Development 105, 665-677 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

Mesoderm induction and mesoderm-inducing factors in early amphibian development

J. C. SMITH

Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Contents

Introduction
Experimental embryology and mesoderm induction
The three-signal model of mesoderm formation
Mesoderm-inducing factors
Mesoderm-inducing factors as growth factors
Xenopus *embryos contain TGF-β-like molecules*
Xenopus *embryos contain FGF*
Elimination of inducing factors from Xenopus *embryos*
Mesoderm-inducing factors and pattern formation
Thresholds
Mesoderm induction, gastrulation and developmental clocks
Modulation of the effects of mesoderm-inducing factors: inhibitors and further cell–cell interactions
Conclusions

Key words: Embryonic induction, amphibian development, *Xenopus laevis*, mesoderm induction, mesoderm-inducing factors, XTC-MIF, TGF-β, FGF.

Introduction

Recently, significant advances have been made in the analysis of mesoderm induction, one of the first inductive interactions in vertebrate development. In this article, I review these advances in the context of earlier work on the subject and go on to discuss what problems remain and how they might be approached. I shall concentrate on cell biological and embryological aspects of mesoderm induction rather than on the transcriptional control of genes which are activated in response to induction. This work has recently been reviewed elsewhere (Gurdon *et al.* 1989).

Mesoderm induction probably occurs during the development of all vertebrates (Nieuwkoop *et al.* 1985; Smith, 1988) but the phenomenon has been most extensively studied in the Amphibia. This is for several reasons: amphibian embryos are large, making microdissection relatively easy; they are accessible to experimental manipulation at all developmental stages, from oogenesis to adulthood; development is rapid: the body plan is established and tissue-specific gene activation occurs within 24 h; amphibian embryos may be obtained in large numbers, which, together with their large size, makes it possible to extract enough material for biochemical analysis; and finally, the embryos do not grow – their early development consists of a series of cleavage divisions (see Fig. 1). This last point means, firstly, that it is possible to construct fate maps by injecting inert lineage tracers into selected blastomeres; because there is no growth the markers do not become diluted during development (see, for example, Jacobson & Hirose, 1978, 1981; Gimlich & Cooke, 1983; Gimlich & Gerhart, 1984; Heasman *et al.* 1984; Cooke & Webber, 1985; Dale & Slack, 1987*a*; Moody, 1987*a*,*b*; see also Fig. 2). Furthermore, since embryonic blastomeres survive on their yolk reserves, they will divide and even differentiate in a simple buffered salts solution. Thus it is possible to test defined molecules for their effects on differentiation without interference by poorly characterized serum components. In view of these advantages, this review deals exclusively with mesoderm induction in Amphibia.

Experimental embryology and mesoderm induction

Mesoderm induction was discovered using the techniques of experimental embryology. At morula stages, the amphibian embryo seems to consist of only two cell types: prospective ectoderm in the animal hemisphere and prospective endoderm in the vegetal hemisphere (Fig. 3A; see Jones & Woodland, 1986). The evidence for this comes from experiments in which different regions of the embryo are dissected and cultured in isolation: even blastomeres of the equatorial 'marginal zone', which give rise to mesoderm in normal development, form epidermis if they are isolated before the 64-cell stage. If, however, they are dissected later than this

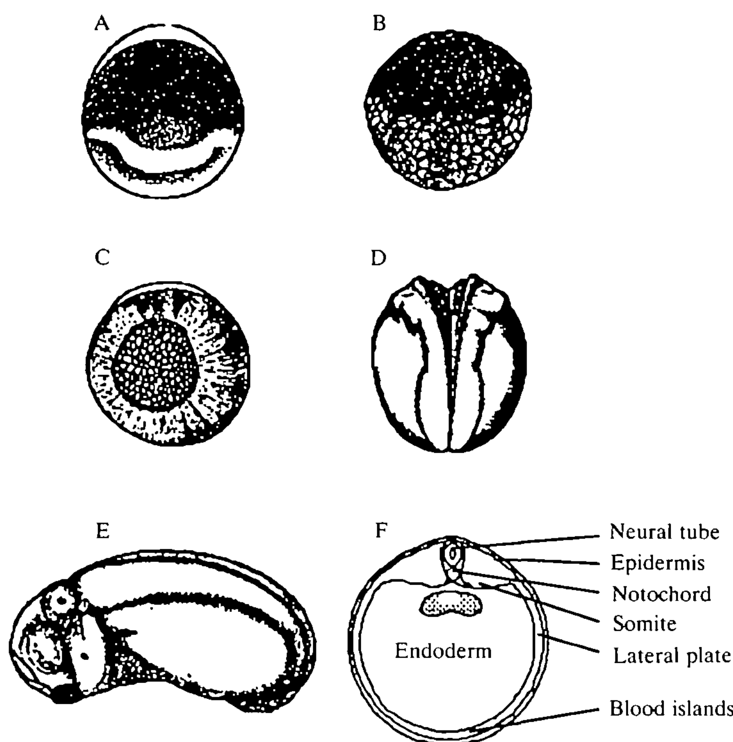

Fig. 1. The early development of *Xenopus laevis*. The *Xenopus* egg (A) has a diameter of about 1·4 mm. The animal hemisphere, which lies upwards by gravity, is heavily pigmented, and the vegetal hemisphere is pale. Sperm entry occurs in the animal hemisphere, and the side on which the sperm enters becomes the ventral–posterior half of the embryo. Ninety minutes after fertilization a rapid series of cleavage divisions begins. As a result, the embryo forms a hollow ball of cells, the blastula (B; 5 h after fertilization). During gastrulation (C; 11 h after fertilization: viewed from the vegetal hemisphere of the embryo) the three germ layers of the embryo take up their definitive positions, a process completed by the neurula stage, by which time tissue-specific gene activation has started (D; 18 h after fertilization). After formation of the neural tube the embryo elongates (E; 27 h after fertilization). The body plan of the embryo is now complete. (F) shows the location of the major cell types. A–E are redrawn from Nieuwkoop & Faber (1967), and are all at the same scale.

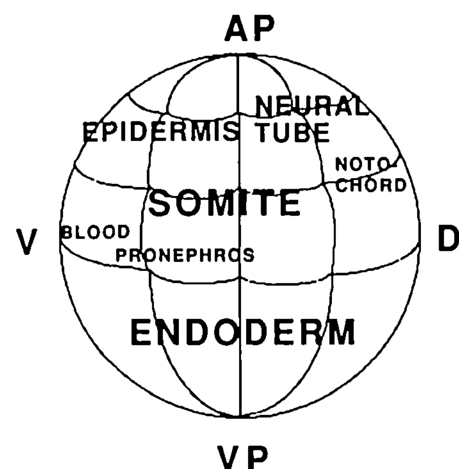

Fig. 2. A fate map of *Xenopus* at the 32-cell stage based on the work of Cooke & Webber (1985), Dale & Slack (1987*a*) and Moody (1987*a*,*b*). AP, Animal pole; VP, Vegetal pole; D, Dorsal; V, Ventral.

they form mesoderm as well as epidermis (Nakamura & Matsuzawa, 1967; Nakamura *et al.* 1970*b*).

One interpretation of this result is that mesoderm formation depends on an interaction between animal and vegetal blastomeres. This conclusion was confirmed by Ogi (1967, 1969) and by Nieuwkoop (1969), both of whom juxtaposed cells from the animal cap of the blastula-staged embryo with cells of the vegetal pole (Fig. 3B). When animal pole cells are cultured alone they form an atypical type of epidermis, while vegetal pole cells alone form poorly differentiated endoderm. Neither make mesoderm. Combinations of animal and vegetal pole cells, however, form a variety of mesodermal cell types.

Neither Ogi nor Nieuwkoop had access to cell lineage markers and they differed in the interpretation of their experiment. Ogi, with tentative support from Nakamura *et al.* (1970*a*), was reminded of work on the sea-urchin embryo indicating the existence of an animal–vegetal double-gradient system (Hörstadius, 1935). According to this view, apposition of animal and vegetal regions should result in regulation of the gradients, with both components giving rise to mesoderm. Nieuwkoop, however, regarded the mesoderm as being formed entirely from the ectodermal component, as a result of induction by prospective endoderm. He subsequently demonstrated that this view was correct by a quantitative analysis of the structures formed from animal–vegetal combinations of blastomeres from *Xenopus* embryos (Sudarwati & Nieuwkoop, 1971) and by using [^{3}H]thymidine to mark the animal pole component of combinations made from axolotl embryos (Nieuwkoop & Ubbels, 1972).

Nieuwkoop made a second major contribution to the understanding of mesoderm induction by demonstrating that the type of mesoderm that forms in animal–vegetal combinations depends on the origin of the vegetal inducing cells (Boterenbrood & Nieuwkoop, 1973). Vegetal pole cells from the dorsal side of the axolotl blastula tended to induce dorsal cell types such as notochord and muscle while lateral and ventral vegetal blastomeres induced blood, a characteristic ventral cell type, along with mesenchyme and mesothelium. Nieuwkoop concluded from this that the pattern of cell types in the mesoderm (Fig. 1) is determined, at least in part, by information derived from underlying vegetal blastomeres.

The three-signal model of mesoderm formation

The most widely used species in the study of amphibian development is *Xenopus laevis*, and the observations of Boterenbrood & Nieuwkoop (1973) on the axolotl have been confirmed for this species by Dale *et al.* (1985) and Dale & Slack (1987*b*). The results, however, appear to contradict the fate map of *Xenopus*. Ventral vegetal blastomeres induce little or no muscle from animal pole cells, yet most of the muscle of the embryo is formed

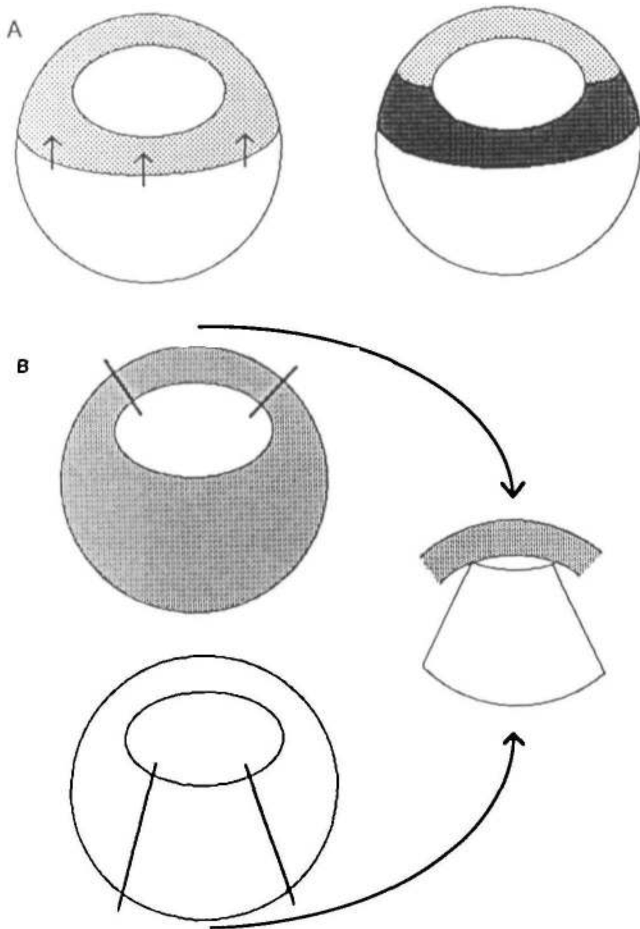

Fig. 3. Mesoderm induction. (A) At early blastula stages, the *Xenopus* embryo can be considered to consist of two cell types: presumptive ectoderm in the animal hemisphere (light stippling) and presumptive endoderm in the vegetal hemisphere (no stippling) (Jones & Woodland, 1986). During mesoderm induction 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 is 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.

from blastomeres of the 'ventral' half of the embryo (Keller, 1976; Cooke & Webber, 1985; Dale & Slack, 1987*a*; Moody, 1987*a*,*b*; see Fig. 2).

This incongruity may be resolved by the 'three signal' model of Slack and his colleagues (Smith & Slack, 1983; Slack *et al.* 1984; Smith *et al.* 1985; Dale & Slack, 1987*b*; see Fig. 4). The first two signals in this model are those of Boterenbrood & Nieuwkoop (1973), and are derived from the vegetal hemisphere of the embryo. One, on the dorsal side, induces predominantly notochord, perhaps with a small amount of muscle. The other, on the ventral side, induces blood, mesenchyme and mesothelium. The third signal, however, originates from

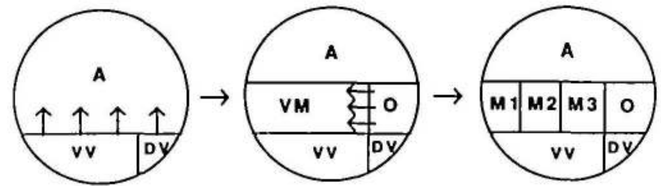

Fig. 4. The three-signal model. Two mesoderm induction signals are assumed to derive from the vegetal region of the early blastula. The dorsal–vegetal (DV) signal induces dorsal mesoderm, or 'organizer' tissue (O) while the ventral vegetal signal (VV) induces general ventral mesoderm (VM). The ventral mesoderm then receives a signal from the organizer, probably during gastrulation, which results in the formation of additional muscle (M3) and perhaps pronephros (M2); only the most remote tissue (M1) remains as ventral blood-forming mesoderm.

newly induced dorsal mesoderm. This signal acts within the prospective mesodermal germ layer to 'dorsalize' adjacent ventral mesoderm such that tissue that would have formed blood forms muscle instead. Only cells near the ventral midline of the embryo are out of range of the dorsalization signal and continue to form blood.

Evidence for the existence of the dorsalization signal comes from experiments in which dorsal and ventral marginal zone regions of the early gastrula are juxtaposed. In isolation, dorsal marginal zone tissue forms notochord, with some muscle and neural tissue, while ventral marginal zone cells form blood, mesenchyme and mesothelium. In combinations, however, although the dorsal tissue continues to differentiate as notochord, the ventral marginal zone forms large amounts of muscle (Slack & Forman, 1980; Dale & Slack, 1987*b*).

Dorsalization may be the major interaction at work in Spemann's 'organizer' graft (Spemann & Mangold, 1924), in which early gastrula dorsal marginal zone tissue (the 'organizer') is grafted into the ventral marginal zone region of a host embryo, causing the formation of a mirror-symmetrical double-dorsal larva. Perhaps the most dramatic aspect of this result is the formation of an additional neural tube, which arises from ventral ectoderm under the influence of a neural induction signal from involuting dorsal mesoderm (see Gimlich & Cooke, 1983; Jacobson, 1984). However, the earlier interaction is probably dorsalization, during which much of the original ventral mesoderm into which the graft was placed is induced to form muscle (Smith & Slack, 1983).

Mesoderm-inducing factors

Much of the recent interest in mesoderm induction stems from the discovery and purification of mesoderm-inducing factors (MIFs: see Smith, 1987; Slack *et al.* 1987; Kimelman & Kirschner, 1987; Rosa *et al.* 1988; Smith *et al.* 1988). It is ironic, therefore, that sources of mesoderm-inducing activity were discovered long before the phenomenon of mesoderm induction was properly defined. In 1953, Toivonen found that guinea-

pig bone marrow could induce mesodermal cell types from isolated newt ectoderm and, a few years later, Saxén & Toivonen (1958) showed that HeLa cells have a similar effect. Indeed the discovery of these inducing factors may have influenced Nieuwkoop (1969) in his interpretation of the results of animal–vegetal combinations.

Since Nieuwkoop's work, other sources of mesoderm-inducing activity have been reported. One such is the carp swim-bladder (Kawakami, 1976; Kawakami *et al.* 1977), but the best-known is Tiedemann's 'vegetalizing factor', which has been purified from 9- to 11-day chick embryos (Born *et al.* 1972*a*). Experiments with this and other factors have provided some significant insights into mesoderm induction in amphibia, but there have been two problems with these inducers that have hindered their use as research tools. One is that they are usually assayed as an insoluble pellet, as the 'filling' of a sandwich in which test animal pole tissue is the bread. This makes dose–response experiments, for example, particularly difficult to interpret. The other problem is that these factors are 'heterogenous', derived from an inappropriate source (see Gurdon, 1987). It is difficult to understand the relevance of a factor from the carp swim-bladder to the events of early frog development.

Mesoderm-inducing factors as growth factors

Recent work is overcoming these problems, but acceptance of the new mesoderm-inducing factors has been facilitated by the fact that they are related to known peptide growth factors. One class of MIFs is related to transforming growth factor type β (TGF-β). This group includes XTC-MIF, which is derived from the *Xenopus* XTC cell line (Smith, 1987; Smith *et al.* 1988) and TGF-β2, obtained from pig platelets (Rosa *et al.* 1988). TGF-β1 has no mesoderm-inducing activity (Slack *et al.* 1987; Kimelman & Kirschner, 1987), but it enhances the effect of the other group of MIFs, the heparin-binding growth factors (Kimelman & Kirschner, 1987). The main members of this second group are acidic and basic fibroblast growth factor (aFGF and bFGF), both of which are equally active in inducing mesoderm from isolated *Xenopus* animal pole tissue (Slack *et al.* 1987; Kimelman & Kirschner, 1987; Slack *et al.* 1988).

The usual test for mesoderm-inducing activity involves culturing isolated animal pole tissue in dilute solutions of the factors. In the absence of MIFs, the test tissue differentiates as epidermis, but in their presence mesodermal cell types arise (see Smith, 1987). Both groups of MIF are active at picomolar concentrations, but the assay reveals a significant difference between them. The TGF-β class of MIF induces a variety of mesodermal cell types including notochord, muscle, kidney, mesenchyme and mesothelium (Smith *et al.* 1988). FGF is capable of inducing all these cell types except notochord, the most dorsal mesodermal tissue (Godsave *et al.* 1988). This has led to the suggestion that the 'dorsal' signal in Slack's model (Fig. 4) is TGF-β-like and that the 'ventral' signal is FGF-like (Dale & Slack, 1987*b*). To confirm suggestions such as this, it is first necessary to demonstrate that TGF-β- and FGF-like molecules are present in the *Xenopus* embryo in the predicted amounts, in the predicted regions, and at the appropriate stages.

Xenopus *embryos contain TGF-β-like molecules*

Definitive TGF-β2 and XTC-MIF transcripts or protein have not yet been identified in *Xenopus* embryos, but a maternal mRNA that is restricted to the vegetal hemisphere of the egg (see Rebagliati *et al.* 1985) has been shown to code for a factor related to TGF-β (Weeks & Melton, 1987). This mRNA, designated Vgl, constitutes 0·05–0·1% of the poly(A)$^+$ RNA pool (Rebagliati *et al.* 1985). An open reading frame of 1080 bases encodes a protein of $41{\cdot}8\times10^3$ M_r of which the carboxy-terminal 120 amino acids show 38% homology to TGF-β1 and in which the positions of the cysteine residues are conserved. Interestingly, Vgl shows greatest similarity to the deduced sequence of the decapentaplegic (*dpp*) gene product of *Drosophila*, another member of the TGF-β family (Padgett *et al.* 1987). There is a 48% match in the carboxy-terminal 114 amino acids, and the two share a potential glycosylation site.

Judging from changes in the spatial distribution of Vgl mRNA, the protein encoded by this message is an excellent candidate for an endogenous mesoderm-inducing factor. During early oogenesis, the message, as revealed by *in situ* hybridization, is distributed uniformly within the oocyte (Melton, 1987). As oogenesis proceeds the RNA moves towards the vegetal pole of the oocyte and eventually is localized as a thin cortical shell. Following fertilization the message is released from its cortical position and it spreads towards the animal pole. This spread is restricted by the cleavage divisions of the embryo such that at the early blastula stage the only cells to contain substantial amounts of Vgl transcripts are the vegetal blastomeres, those responsible for inducing adjacent equatorial cells to form mesoderm (Weeks & Melton, 1987).

Although the circumstantial evidence is compelling, there is no direct evidence at present that Vgl mRNA does code for a mesoderm-inducing factor. It is possible, for example, that it is an inactive homologue of an endogenous inducing factor in the way that TGF-β1 is an inactive homologue of the inducing factor TGF-β2. One way to investigate this might be to make Vgl protein by amplified expression in Chinese Hamster ovary cells, as described for TGF-β1 by Gentry *et al.* (1987). Alternatively, Vgl RNA might be microinjected into the animal hemisphere of early *Xenopus* embryos. This should cause the formation of ectopic mesoderm in a manner similar to microinjected XTC cell poly (A)$^+$ RNA (Cooke *et al.* 1987; Woodland & Jones, 1987).

Even if Vgl protein is not a mesoderm-inducing factor, study of the message will yield important results concerning the mechanisms by which mRNAs are localized within early embryos. For example, Pondel & King (1988) have recently shown that Vgl RNA is concentrated 35- to 50-fold in detergent-insoluble ex-

tracts of *Xenopus* oocytes, whereas histone H3 mRNA is equally distributed between detergent-insoluble and -soluble fractions. The detergent-insoluble extracts are enriched for cytokeratins and vimentin, suggesting that Vgl RNA may be localized through an interaction with the cytoskeleton. In support of this, Vgl RNA is found to be released to the detergent-soluble fraction after ovulation; this is when cortical cytokeratin filaments break down (Klymkowsky *et al.* 1987) and Vgl RNA spreads towards the animal pole (Weeks & Melton, 1987). Using a different approach, Yisraeli & Melton (1988) have shown that Vgl message transcribed *in vitro* is translocated to the vegetal hemisphere after being injected into immature *Xenopus* oocytes. Localization does not require translation of Vgl. This technique should make it possible to map the 'vegetal translocation' sequence on the Vgl message, which may, for example, recognize intermediate filaments. It will then be interesting to screen *Xenopus* oocyte cDNA libraries for clones with similar sequences. Analysis of such clones may reveal additional localized mRNAs which could code for mesoderm-inducing factors.

Xenopus *embryos contain FGF*

Work on FGF indicates that both the mRNA and the protein are present in the early embryo. In their original paper, Kimelman & Kirschner (1987) described a cDNA from a *Xenopus* oocyte library that contained sequences closely related to human and bovine basic FGF. This cDNA hybridized to a 1·5 kb RNA in *Xenopus* oocytes, eggs and early embryos, but the level of this transcript varied through early development. The message was abundant in oocyte preparations (which included follicle cells), but had decreased by 95 % at the fertilized egg stage. It increased again at the midblastula transition, when embryonic transcription commences (Newport & Kirschner, 1982), and remained fairly steady at least until the late neurula stage.

The RNA detected by Kimelman & Kirschner's original cDNA is large enough to encode a protein the size of bFGF but, although it contains a short open reading frame encoding a peptide domain homologous to the third exon of mammalian bFGFs, sequences homologous to the first and second exons are absent. Recently, however, use of a different probe has revealed two additional RNA species (Kimelman *et al.* 1988). One of these, of approximately 2·1 kb, is present continuously from the oocyte to midgastrula stages. The other is a 4·2 kb species which is present in the oocyte and is transcribed again at neurula stages. A 4·3 kb cDNA corresponding to this larger species was isolated and sequence analysis indicated that the primary translation product is a 155-residue protein with an overall homology to human bFGF of 89 %. When this protein was synthesized in a T7 expression system and purified by heparin–Sepharose chromatography it was shown to be as effective as bovine bFGF in inducing muscle from isolated *Xenopus* animal pole regions.

To demonstrate that bFGF protein is present in the *Xenopus* embryo, both Kimelman *et al.* (1988) and Slack & Isaacs (1989) have passed extracts of eggs and blastulae through heparin–Sepharose columns before eluting the bound material with high concentrations of NaCl. Slack & Isaacs (1989) were able to show that the eluted material had mesoderm-inducing activity which could be blocked by antibodies to bFGF but not to aFGF or TGF-β. Furthermore, active fractions from an HPLC heparin affinity column contained material identifiable as bFGF by use of specific antibodies after Western blotting. Kimelman *et al.* (1988) did not assay their heparin–Sepharose eluates for mesoderm-inducing activity, but they also showed by immunological criteria that FGF-like-protein was present. Both groups estimated the total amount of FGF present in the *Xenopus* embryo by inspection of Western blots reacted with anti-FGF antibodies, but they differed in their conclusions. Kimelman *et al.* (1988) calculated that a single embryo contains 100 pg FGF, a concentration of approximately 70 ng ml^{-1}. Slack & Isaacs (1989), however, estimate a total concentration of about 7 ng ml^{-1} bFGF. Both concentrations are certainly high enough for FGF to be a natural mesoderm inducer, but further work is required to discover which estimate is nearer the mark. Calculations of amounts of protein from the intensities of bands on gels are probably subject to errors of at least a factor of three, so the apparent difference may not be significant. It is noteworthy, however, that the antibody used by Slack & Isaacs (1989) may not recognize *Xenopus* FGF as efficiently as human; on the other hand, their figure is corroborated by the levels of biological activity that can be recovered from the embryo.

The spatial distribution of FGF in the *Xenopus* embryo has not yet been studied. The obvious prediction is that, like Vgl, the factor is concentrated in the vegetal hemisphere of the embryo. There is, however, one difficulty with understanding how localized FGF might act as an inducing factor. TGF-β-like molecules, including Vgl (Weeks & Melton, 1987), carry a signal sequence which directs their secretion from the cell, thus allowing them to act on their neighbours (reviewed by Massagué, 1987). Bovine and *Xenopus* FGF carry no such sequence (Abraham *et al.* 1986; Kimelman *et al.* 1988) and cannot be secreted from cells through the usual pathway. For example, NIH 3T3 cells transfected with a plasmid containing cDNA that encodes bovine bFGF synthesize large amounts of bFGF, but this remains associated with the cells in an inactive form, and the cells themselves appear morphologically normal. However, when a heterologous secretory signal sequence is fused to the bFGF cDNA, the cells become morphologically transformed and tumorigenic, although no bFGF can be detected in the culture medium (Rogelj *et al.* 1988). A similar experiment has been carried out with acidic FGF and Swiss 3T3 cells (Jaye *et al.* 1988). Again, no FGF could be detected in medium conditioned by the transfected cells but, even in the absence of a signal peptide, several traits characteristic of the transformed phenotype were expressed. The authors suggest, as has also been suggested for cells expressing PDGF encoded by v-*sis*, that the growth

factor may stimulate its receptor in an internal compartment (Leal *et al.* 1985; Keating & Williams, 1988).

It is not possible to draw conclusions about the way FGF acts in *Xenopus* embryos from these experiments on tissue-culture cells, although there are several intriguing possibilities. For example, FGF may act during normal development in an autocrine fashion. In this case, prospective mesodermal cells would both synthesize and respond to the factor, perhaps on receipt of a TGF-β-like factor. The observation that exogenous FGF acts as an inducing factor on isolated animal pole regions might then be coincidental, not implying anything about the nature of the vegetal inducer.

Elimination of inducing factors from Xenopus *embryos*

It is encouraging that FGF and TGF-β-like molecules are present in the *Xenopus* embryo, but much work remains to be done before either is proved to be a natural mesoderm inducer. In the case of TGF-β, the first task is to identify and characterize a member of the family which is both present in the embryo and which has mesoderm-inducing activity. There are three candidates at present: the Vg1 protein, the *Xenopus* equivalent of TGF-β2, and XTC-MIF. The mRNA for the first of these is present in the embryo at the right time and place but it is not yet known whether the protein has inducing activity or, indeed, whether the RNA is translated. The other two are known to be mesoderm-inducing factors, but their presence in the embryo must be established, followed by an analysis of their spatial distribution. FGF is known to be present in the embryo and to be active as a mesoderm inducer, but its spatial distribution has not yet been studied.

Definitive proof that bFGF or TGF-β-like factors are the natural mesoderm inducers requires the elimination of these factors from the embryo. Recently, techniques have been developed that should make this possible. Embryological experiments indicate that the vegetal hemisphere acquires the ability to induce mesoderm from animal pole regions at least as early as the 64-cell stage (Jones & Woodland, 1987). This is about 3 h earlier than the midblastula transition, when embryonic transcription begins (Newport & Kirschner, 1982), so the mRNA for inducing factors must be synthesized during oogenesis. Shuttleworth & Colman (1988) have demonstrated that such maternal messages can specifically be eliminated from the oocyte simply by microinjecting appropriate antisense oligonucleotides. The oligonucleotides form a DNA–RNA duplex with the target message and this acts as a substrate for an endogenous RNase H activity. The technique works most effectively when oligonucleotides are injected into oocytes rather than fertilized eggs or early embryos, because an RNA duplex unwinding activity appears soon after fertilization (Rebagliati & Melton, 1987; Bass & Weintraub, 1987). However, it is possible to overcome this problem by ablating mRNA in oocytes as outlined above and then maturing the oocytes *in vivo* before fertilizing them *in vitro*, as described by Holwill *et al.* (1987). The result of such experiments, if mRNAs for endogenous mesoderm-inducing factors are ablated early enough, should be the development of mesodermless embryos.

It may not be possible to eliminate mRNAs for inducing factors before synthesis of substantial amounts of the factors themselves. If this is the case it may be possible to interfere with induction *in vivo* by microinjecting specific antibodies to MIFs into the vegetal hemisphere of early embryos.

Mesoderm-inducing factors and pattern formation

It is important to discover the identity and analyse the spatial distribution of endogenous mesoderm-inducing factors in the amphibian embryo, but to explain how the right mesodermal cell type forms in the right place it is also necessary to study the responses of animal pole cells to these factors. Most recent studies of mesoderm induction in *Xenopus* have used muscle, the most abundant mesodermal cell type (Cooke, 1983), as a marker of mesoderm formation (for example, Gurdon *et al.* 1985; Sargent *et al.* 1986; Kimelman & Kirschner, 1987; Rosa *et al.* 1988; Gurdon, 1988, 1989). However, as mentioned above, many other mesodermal cell types are formed in response to induction, both in animal–vegetal combinations (Dale *et al.* 1985) and in response to purified mesoderm-inducing factors (Smith *et al.* 1988; Godsave *et al.* 1988; J. Cooke & J. C. Smith, unpublished observations). The only mesodermal cell type that is not formed in response to MIFs is blood (Smith, 1987; K. Symes & J. C. Smith, unpublished observations), and it seems likely that this is because erythrocyte differentiation requires a late-acting permissive signal from hepatic endoderm, not because MIFs do not specify ventral mesoderm (Deparis & Jaylet, 1984).

The embryological experiments already described indicate that different mesodermal cell types are formed in response to different regions of the vegetal hemisphere of the embryo. Dorsal mesodermal cell types are formed in response to dorsal vegetal pole blastomeres and intermediate or ventral cell types in response to ventral vegetal pole blastomeres. This series of cell types is also observed in response to decreasing concentrations of mesoderm-inducing factors. Thus high concentrations (10–25 ng ml^{-1}) of XTC-MIF induce notochord, and progressively lower concentrations induce muscle, followed by mesenchyme and mesothelium (Smith *et al.* 1988). Like Godsave *et al.* (1988) I consider mesenchyme, and particularly mesothelium, to represent ventral differentiation, even though large amounts of mesenchyme are found, for example, in the head of *Xenopus*. This is because mesenchyme and mesothelium form more frequently in combinations of ventral vegetal blastomeres with animal pole regions than in combinations involving dorsal vegetal blastomeres (Dale & Slack, 1987*b*). A similar dorsal-to-ventral response curve is seen with bFGF, with the significant difference that even the highest concentrations of this factor only rarely induce notochord (Godsave *et al.* 1988).

One conclusion from these results, already mentioned above, is that a TGF-β-like factor may act as the 'dorsal' component of Slack's three-signal model (Fig. 4), with bFGF, being unable to induce notochord, representing the ventral component. The data also suggest that the vegetal hemisphere of the embryo might contain graded distributions of one or both factors, with the highest concentrations at the dorsal side. However, there is no evidence for a graded distribution of the mRNA for the only potential MIF for which such information is available: *in situ* hybridization studies with Vg1 probes have not revealed an asymmetrical distribution in the dorsoventral axis (D. A. Melton, personal communication). Such studies need to be repeated with the other candidates for endogenous mesoderm-inducing factors, but embryological evidence suggests that the gradient hypothesis in its simplest form, in which mRNAs for inducing factors are arranged in an asymmetric fashion, is unlikely to be true. The dorsoventral axis of *Xenopus* is established at fertilization, with dorsal structures usually forming opposite the site of sperm entry (Black & Gerhart, 1985). This determinative event is linked to a series of cytoplasmic shifts in which the entire subcortical cytoplasm rotates about 30° with respect to the cell membrane, with that in the vegetal hemisphere moving away from the dorsal side (Vincent *et al.* 1986). According to a strict localization model these shifts might move determinants to the dorsal side of the embryo. However, this interpretation is not consistent with experiments in which fertilized *Xenopus* eggs are tilted or centrifuged, so as to displace the contents of the cytoplasm. Embryos subjected to this treatment become either double-dorsal or 'head-heavy' (Black & Gerhart, 1986; Cooke, 1986, 1987) and this cannot be explained by the redistribution of existing determinants for two reasons. First, in the formation of twinned embryos, it is not clear why centrifugation would only move half of the axis-forming molecules. Secondly, in both experiments, the results imply that centrifugation has caused greater amounts of XTC-MIF-like factors to be created. This would not be predicted by a simple gradient model. It may be that regions specified by centrifugation as dorsal translate mRNA for inducing factors more efficiently. Alternatively, there may be graded post-translational modifications of proteins which affect their activity, or the receptors for inducing factors may become differentially localized. These possibilities require investigation.

Thresholds

Another difficult problem is that of how different concentrations of MIFs induce different types of cell differentiation. This question is not restricted to mesoderm induction in the amphibian embryo. A similar problem exists in systems as different as the chick limb, where a graded signal from the posterior margin of the limb bud (perhaps retinoic acid: Tickle *et al.* 1982; Thaller & Eichele, 1987) specifies different digits along the anteroposterior axis (Tickle *et al.* 1975; Smith *et al.* 1978), and in the insect egg, where position along the anteroposterior axis is specified by the concentration of the *bicoid* gene product (Driever & Nüsslein-Volhard, 1988).

A measure of our ignorance on this topic, that of the interpretation of positional information (Wolpert, 1969), is that it is not even clear whether such threshold phenomena operate at the single cell level or at the level of cell populations. That is, does an individual cell have several thresholds, such that at a low concentration of a factor it will form mesothelium, and at a higher concentration muscle, or will mesothelium be formed by a population of cells if 10 % of them undergo an initial response to induction, and muscle be formed if 50 % of them do so? An experiment by Cooke *et al.* (1987) suggests that the latter model is more likely to be correct. In this work, animal pole explants were allowed to 'round up' before treatment with high concentrations of XTC-MIF. The outer surface of rounded explants is not responsive to soluble inducing factors, so the procedure reduces the number of cells in the explants that are exposed to the factor. If individual cells have several thresholds, the explants would be expected to contain small patches of notochord. However, mesenchyme and mesothelium were formed, suggesting that the threshold phenomenon is a cell population effect. We are further testing the idea that it is the proportion of induced cells within an explant that determines which cell types differentiate by mixing populations of induced and uninduced cells in different proportions (M. Yaqoob, K. Symes, J. B. A. Green & J. C. Smith, work in progress).

If it is the proportion of induced cells that determines which cell types form in response to MIFs, there must be communication between cells after the initial inductive stimulus. Work by Symes *et al.* (1988) indicates that this is the case. This series of experiments was initiated by the results of Sargent *et al.* (1986), who cultured *Xenopus* embryos in calcium- and magnesium-free medium (CMFM). Under these conditions, the blastomeres of the embryo lose adhesion, but continue dividing to form a loose heap of cells (Fig. 5A). If divalent cations are restored at the early gastrula stage the cells readhere and eventually form muscle (Fig. 5B) as well as epidermis (Fig. 5C). If, however, the cells are dispersed during culture in CMFM (Fig. 5D), muscle does not form following reaggregation (Fig. 5E), although epidermis formation is enhanced (Fig. 5F). This suggests that culturing blastomeres in a heap allows the transmission of mesoderm-induction signals, while dispersion effectively dilutes the signal. Symes *et al.* (1988) attempted to substitute for cell proximity by culturing dispersed blastomeres in XTC-MIF (Fig. 5G). The surprising result was that dispersed cells did not respond to XTC-MIF by forming muscle, or indeed any mesodermal cell type, after reaggregation, but the factor did inhibit epidermal differentiation (Fig. 5H,I).

Two conclusions may be drawn from this result. One is that an early stage in mesoderm induction is the suppression of epidermal differentiation. This inhibition is not due to any toxic action of XTC-MIF (Symes *et al.* 1988) and is a specific effect of mesoderm-

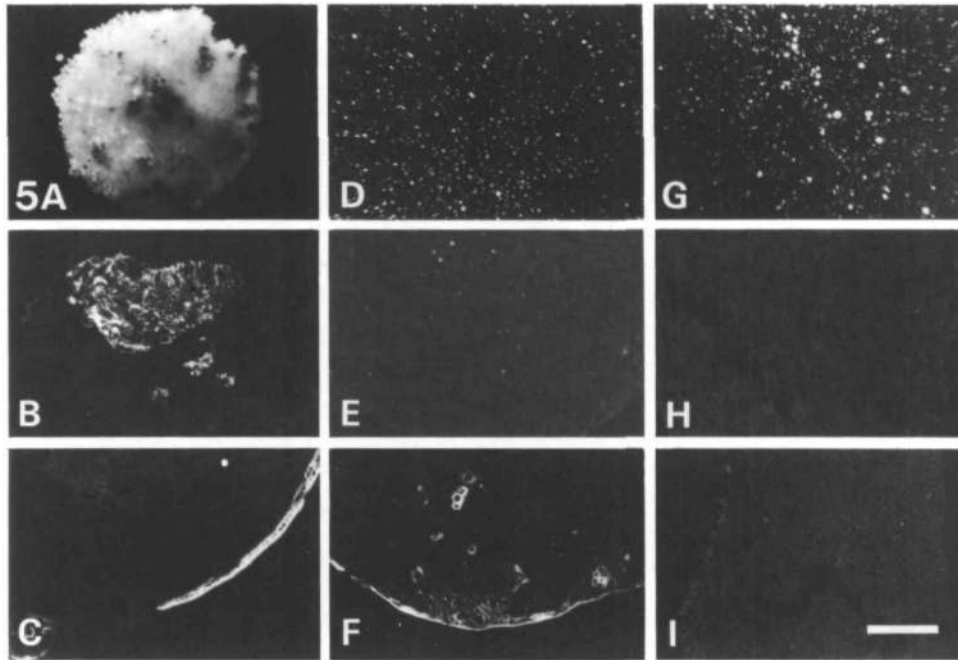

Fig. 5. Evidence for additional signals involved in mesoderm induction. (A) *Xenopus* embryos cultured in calcium- and magnesium-free medium (CMFM) disaggregate to form a loose heap of cells. If divalent cations are restored at the early gastrula stage, both muscle (B) and epidermis (C) are formed. If disaggregated blastomeres are dispersed during culture in CMFM before reaggregation at the early gastrula stage (D), they do not form muscle (E) but epidermal differentiation is enhanced (F). If dispersed embryos are cultured in the presence of XTC-MIF (G) they do not form muscle on reaggregation (H), but neither do they form epidermis (I). Scale bar in (I) is 200 μm, and also applies to (B), (C), (E), (F) and (H).

inducing factors: bFGF also suppresses epidermal differentiation, but EGF and fetal calf serum have no effect. The suppression of epidermal differentiation at the single cell level is all-or-nothing, but the proportion of cells that are diverted from epidermal differentiation depends on the concentration of XTC-MIF or bFGF and on the stage of the responding tissue. This result allowed the first real quantitative analysis of the effects of MIFs (Symes *et al.* 1988). The second conclusion is that formation of mesoderm in response to XTC-MIF requires further signals that are produced in response to the first. Dispersion of blastomeres during exposure to XTC-MIF effectively dilutes these signals and prevents mesoderm forming. The nature of these 'second signals' is under investigation in this laboratory. One possibility is that XTC-MIF and other inducing factors act by converting animal pole cells to vegetal blastomeres, which then produce their natural inducing signal (see Minuth & Grunz, 1980). However, one argument that this is not the case is that the inducing activity of *Xenopus* vegetal pole regions is lost around the beginning of gastrulation, or soon afterwards (Nakamura *et al.* 1970*a*; Dale *et al.* 1985; Gurdon *et al.* 1985; Jones & Woodland, 1987), while *Xenopus* animal pole regions are responsive to XTC-MIF at this stage and even later (K. Symes and J. C. Smith, unpublished observations). If XTC-MIF were to act by converting some cells to endoderm, even if this transformation were instantaneous, there would barely be enough time for the subsequent mesoderm induction to occur. It seems more likely that the proposed second signal is part of a cascade of events that follows mesoderm induction and is required for the activation of mesoderm-specific genes. Such an idea would also explain Gurdon's (1987, 1988) observation that small numbers (tens) of animal pole cells do not form muscle in response to vegetal pole regions, whereas larger numbers (hundreds) do. This phenomenon, described by Gurdon as a 'community effect', might occur because small numbers of responding cells cannot build up sufficient levels of second signal.

This, of course, does not solve the problem of how blastomeres become specified to form different cell types – it simply inserts another step in the process. Thus the question remaining is: how can the proportion of cells responding to induction influence the cell types that arise? One suggestion might 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 & 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 they can produce notochord or muscle 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 would 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 & Smith, 1989).

Mesoderm induction, gastrulation and developmental clocks

Gurdon (1987) has emphasized that a significant difference between embryonic induction and other cell signalling systems is that the timing of the response is dictated by the stage of the responding tissue and not by the time at which the signal was received. Gurdon himself has illustrated this with reference to the timing of muscle-specific actin gene activation in response to mesoderm induction: irrespective of the stage at which animal and vegetal pole regions are placed in contact, actin gene activation always occurs at the mid- to late-gastrula stage; if the combination is made at the early blastula stage, transcription occurs some 9·5 h later, but if the tissues are placed in contact at the early gastrula stage, the interval is between 5 and 7 h (Gurdon *et al.* 1985). Similar results have been reported by Kimelman & Kirschner (1987). Actin gene activation in response to mesoderm induction is the earliest molecular marker of induction yet identified. However, an even earlier response is a visible one. *Xenopus* animal pole explants exposed to XTC-MIF undergo changes in shape, involving elongation and constriction, that mimic the gastrulation movements of isolated dorsal mesoderm (Smith, 1987; Symes & Smith, 1987; see Fig. 6). These movements provide a novel system for the study of gastrulation itself (Symes & Smith, 1987; D. DeSimone, J. C. Smith & K. Symes, work in progress), but they also supply further information on timing mechanisms in early development. Thus, within the limits of observation, the XTC-MIF-induced gastrulation movements of animal pole regions always commence at the equivalent of the early gastrula stage, irrespective of the stage at which explants were exposed to the factor (Symes & Smith, 1987).

Animal pole explants exposed to bFGF also undergo

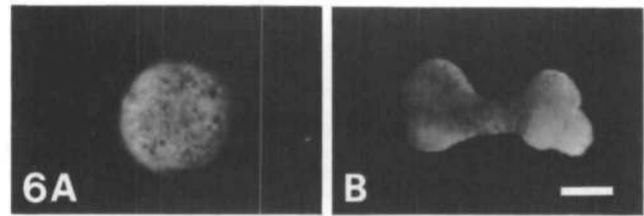

Fig. 6. XTC-MIF induces gastrulation-like movements in animal pole explants. (A) A control explant. (B) An induced explant 15 h after treatment with XTC-MIF. Scale bar in (B) is 200 μm, and also applies to (A).

gastrulation-like movements, but these are less dramatic (Slack *et al.* 1987; Kimelman & Kirschner, 1987; Godsave *et al.* 1988; Slack *et al.* 1988), making it harder to estimate their time of onset. A technique introduced by Cooke *et al.* (1987) and Cooke & Smith (1989) overcomes this problem by microinjecting mesoderm-inducing factors into the blastocoels of *Xenopus* embryos. This results in wide-spread changes in cell shape, locomotion and adhesion that correspond to those that occur in a spatially and temporally organized fashion around the marginal zone of the embryo (Keller, 1976, 1986; Keller & Schoenwolf, 1977). It is as if the entire blastocoel roof is induced to form mesoderm. These events can be observed and timed with great precision by dissection of fixed embryos, and the results confirm those of Symes & Smith (1987) in that the time of transformation of cell behaviour is independent of the time of injection of inducing factor. However, the results also indicate that the time of onset does not depend on the intrablastocoelic concentration of factor, and that the response is extraordinarily rapid: if XTC-MIF is injected at the early gastrula stage, the transformation of cell behaviour occurs within 30 min (Cooke & Smith, 1989). But perhaps the most significant observation is that the changes in cell behaviour induced by XTC-MIF and by bFGF start at different times: those induced by XTC-MIF begin at the early gastrula stage, whereas those induced by bFGF occur over 1 h later, at the midgastrula stage.

This result is significant for at least three reasons. First, in relation to the threshold model outlined above, it demonstrates that at least one response to bFGF occurs later than a similar response to XTC-MIF. It is possible, therefore, that production of a second inductive signal also begins later in response to bFGF than it does to XTC-MIF or other TGF-β-like factors. Second, the result is consistent with the simple model that XTC-MIF represents the 'dorsal' mesoderm induction signal and bFGF the 'ventral' (see Fig. 4), because in normal development dorsal–anterior structures such as notochord start gastrulating before ventral–posterior structures (see Keller, 1986). Finally, it suggests that a particular cell type induced by XTC-MIF may differ in some way from the same cell type induced by bFGF; in the terminology of Lewis & Wolpert (1976) the cells may be 'non-equivalent'. This difference may relate, for example, to the position of the tissue within the embryo.

Modulation of the effects of mesoderm-inducing factors: inhibitors and further cell–cell interactions

The *Xenopus* embryo may thus employ two mesoderm-inducing factors. Both of these induce different cell types at different concentrations, and each causes the onset of gastrulation-like behaviour at a time appropriate to a specific position in the embryo. Such a combination of effects should be capable of establishing a complex yet consistent pattern of cell differentiation in the mesoderm, but even with our present limited understanding we know of at least three ways in which the initial response to mesoderm induction can be modified. First, the embryological evidence discussed at the beginning of this article predicts that at least three signals are required to establish the pattern of mesodermal differentiation in *Xenopus*. Two of these derive from the vegetal hemisphere of the embryo, and might be represented by XTC-MIF (or another TGF-β-like molecule) and bFGF. The third signal, produced by dorsal marginal zone cells somewhat later in development, 'dorsalizes' adjacent ventral mesoderm. Little is known about the nature of the dorsalization signal, except for an unconfirmed report by Flickenger (1980) that heparin and heparan sulphate can have dorsalizing effects on ventral mesoderm. However, an alternative strategy to identify the signal might take advantage of recent observations that animal pole cells treated with XTC-MIF, but not bFGF, can act as Spemann's organizer (Cooke *et al.* 1987; J. Cooke, unpublished observations); it may thus be possible to identify the dorsalization signal through the differential screening of cDNA libraries.

A second way in which the effects of mesoderm-inducing factors might be modulated is through specific inhibitors. Direct evidence for the existence of such inhibitors comes from experiments in which XTC-MIF or bFGF is injected into the blastocoels of host embryos (Cooke *et al.* 1987; Cooke & Smith, 1989). The concentration of XTC-MIF or bFGF required to induce ectopic mesoderm under such circumstances is 20–50 times higher than the concentration required to induce mesoderm from isolated animal pole regions. Inhibitors of mesoderm induction may play a role in normal development by preventing mesoderm induction from spreading too far towards the animal pole or across the blastocoel (see Smith, 1987; Gurdon, 1989) or by acting in concert with inducing factors in a reaction–diffusion patterning system (see Meinhardt, 1982). No inhibitor of mesoderm induction has been isolated from amphibian embryos, but Born, Tiedemann & Tiedemann (1972*b*) have isolated an inhibitor of the 'vegetalizing factor' from chicken embryos. Another candidate for an inhibitor of mesoderm induction is heparin, which inhibits induction both in animal–vegetal conjugates and that mediated by exogenous bFGF (Slack *et al.* 1987). α_2-macroglobulin, which inhibits binding of TGF-β to its receptor (O'Connor-McCourt & Wakefield, 1987), does not inhibit the action of XTC-MIF, and nor does its presence in the blastocoel affect normal *Xenopus* development (J. C. Smith & J. Cooke, unpublished work).

Finally, mesoderm can also be induced from ectoderm through the phenomenon of homeogenetic induction. In its 'strong' form homeogenetic induction refers to the induction of like by like: for example, muscle-forming cells might induce muscle from adjacent ectoderm. However, as discussed below, the term can also be used to describe a form of induction in which one type of mesoderm induces another.

The existence of homeogenetic induction in amphibian development has been inferred from grafting experiments in the newt *Cynops pyrrhogaster* (Kaneda, 1981; Kaneda & Suzuki, 1983). However, the phenomenon has been demonstrated more directly by Kurihara & Sasaki (1981), who have shown that ectoderm induced to become muscle and notochord by exposure to carp swim-bladder can itself induce adjacent ectoderm to become mesenchyme and mesothelium. A more recent experiment to demonstrate homeogenetic induction in *Xenopus* takes advantage of the observation that animal pole cells require only a brief exposure to inducing factors in order to form mesoderm (Cooke *et al.* 1987; Slack *et al.* 1988). Lineage-labelled uninduced *Xenopus* animal pole ectoderm was juxtaposed with unlabelled ectoderm which had been exposed to XTC-MIF for 1 h and was then thoroughly washed. The combinations formed elongated structures surrounded by epidermis derived from both components. The unlabelled, originally induced, tissue formed large masses of muscle, occasionally with notochord. However, significant numbers of lineage-labelled, originally uninduced, cells also formed muscle, with much of the remainder differentiating as mesenchyme and mesothelium (Cooke *et al.* 1987). These results, along with those of Kurihara & Sasaki (1981), not only indicate that both 'weak' and 'strong' forms of homeogenetic induction can occur, but also show that a form of pattern formation takes place, in which responding ectoderm forms more ventral structures with increasing distance from the inducing tissue. The relevance of this observation to normal development is unclear at present, but there is indirect evidence that homeogenetic induction does occur during embryogenesis. Clonal analysis of animal pole blastomeres (Gimlich & Cooke, 1983; Dale & Slack, 1987*a*) shows that cells may form substantial amounts of mesoderm without making direct contact with vegetal pole cells; furthermore, these cells must receive their inductive signal relatively late in development, because even if they are isolated at the late blastula stage they form only epidermis (see Cooke *et al.* 1987). It seems likely that formation of mesoderm by these cells occurs due to homeogenetic induction.

Conclusions

The analysis of mesoderm induction has made great advances over the last two or three years due to the discovery of soluble mesoderm-inducing factors which have been shown to be related to peptide growth

factors. Over the next two or three years it should be possible to show which of these factors are present in the embryo, to define their location and to ablate the messages from the embryo to confirm their involvement in mesoderm induction.

The challenge will then be: how do mesoderm-inducing factors establish the correct spatial pattern of cellular differentiation in the mesoderm of *Xenopus*? I believe that the answer to this question will depend on a detailed understanding of the early embryological and cell biological aspects of mesoderm induction. Results from this laboratory indicate that MIFs merely establish the initial conditions for pattern formation in the mesoderm. There is evidence that FGF or XTC-MIF alone cannot induce mesodermal cell types: further signals produced by responding cells are also required, but the identity of these is not yet known. It may be the level of these second signals which determines which mesodermal cell types eventually form, and this decision may depend on a timing mechanism.

Cell differentiation in the mesoderm may also be influenced by an inhibitor of mesoderm induction, by a 'dorsalization' signal which converts prospective blood-forming tissue into muscle, and by homeogenetic induction. It will be a formidable problem to discover the relative importance of each of these components of the system and to explain how they interact with each other and feed back to produce the remarkably constant mesodermal pattern of *Xenopus* (Cooke & Smith, 1987).

I am very grateful to Jonathan Cooke, Jeremy Green, Geoff Howes, Jack Price and Peter Vize for their helpful comments.

References

Abraham, J. A., Mergia, A., Whang, J. L., Tumolo, A., Friedman, J., Hjerrild, K. A., Gospodarowicz, D. & Fiddes, J. C. (1986). Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. *Science* **233**, 545–548.

Banville, D. & Williams, J. G. (1985). Developmental changes in the pattern of larval β-globin mRNA sequences, *J. mol. Biol.* **184**, 611–620.

Bass, B. L. & Weintraub, H. (1987). A developmentally regulated activity that unwinds RNA duplexes. *Cell* **48**, 607–613.

Black, S. D. & Gerhart, J. C. (1985). Experimental control of the site of embryonic axis formation in *Xenopus laevis* eggs centrifuged before first cleavage. *Devl Biol.* **108**, 310–324.

Black, S. D. & Gerhart, J. C. (1986). High frequency twinning of *Xenopus* embryos from eggs centrifuged before first cleavage. *Devl Biol.* **116**, 228–240.

Born, J., Geithe, H. P., Tiedemann, H., Tiedemann, H. & Kocher-Becker, U. (1972*a*). Isolation of a vegetalizing inducing factor. *Z. Physiol. Chem.* **353**, 1075–1084.

Born, J., Tiedemann, H. & Tiedemann, H. (1972*b*). The mechanism of embryonic induction: isolation of an inhibitor for the vegetalizing factor. *Biochim. biophys. Acta* **279**, 175–183.

Boterenbrood, E. C. & Nieuwkoop, P. D. (1973). The formation of the mesoderm in urodelean amphibians. V. Its regional induction by the endoderm. *Wilhelm Roux' Arch. devl Biol.* **173**, 319–332.

Cooke, J. (1983). Evidence for specific feedback signals underlying pattern control during vertebrate embryogenesis. *J. Embryol. exp. Morph.* **76**, 95–114.

Cooke, J. (1986). Permanent distortion of positional system of *Xenopus* embryo by brief early perturbation in gravity. *Nature, Lond.* **319**, 60–63.

Cooke, J. (1987). Dynamics of the control of body pattern in the development of *Xenopus laevis*. IV. Timing and pattern in the development of twinned bodies after reorientation of eggs in gravity. *Development* **99**, 417–427.

Cooke, J. & Smith, J. C. (1987). The midblastula cell cycle transition and the character of mesoderm in u.v.-induced non-axial *Xenopus* development. *Development* **99**, 197–210.

Cooke, J. & Smith, J. C. (1989). Gastrulation and larval pattern in *Xenopus* after blastocoelic injection of a *Xenopus* inducing factor: experiments testing models for the normal organization of mesoderm, *Devl Biol.* (in press).

Cooke, J., Smith, J. C., Smith, E. J. & Yaqoob, M. (1987). The organization of mesodermal pattern in *Xenopus laevis*: experiments using a *Xenopus* mesoderm-inducing factor. *Development* **101**, 893–908.

Cooke, J. & Webber, J. A. (1985). Dynamics of the control of body pattern in the development of *Xenopus laevis*. I. Timing and pattern in the development of dorso-anterior and of posterior blastomere pairs isolated at the 4-cell stage. *J. Embryol. exp. Morph.* **88**, 85–112.

Dale, L. & Slack, J. M. W. (1987*a*). Fate map for the 32-cell stage of *Xenopus laevis*. *Development* **99**, 527–551.

Dale, L. & Slack, J. M. W. (1987*b*). Regional specification within the mesoderm of early embryos of *Xenopus laevis*. *Development* **100**, 279–295.

Dale, L., Smith, J. C. & Slack, J. M. W. (1985). Mesoderm induction in *Xenopus laevis*: a quantitative study using a cell lineage label and tissue-specific antibodies. *J. Embryol. exp. Morph.* **89**, 289–312.

Deparis, P. & Jaylet, A. (1984). The role of endoderm in blood cell ontogeny in the newt *Pleurodeles waltl*. *J. Embryol. exp. Morph.* **81**, 37–47.

Driever, W. & Nüsslein-Volhard, C. (1988). A gradient of *bicoid* protein in Drosophila embryos. *Cell* **54**, 83–93.

Flickenger, R. A. (1980). The effect of heparin upon differentiation of ventral halves of frog gastrulae. *Wilhelm Roux' Arch. devl Biol.* **188**, 9–11.

Gentry, L. E., Webb, N. R., Lim, G. J., Brunner, A. M., Ranchalis, J. E., Twardzik, D. R., Lioubin, M. N., Marquardt, H. & Purchio, A. F. (1987). Type 1 transforming growth factor beta: amplified expression and secretion of mature and precursor polypeptides in Chinese Hamster Ovary cells. *Mol. cell. Biol.* **7**, 3418–3427.

Gimlich, R. L. & Cooke, J. (1983). Cell lineage and the induction of second nervous systems in amphibian development. *Nature, Lond.* **306**, 471–473.

Gimlich, R. L. & Gerhart, J. C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. *Devl Biol.* **104**, 117–130.

Godsave, S. F., Isaacs, H. V. & Slack, J. M. W. (1988). Mesoderm inducing factors: a small class of molecules. *Development* **102**, 555–566.

Gurdon, J. B. (1987). Embryonic induction – molecular prospects. *Development* **99**, 285–306.

Gurdon, J. B. (1988). Cell movements and a community effect in tissue morphogenesis. *Nature, Lond.* **336**, 772–774.

Gurdon, J. B. (1989). The localization of an inductive response. *Development* **105**, 27–33.

Gurdon, J. B., Fairman, S., Mohun, T. J. & Brennan, S. (1985). The activation of muscle-specific actin genes in *Xenopus* development by an induction between animal and vegetal cells of a blastula. *Cell* **41**, 913–922.

Gurdon, J. B., Mohun, T. J., Sharpe, C. R. & Taylor, M. V. (1989). Embryonic induction and muscle gene activation. *Trends in Genetics*, (in press).

Heasman, J., Wylie, C. C., Hausen, P. & Smith, J. C. (1984). Fates and states of determination of single vegetal pole blastomeres of X. laevis. *Cell* **37**, 185–194.

Holwill, S., Heasman, J., Crawley, C. R. & Wylie, C. C. (1987). Axis and germ line deficiencies caused by u.v. irradiation of *Xenopus* oocytes cultured *in vitro*. *Development* **100**, 735–743.

Hörstadius, S. (1935). Über die Determination im Verlaufe der Eiachse bei Seeigeln. *Publ. Staz. Zool. Napoli* **14**, 132–179.
Jacobson, M. (1984). Cell lineage analysis of neural induction: origins of cells forming the induced nervous system. *Devl Biol.* **102**, 122–129.
Jacobson, M. & Hirose, G. (1978). Origin of the retina from both sides of the embryonic brain: a contribution to the problem of crossing at the optic chiasma. *Science* **202**, 637–639.
Jacobson, M. & Hirose, G. (1981). Clonal organization of the central nervous system of the frog. II. clones stemming from individual blastomeres of the 32- and 64-cell stages. *J. Neurosci.* **1**, 271–284.
Jaye, M., Lyall, R. M., Mudd, R., Schlessinger, J. & Sarver, N. (1988). Expression of acidic fibroblast growth factor cDNA confers growth advantage and tumorigenesis to Swiss 3T3 cells. *EMBO J.* **7**, 963–969.
Jones, E. A. & Woodland, H. R. (1986). Development of the ectoderm in Xenopus laevis: the definition of a monoclonal antibody to an epidermal marker. *Cell* **44**, 345–355.
Jones, E. A. & Woodland, H. R. (1987). The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. *Development* **101**, 557–563.
Kaneda, T. (1981). Studies on the formation and state of determination of the trunk organizer in the newt *Cynops pyrrhogoster*. III. Tangential induction in the dorsal marginal zone. *Dev. Growth Diff.* **23**, 553–564.
Kaneda, T. & Suzuki, A. S. (1983). Studies on the formation and state of determination of the trunk organizer in the newt *Cynops pyrrhogaster*. IV. The association of the neural-inducing activity with the mesodermization of the trunk organizer. *Wilhelm Roux' Arch. devl Biol.* **192**, 8–12.
Kawakami, I. (1976). Fish swimbladder: an excellent mesodermal inductor in primary embryonic induction. *J. Embryol. exp. Morph.* **36**, 315–320.
Kawakami, I., Noda, S., Kurihara, K. & Okuma, K. (1977). Vegetalizing factor extracted from the fish swimbladder and tested on presumptive ectoderm of *Triturus* embryos. *Wilhelm Roux' Arch. devl Biol.* **182**, 1–7.
Keating, M. T. & Williams, L. T. (1988). Autocrine stimulation of intracellular PDGF receptors in v-*sis*-transformed cells. *Science* **239**, 914–916.
Keller, R. E. (1976). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movements of the deep layer. *Devl Biol.* **51**, 118–137.
Keller, R. E. (1986). The Cellular Basis of Amphibian Gastrulation. In *Developmental Biology: A Comprehensive Synthesis*, vol. 2, *The Cellular Basis of Morphogenesis* (ed. L. Browder), pp. 241–327. New York: Plenum Press.
Keller, R. E., Danilchik, M., Gimlich, R. & Shih, J. (1985). The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. exp. Morph* **89 Supplement**, 185–209.
Keller, R. E. & Schoenwolf, G. C. (1977). An SEM study of cellular morphology, contact, and arrangement, as related to gastrulation in *Xenopus laevis*. *Wilhelm Roux's Arch. devl Biol.* **182**, 165–186.
Kimelman, D., Abraham, J. A., Haaparanta, T., Palisi, T. M. & Kirschner, M. (1988). The presence of FGF in the frog egg: its role as a natural mesoderm inducer. *Science* **242**, 1053–1056.
Kimelman, D. & Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGFβ and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell* **51**, 369–377.
Klymkowsky, M. W., Maynell, L. A. & Polson, A. G. (1987). Polar asymmetry in the organization of the cortical cytokeratin system of *Xenopus laevis* oocytes and embryos. *Development* **100**, 543–557.
Kurihara, K. & Sasaki, N. (1981). Transmission of homoiogenetic induction in presumptive ectoderm of newt embryo. *Dev. Growth Diff.* **23**, 361–369.
Leal, F., Williams, L. T., Robbins, K. C. & Aaronson, S. A. (1985). Evidence that the v-*sis* gene product transforms by interaction with the receptor for platelet-derived growth factor. *Science* **230**, 327–330.
Lewis, J. H. & Wolpert, L. (1976). The principle of non-equivalence in development. *J. theor. Biol.* **62**, 479–490.
Massagué, J. (1987). The TGF-β family of growth and differentiation factors. *Cell* **49**, 437–438.
Meinhardt, H. (1982). *Models of Biological Pattern Formation.* London: Academic Press.
Melton, D. A. (1987). Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. *Nature, Lond.* **328**, 80–82.
Minuth, M. & Grunz, H. (1980). The formation of mesodermal derivatives after induction with vegetalizing factor depends upon secondary cell interactions. *Cell Diff.* **9**, 229–238.
Moody, S. A. (1987*a*). Fates of the blastomeres of the 16-cell-stage *Xenopus* embryo. *Devl Biol.* **119**, 560–578.
Moody, S. A. (1987*b*). Fates of the blastomeres of the 32-cell-stage *Xenopus* embryo. *Devl Biol.* **122**, 300–319.
Nakamura, O. & Matsuzawa, T. (1967). Differentiation capacity of the marginal zone in the morula and blastula of *Triturus pyrrhogaster* *Embryologia* **9**, 223–237.
Nakamura, O., Takasaki, H. & Ishihara, M. (1970*a*). Formation of the organizer from combinations of presumptive ectoderm and endoderm. I. *Proc. Japan Acad.* **47**, 313–318.
Nakamura, O., Takasaki, H. & Mizohata, T. (1970*b*). Differentiation during cleavage in *Xenopus laevis*. I. Acquisition of self-differentiation capacity of the dorsal marginal zone. *Proc. Japan Acad.* **46**, 694–699.
Newport, J. & Kirschner, M. (1982). A major developmental transition in early *Xenopus* embryos: I. Characterization and timing of cellular changes at the midblastula stage. *Cell* **30**, 675–686.
Nieuwkoop, P. D. (1969). The formation of mesoderm in Urodelean amphibians. I. Induction by the endoderm. *Wilhelm Roux' Arch. EntwMech. Org.* **162**, 341–373.
Nieuwkoop, P. D. & Faber, J. (eds) (1967). *Normal Table of* Xenopus laevis *(Daudin)*, 2nd ed. Amsterdam: North-Holland.
Nieuwkoop, P. D., Johnen, A. G. & Albers, B. (1985). *The Epigenetic Nature of Early Chordate Development. Inductive Interaction and Competence.* Cambridge: Cambridge University Press.
Nieuwkoop, P. D. & Ubbels, G. A. (1972). The formation of mesoderm in Urodelean amphibians. IV. Quantitative evidence for the purely "ectodermal" origin of the entire mesoderm and of the pharyngeal endoderm. *Wilhelm Roux' Arch. EntwMech. Org.* **169**, 185–199.
O'Connor-McCourt, M. D. & Wakefield, L. M. (1987). Latent transforming growth factor-β in serum. A specific complex with α_2-macroglobulin. *J. biol. Chem.* **262**, 14090–14099.
Ogi, K.-I. (1967). Determination in the development of the amphibian embryo. *Sci. Rep. Tohoku Univ. Ser. IV (Biol.)* **33**, 239–247.
Ogi, K.-I. (1969). Regulative capacity in the early amphibian embryo. *Res. Bull. Dept. Gen. Ed. Nagoya University* **13**, 31–40.
Padgett, R. W., St Johnston, R. D. & Gelbart, W. M. (1987). A transcript from a *Drosophila* pattern gene predicts a protein homologous to the transforming growth factor-β family. *Nature, Lond.* **325**, 81–84.
Pondel, M. D. & King, M. L. (1988). Localized maternal mRNA related to transforming growth factor β mRNA is concentrated in a cytokeratin-enriched fraction from *Xenopus* oocytes. *Proc. natn. Acad. Sci. U.S.A.* **85**, 7612–7616.
Rebagliati, M. R. & Melton, D. A. (1987). Antisense RNA injections in fertilized frog eggs reveal an RNA duplex unwinding activity. *Cell* **48**, 599–605.
Rebagliati, M. R., Weeks, D. L., Harvey, R. P. & Melton, D. A. (1985). Identification and cloning of localized maternal RNAs from *Xenopus* eggs. *Cell* **42**, 769–777.
Rogelj, S., Weinberg, R. A., Fanning, P. & Klagsbrun, M. (1988). Basic fibroblast growth factor fused to a signal peptide transforms cells. *Nature, Lond.* **331**, 173–175.
Rosa, F., Roberts, A. B., Danielpour, D., Dart, L. L., Sporn, M. B. & Dawid, I. B. (1988). Mesoderm induction in amphibians: the role of TGF-β2-like factors. *Science* **239**, 783–785.
Sargent, T. D., Jamrich, M. & Dawid, I. B. (1986). Cell

interactions and the control of gene activity during early development of *Xenopus laevis*. *Devl Biol.* **114**, 238–246.
SAXÉN, L. & TOIVONEN, S. (1958). The dependence of the embryonic induction action of HeLa cells on their growth media. *J. Embryol. exp. Morph.* **6**, 616–633.
SHUTTLEWORTH, J. & COLMAN, A. (1988). Antisense oligonucleotide-directed cleavage of mRNA in *Xenopus* oocytes and eggs. *EMBO J.* **7**, 427–434.
SLACK, J. M. W. (1983). *From Egg to Embryo. Determinative Events in Early Development.* Cambridge: Cambridge University Press.
SLACK, J. M. W., DALE, L. & SMITH, J. C. (1984). Analysis of embryonic induction by using cell lineage markers. *Phil. Trans. R. Soc. Lond. B* **307**, 331–336.
SLACK, J. M. W., DARLINGTON, B. G., HEATH, J. K. & GODSAVE, S. F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature, Lond.* **326**, 197–200.
SLACK, J. M. W. & FORMAN, D. (1980). An interaction between dorsal and ventral regions of the marginal zone in early amphibian embryos. *J. Embryol exp. Morph.* **56**, 283–299.
SLACK, J. M. W. & ISAACS, H. (1989). Presence of basic fibroblast growth factor in the early *Xenopus* embryo. *Development* **105**, 147–153.
SLACK, J. M. W., ISAACS, H. V. & DARLINGTON, B. G. (1988). Inductive effects of fibroblast growth factor and lithium ion on *Xenopus* blastula ectoderm. *Development* **103**, 581–590.
SMITH, J. C. (1987). A mesoderm-inducing factor is produced by a *Xenopus* cell line. *Development* **99**, 3–14.
SMITH, J. C. (1988). Cellular interactions in establishment of regional patterns of cell fate during development. In *Developmental Biology: A Comprehensive Synthesis*, vol. 5 (ed. L. Browder) pp. 79–125. New York: Plenum Press.
SMITH, J. C., DALE, L. & SLACK, J. M. W. (1985). Cell lineage labels and region-specific markers in the analysis of inductive interactions. *J. Embryol. exp. Morph.* **89 Supplement**, 317–331.
SMITH, J. C. & SLACK, J. M. W. (1983). Dorsalization and neural induction: properties of the organizer in *Xenopus laevis*. *J. Embryol. exp. Morph.* **78**, 299–317.
SMITH, J. C., TICKLE, C. & WOLPERT, L. (1978). Attenuation of positional signalling in the chick limb by high doses of γ-radiation. *Nature, Lond.* **272**, 612–613.
SMITH, J. C., YAQOOB, M. & SYMES, K. (1988). Purification, partial characterization and biological effects of the XTC mesoderm-inducing factor. *Development* **103**, 591–600.
SPEMANN, H. & MANGOLD, H. (1924). Uber Induktion von Embryonenanlagen durch Implantation artfremder Organisatoren. *Wilhelm Roux' Arch. EntwMech. Org.* **100**, 599–638.
SUDARWATI, S. & NIEUWKOOP, P. D. (1971). Mesoderm formation in the Anuran *Xenopus laevis* (Daudin). *Wilhelm Roux' Arch. EntwMech. Org.* **166**, 189–204.
SYMES, K. & SMITH, J. C. (1987). Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis*. *Development* **101**, 339–349.
SYMES, K., YAQOOB, M. & SMITH, J. C. (1988). Mesoderm induction in *Xenopus laevis*: responding cells must be in contact for mesoderm formation but suppression of epidermal differentiation can occur in single cells. *Development* **104**, 609–618.
THALLER, C. & EICHELE, G. (1987). Identification and spatial distribution of retinoids in the developing chick limb bud. *Nature, Lond.* **327**, 625–628.
TICKLE, C., ALBERTS, B. M., WOLPERT, L. & LEE, J. (1982). Local application of retinoic acid to the limb bud mimics the action of the polarizing region. *Nature, Lond.* **296**, 564–565.
TICKLE, C., SUMMERBELL, D. & WOLPERT, L. (1975). Positional signalling and specification of digits in chick limb morphogenesis. *Nature, Lond.* **254**, 199–202.
TOIVONEN, S. (1953). Bone-marrow of the guinea-pig as a mesoderm inductor in implantation experiments with embryos of *Triturus*. *J. Embryol. exp. Morph.* **1**, 97–104.
VINCENT, J.-P., OSTER, G. & GERHART, J. C. (1986). Kinematics of gray crescent formation in amphibian eggs: the displacement of sub-cortical cytoplasm relative to the egg surface. *Devl Biol.* **113**, 484–500.
WEEKS, D. L. & MELTON, D. A. (1987). A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGFβ. *Cell* **51**, 861–867.
WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1–47.
WOODLAND, H. R. & JONES, E. A. (1987). The development of an assay to detect mRNAs that affect early development. *Development* **101**, 925–930.
YISRAELI, J. K. & MELTON, D. A. (1988). The maternal mRNA Vg1 is correctly localized following injection into *Xenopus* oocytes. *Nature, Lond.* **336**, 592–595.

(*Accepted 4 January 1989*)