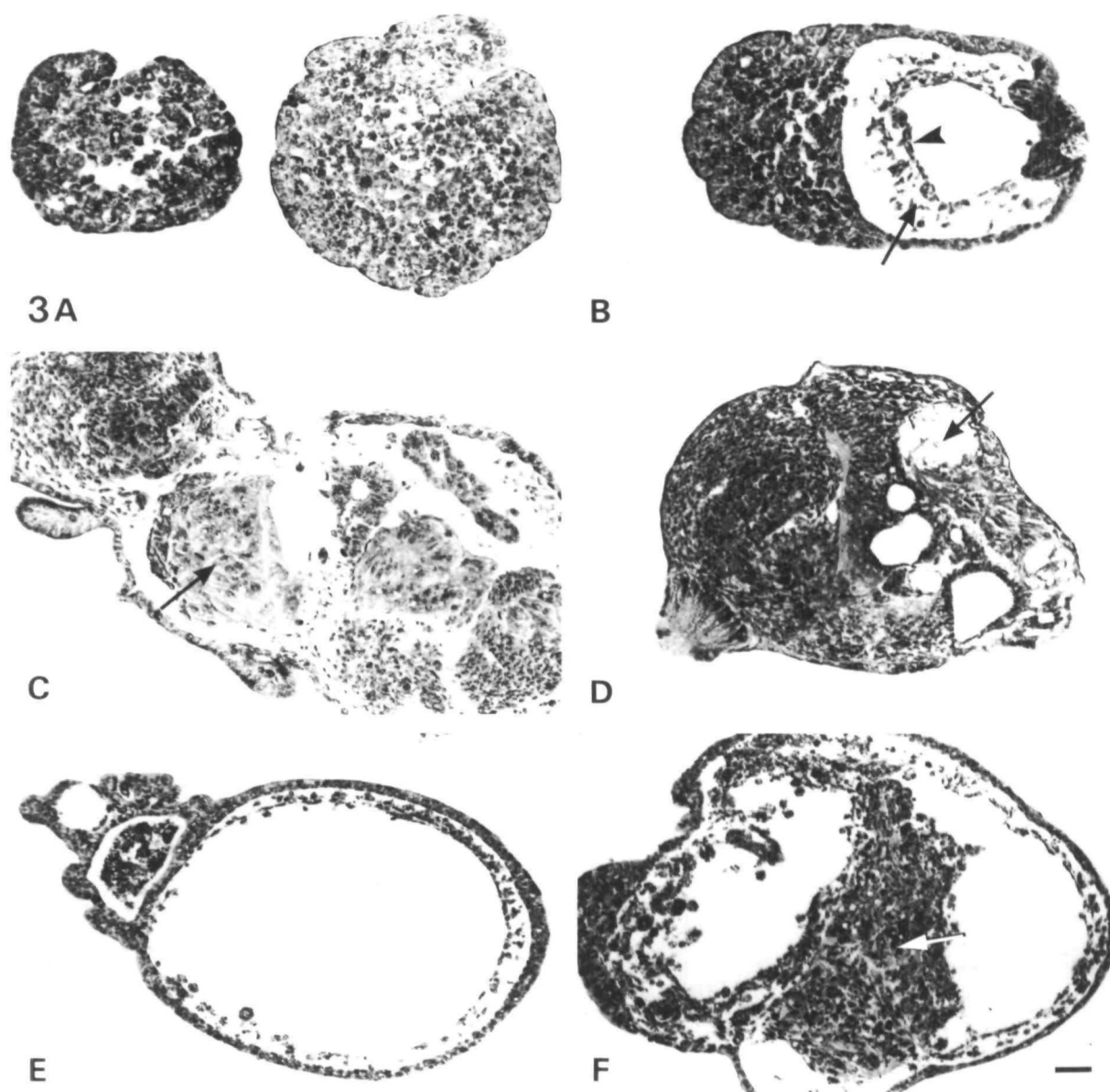


Fig. 3. Cell types formed in response to bFGF and XTC-MIF. (A) Animal pole regions cultured in the absence of mesoderm-inducing factors form 'atypical epidermis'. (B) 0.2 ng ml^{-1} XTC-MIF causes the formation of 'mesenchyme' (arrow) and 'mesothelium' (arrowhead). (C) $1\text{--}2 \text{ ng ml}^{-1}$ XTC-MIF is sufficient to induce quite large amounts of muscle from animal pole regions (arrow). (D) In some experiments with high concentrations of XTC-MIF, notochord is formed (arrow). Notice the cement gland at the opposite side of the explant. (E) $1\text{--}10 \text{ ng ml}^{-1}$ *Xenopus* bFGF causes the formation of mesenchyme and mesothelium. (F) Concentrations of approximately 50 ng ml^{-1} bFGF are required to induce muscle (arrow). Scale bar in (F) is $50 \mu\text{m}$, and also applies to frames (A) to (E).

Thresholds in mesoderm induction

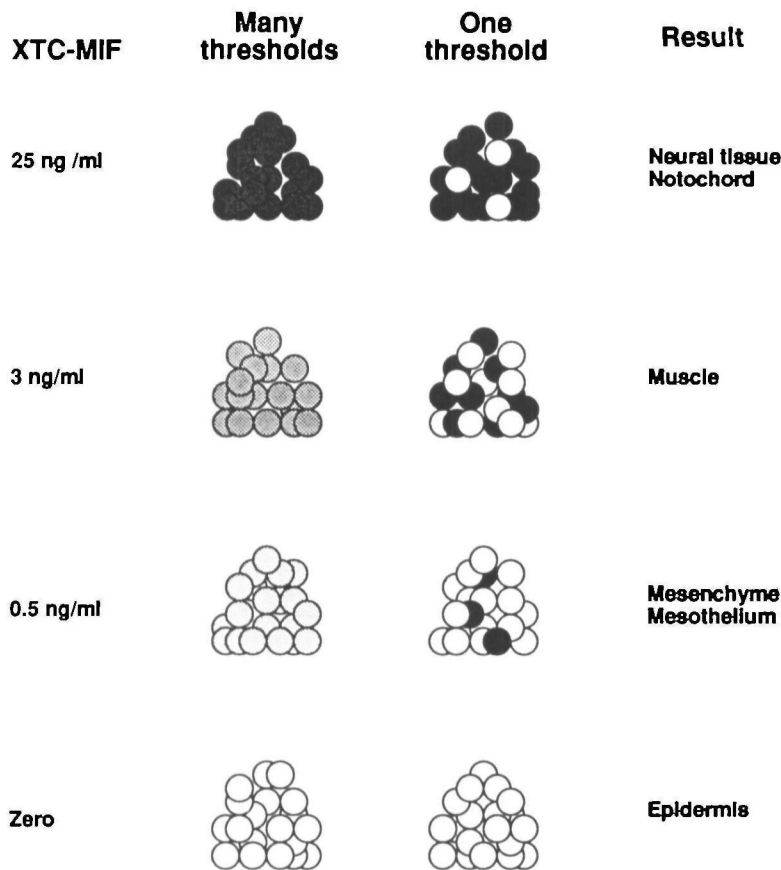


Fig. 4. Two models for thresholds in mesoderm induction. In one model (left), each individual cell has a qualitatively different response to different concentrations of XTC-MIF; that is, each cell has many thresholds. The levels of response directly determines how the cell eventually differentiates. In the other model (right) each cell has only one threshold, and the cell types that eventually form depend on the proportion of cells that exceed that threshold. This model requires that there be communication between cells during, or shortly after induction by XTC-MIF.

XTC-MIF tend to cause the formation of different cell types raises the question of thresholds in development (Lewis *et al.* 1977). What form might these thresholds take? According to one view, each responding animal pole cell would have many thresholds, each activated at a certain concentration of XTC-MIF (Fig. 4; see Smith, 1989). Thus at 0.5 ng ml^{-1} XTC-MIF the first threshold would be exceeded and mesenchyme and mesothelium would form. Higher concentrations would cause further thresholds to be exceeded so that at, say, 25 ng ml^{-1} the cells would be programmed to form notochord and neural tissue. The following experiment, however, argues against such a model, and we go on to suggest an alternative view that the threshold phenomenon represents a cell population effect.

It is possible to disaggregate animal pole blastomeres by culturing animal caps in calcium- and magnesium-free medium. Such cells can be kept as a small heap or they can be dispersed over the culture dish, essentially as described by Sargent *et al.* (1986). If either heaped or dispersed cells are reaggregated at the early gastrula stage, by addition of divalent cations, they go on to form epidermis, their normal fate. If heaped cells are exposed to XTC-MIF, washed, and then reaggregated at the early gastrula stage, when mesodermal competence is almost over, they form muscle, epidermis, and sometimes notochord (Fig. 5; Symes, 1988). This

indicates that deprivation of divalent cations does not affect the primary response to XTC-MIF. By contrast, when cells are dispersed during exposure to XTC-MIF, muscle formation does not occur after reaggregation, although epidermal differentiation is suppressed (Fig. 5; Symes, 1988). Several experiments, including measurements of rates of RNA and protein synthesis, indicate that this response is not due to toxicity of XTC-MIF (Symes *et al.* 1988).

These results suggest that mesoderm formation after exposure to XTC-MIF is not a direct response, but one which requires contact-dependent cell-cell interactions during induction. If this is so, the changes in patterns of cell differentiation with different concentrations of XTC-MIF might occur through these interactions, and it may only be necessary for blastomeres to have one threshold to XTC-MIF. Indirect evidence for a single threshold is that the time of onset of gastrulation movements in response to XTC-MIF, one of the earliest responses to induction yet observed (Symes and Smith, 1987; Cooke and Smith, 1989), and one that relates directly to mesodermal cell type, does not depend on the concentration of factor (Cooke and Smith, 1989; see below).

An alternative view of thresholds in mesoderm induction is that the phenomenon represents a cell population effect, with the proportion of cells responding to

XTC-MIF does not act on single cells to induce mesoderm

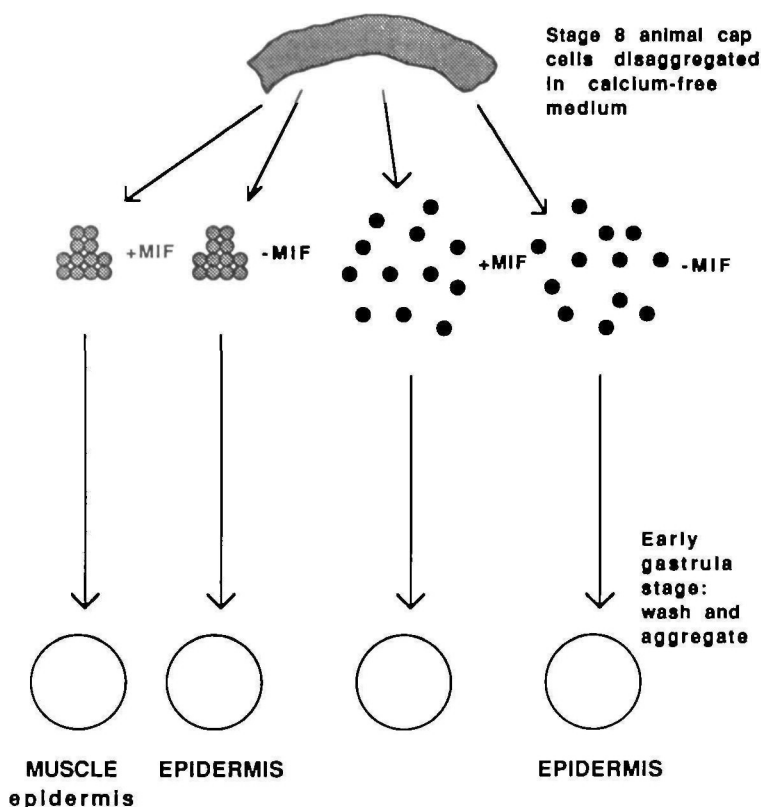


Fig. 5. Design of experiment to show that cell contact is required during treatment with XTC-MIF if muscle (and probably other mesodermal cell types) is to form. Animal caps are dissociated at the early blastula stage and cultured as indicated, either in a small heap or dispersed over the surface of an agar-coated Petri dish. Some cultures contain XTC-MIF. At the beginning of gastrulation, near the end of the period of competence to respond to XTC-MIF, the cultured blastomeres are thoroughly washed, reaggregated, and cultured until sibling embryos reached stage 40. The results were obtained by immunofluorescence analysis of specimens. Note that in the absence of XTC-MIF both 'heaped' and 'dispersed' cultures form epidermis and no muscle. When 'heaped' cells are exposed to XTC-MIF they eventually form epidermis and muscle, but neither cell type is detected if blastomeres are exposed to XTC-MIF while dispersed.

induction determining which cell types eventually form. Each cell, for example, could have a single binary on/off switch which is tripped by the primary inducing factor. Secondary signals, whose local strength would depend on the proportion of nearby 'switched on' cells, would then determine cell fate. Thus if 10% of the cells respond to the primary signal, the population as a whole might form mesenchyme and mesothelium; if 50% respond, muscle might arise; and, if 90%, the cells might form notochord and neural tissue (Fig. 4). In future work, we hope to test this idea by mixing induced and uninduced cells in different proportions.

One way in which the proportion of cells responding to induction might influence cell differentiation could involve a timing mechanism. The sequence of cell differentiation in the mesoderm of *Xenopus* seems to follow the dorsoventral axis. Thus the notochord, the most dorsal mesodermal cell type, becomes visible as a separate group of cells at the late gastrula stage (Keller *et al.* 1985), and presumably this is preceded by transcription-dependent changes in the cell surfaces of these cells. Activation of muscle-specific actin genes occurs at the late gastrula stage (Gurdon *et al.* 1985), while transcription of globin genes, which characterize the most ventral cell type, blood, does not start until the tailbud stage (Banville and Williams, 1985). According to one model, similar to Cooke's (1983) 'serial diversion theory', cells induced by XTC-MIF or FGF might pass through phases of development during which they are

capable of differentiating first as notochord, then as muscle, and then as successively more ventral cell types. For differentiation to occur there must be a threshold level of a second signal, whose concentration depends upon the proportion of cells that are induced. If a small number of cells initially responded to induction, the concentration of second signal would be slow to build up, so that the phases during which notochord or muscle pathways can be entered will have passed, and their only option is to form ventral cell types. If many cells responded to induction, the second signal would accumulate rapidly, so that notochord could be formed. One reason why XTC-MIF but not bFGF can induce notochord might be that they produce different second signals which build up at different rates or have different specific activities. Alternatively, the accumulation of second signal in response to FGF may start later in development. Recent results on the timing of gastrulation movements induced by XTC-MIF and bFGF, described below, may support the latter idea (Cooke and Smith, 1989).

The requirement for cell contact between animal pole blastomeres during induction by XTC-MIF, if mesoderm is to form, bears upon some recent results of Gurdon (1988). Gurdon finds that the ability of an animal pole cell to form muscle in response to vegetal pole cells is dependent on the presence of other, similarly induced, animal pole cells. He calls this phenomenon the 'community effect'. It is possible that

the effect is merely permissive; that is, that cells specified as muscle simply cannot express their appropriate phenotype as isolated individuals. Alternatively, cell contact could be instructive in the sense that the disposition and abundance of induced cells could decide which and how much of several fates eventually appear.

XTC-MIF-treated animal cap cells behave as Spemann's organizer

The above results suggest that animal pole cells exposed to XTC-MIF should be regarded as dorsal mesoderm, because they form notochord. A further property of dorsal mesoderm, however, is that it should also produce the dorsalizing signal mentioned above (see Fig. 2). We have tested this prediction by microinjecting XTC-MIF, bFGF or a control solution into the blastocoels of *Xenopus* embryos at the early blastula stage. Two hours later, small pieces of animal pole tissue are removed from these embryos, washed carefully, and grafted to the ventral marginal zones of host embryos (Fig. 6). The results show that ectoderm treated with XTC-MIF, but not with bFGF or a control solution, does indeed behave as Spemann's organizer and therefore produces a dorsalization signal (Cooke *et al.* 1987; Cooke, 1989). Experiments using lineage-labelled grafts confirm that the secondary axes in the

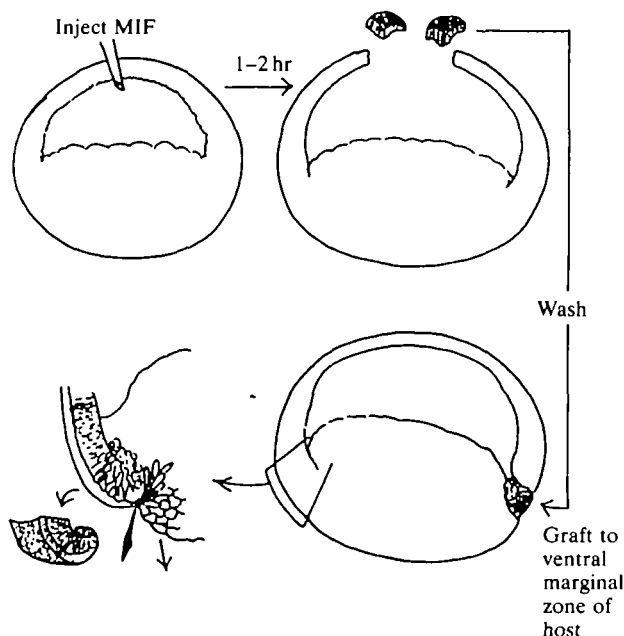


Fig. 6. Experiment to show that XTC-MIF-treated animal caps behave like Spemann's organizer. XTC-MIF, bFGF, or a control solution are injected into the blastocoel of a *Xenopus* embryo at the mid-blastula stage. 1–2 h later small pieces of animal cap tissue are dissected from the treated embryo, washed, and grafted to the ventral side of a host embryo.

The drawing at bottom left illustrates the architecture of the dorsal lip of the blastopore, the natural organizer. Only tissue above the bottle cells, consisting of dorsal mesoderm and suprablastoporal endoderm, has organizer activity; more vegetal endoderm lacks activity. Presumably XTC-MIF induces tissue of the former type.

double-dorsal embryos do consist largely of host tissue and have not arisen through self-differentiation of the graft (Cooke, 1989).

Interestingly, as would be predicted by the work of Gerhart *et al.* (this volume), the more 'powerful' the organizer (whether in terms of the concentration of XTC-MIF injected into the blastocoel or in terms of the size of the graft), the more complete the secondary axis formed, provided the cells having organizer activity are adequately localized within the tissue. Thus, presumptive ectoderm that has been exposed to a high concentration of XTC-MIF induces head, trunk and tail structures, while that exposed to lower concentrations might only induce trunk and tail. Finally, at the lowest concentrations of factor only tail is formed (Cooke, 1989). As with the concentration 'threshold' effects for XTC-MIF-induced cell differentiation, we do not yet know whether this gradation is mediated by a series of distinct organizer states in the cells, or through variation in the total number of cells in the graft that have been induced into an organizer 'state'.

These observations are of interest for three reasons. First, they confirm that XTC-MIF-treated ectoderm does indeed resemble dorsoanterior mesoderm, while bFGF-treated tissue is more similar to ventroposterior mesoderm, which lacks organizer activity (Smith and Slack, 1983). Second, the results offer an opportunity to screen for factors that represent the elusive organizer substance. It should be possible to conduct a differential screen to identify secreted molecules that are produced in response to XTC-MIF but not to bFGF. And finally, it may be possible to design experiments to explain how positional values are established along the anteroposterior axis of the embryo.

Inducing factors, gastrulation and the developmental timer

The first visible manifestation of mesoderm induction is gastrulation. In *Xenopus*, as in other Amphibia, gastrulation is under precise temporal and spatial control. This control is exerted through the cells of the mesoderm, which provide the motive force for gastrulation (Keller, 1986). Mesoderm-inducing factors, such as XTC-MIF and bFGF, induce gastrulation-like movements in animal pole tissue (Fig. 7) (Symes and Smith, 1987; Cooke *et al.* 1987; Rosa *et al.* 1988; Cooke and Smith, 1989), and this provides a new approach to the study of gastrulation (Smith, Symes, Hynes and DeSimone, in preparation).

One particularly interesting aspect of MIF-induced gastrulation movements concerns their timing. In a preliminary study Symes and Smith (1987) estimated the time of onset of gastrulation movements in isolated animal pole regions exposed to XTC-MIF. Within the limits of observation, the explants started to elongate at the equivalent of the early gastrula stage, irrespective of the stage at which they were exposed to the factor. This suggested that cells of the animal hemisphere contain a gastrulation 'timer', which is running even in those cells that would not normally need to refer to it.

Gastrulation movements induced by bFGF are less

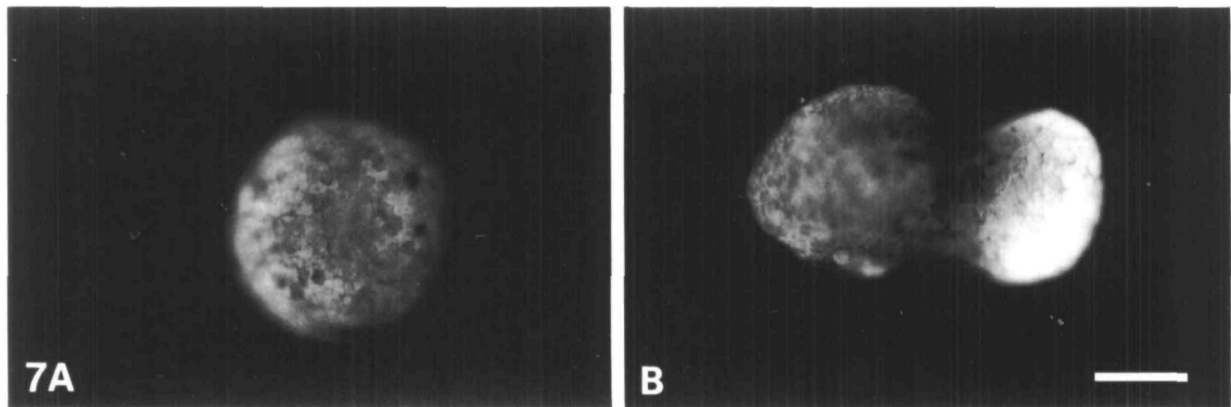


Fig. 7. XTC-MIF induces gastrulation-like movements in animal pole explants. (A) A control explant. (B) An induced explant 15 h after treatment with XTC-MIF. Scale bar in (B) is 200 μ m, and also applies to (A).

dramatic (Slack *et al.* 1987), making it harder to estimate their time of onset. However, Cooke *et al.* (1987) and Cooke and Smith (1989) have introduced a technique to overcome this problem. This involves microinjecting MIFs into the blastocoels of *Xenopus* embryos so that the entire blastocoel roof becomes converted to mesoderm. As a result the whole of this tissue undergoes changes in cell shape and adhesion that mimic those occurring in the marginal zone of the embryo. The timing of these events can be estimated very precisely, by dissection of living and fixed embryos, and the first results confirmed those of Symes and Smith (1987) in showing that the time of onset of gastrulation-like movements is independent of the time of receipt of inducing factor. The data showed, however, that the time of onset was also independent of the concentration of factor and that the response could be very rapid. Thus if microinjection of the factor was delayed until just before the onset of gastrulation in the host embryo, the minimum possible interval between microinjection and onset of gastrulation movements in the ectopically induced mesoderm was at most 30 min (Cooke and Smith, 1989).

Microinjection of bFGF into the blastocoels of *Xenopus* embryos confirmed that the time of onset of the resulting ectopic gastrulation movements is, similarly, independent both of time of injection and of concentration of factor. However, the time at which bFGF-induced movements commence is significantly later, within the progress of the embryos 'own' gastrulation movements, than the time at which XTC-MIF-induced movements start. The interval, about 60 min, resembles the difference in time of gastrulation of dorsal and ventral mesoderm (Gerhart and Keller, 1986).

Here, then, is the third piece of evidence that XTC-MIF induces dorsal mesoderm and bFGF ventral; the gastrulation movements induced by the two factors differ in timing in a way that corresponds to the dorsal and ventral mesoderm of the early gastrula. But these results are also of great interest in the more general question of 'developmental timers'. Gurdon (1987) has emphasized that a significant difference between embryonic induction and other cellular interactions is

indeed that in induction the time of response depends upon the responding tissue and not on the time of receipt of the signal. The example used by Gurdon was actin gene activation: Gurdon *et al.* (1985) showed that in animal-vegetal combinations this always occurs at the midgastrula stage, irrespective of the stage at which the combination was made. Thus, if the tissues were apposed at the early blastula stage, transcription occurred some 9.5 h later, but, if they were placed in contact at the early gastrula stage, the interval was only 5–7 h. In the molecular analysis of developmental time measurement, it may be more profitable to study the control of gastrulation than that of actin synthesis, because this is a much earlier response and there are likely to be fewer intervening steps between signal and response. In particular, it is noteworthy that one gastrulation-specific response, the ability to spread on a fibronectin-coated substrate, can occur in single cells (Smith, Symes, Hynes and DeSimone, in preparation), whereas muscle formation may require interactions between groups of cells (Gurdon, 1988; Symes *et al.* 1988). In the analysis of induction, it is a great advantage to be able to study single cells (Gurdon, 1987).

Conclusions

The work described in this paper summarizes the evidence that, of the two classes of mesoderm-inducing factor that have been discovered, members of the FGF family are likely to constitute the ventral vegetal signal, and members of the TGF- β family the dorsal vegetal signal. We think this is an important conclusion which should assist the design of future experiments aimed at understanding pattern formation in the mesoderm of *Xenopus* and perhaps other organisms.

Much, however, remains to be done. First, and seemingly most straightforward, it is necessary to establish just which of the inducing factors listed in Table 1 are present and active in the *Xenopus* embryo. It is then necessary to discover which of these are *required* for mesoderm formation, by inactivating the endogenous factors. At present it is not completely clear how this

Table 1. Mesoderm-inducing factors in *Xenopus*

Factor	Family	Active?	Present in early embryo?		
			RNA	Protein	Localized?
Vg1 ¹	TGF- β	Unknown	Yes	Yes	Yes
XTC-MIF ²	TGF- β	Yes	Unknown	Unknown	Unknown
TGF- β 1 ³	TGF- β	With FGF	Unknown	Unknown	Unknown
TGF- β 2 ⁴	TGF- β	Yes	Unknown	Unknown	Unknown
aFGF ⁵	FGF	Yes	Unknown	No	Unknown
bFGF ⁶	FGF	Yes	Yes	Yes	Unknown
ECDGF ⁷	FGF	Yes	Unknown	Unknown	Unknown
INT-2 ⁸	FGF	Yes	Unknown	Unknown	Unknown
kFGF ⁸	FGF	Yes	Unknown	Unknown	Unknown

¹ Rebagliati *et al.* (1985); Weeks and Melton (1987); Melton (1987); Yisraeli and Melton (1988); Yisraeli *et al.* (this volume); Dale *et al.* (1989); Tannahill and Melton (1989).

² Smith (1987); Rosa *et al.* (1988); Smith *et al.* (1988).

³ Kimelman and Kirschner (1987).

⁴ Rosa *et al.* (1988).

⁵ Slack *et al.* (1987); Slack *et al.* (1988); Slack and Isaacs (1989).

⁶ Slack *et al.* (1987); Kimelman and Kirschner (1987); Kimelman *et al.* (1988); Slack and Isaacs (1989).

⁷ Slack *et al.* (1987).

⁸ Paterno *et al.* (1989).

might best be done. The most obvious approach is to microinject antisense oligonucleotides into the mature oocyte to destroy endogenous inducing factor mRNA (Shuttleworth and Colman, 1988), followed by maturing the oocyte by passage through a female frog and then fertilizing it (Holwill *et al.* 1987). However, this may not be effective because most Vg1 and bFGF protein seems to be synthesized during earlier oogenesis (Kimelman *et al.* 1988; Dale *et al.* 1989; Slack and Isaacs, 1989; Tannahill and Melton, 1989). An alternative approach might involve microinjection of antibodies against inducing factors into the fertilized egg. However, experiments such as these are difficult to control, and must be interpreted with care. When it is established which factors are involved in mesoderm induction it will be important to discover whether, like Vg1 (see Yisraeli *et al.* this volume), they are localized to the vegetal hemisphere, how that localization occurs, and, most importantly, how at least one of the factors becomes differentially activated on the dorsal side of the embryo in response to the postfertilization cortical rotation (see Gerhart *et al.* this volume).

It is also important to understand how factors like XTC-MIF and *Xenopus* bFGF exert different embryological, cellular and molecular effects, as outlined in this paper and elsewhere (Rosa, 1989; Green *et al.* in preparation). One possibility is that these differences are caused by the two factors activating different second messenger pathways, although the fact that the activity of both XTC-MIF and bFGF is enhanced by treatment of responding animal cap tissue with LiCl (Slack *et al.* 1988; Cooke *et al.* 1989) might be thought to argue that some of the signal transduction pathways are shared: one effect of lithium is to inhibit inositol monophosphatase (Hallcher and Sherman, 1980), thus blocking the recycling of InsP₃ to inositol and perhaps modulating the effects of any factor acting through inositol lipid-linked signalling systems (Drummond, 1987). This view of the effects of lithium is strengthened by the obser-

vation that the 'dorsalizing' effect of lithium, when microinjected into ventral blastomeres of the 32-cell stage embryo, is prevented by coinjection of equimolar *myo*-inositol (Busa and Gimlich, 1989). However, although these results are consistent with the suggestion that XTC-MIF and bFGF act through a similar second messenger pathway, lithium has so many other effects on cells that other interpretations are possible (see Slack *et al.* 1988).

A second aspect of the early response to inducing factors is that, as we discussed above, this is unlikely to specify cell differentiation directly; communication between the cells of an induced population seems to be required to define particular cell types (Symes *et al.* 1988). A signalling process of this sort seems to occur during gastrulation, and to be involved in establishing positional values along the anteroposterior axis of the embryo (see Gerhart *et al.* this volume). The nature of the molecules involved in this interaction is unknown at present, but it is possible that one of the early genes activated by XTC-MIF (Rosa, 1989) encodes a further signalling molecule.

Gastrulation itself may be controlled through a post-transcriptional response to mesoderm-inducing factors (Smith, Symes, Hynes and DeSimone, in preparation); animal pole cells can commence gastrulation-like movements in response to XTC-MIF within 30 min (Cooke and Smith, 1989), during which time even the early gene *Mix.1* is only weakly activated (Rosa, 1989). Post-transcriptional events following treatment with XTC-MIF and bFGF are currently under investigation in this laboratory.

It is clear that mesoderm induction and mesodermal patterning in Amphibia are complicated processes that must involve sequential inductive interactions, different patterns of cell movement, post-transcriptional and post-translational controls, and the activation of many genes. At present our understanding is limited, but the identification of mesoderm-inducing factors, and the

suggestion that the two groups of factors are active in different regions of the embryo, should allow us to improve our understanding quite rapidly.

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