Mesoderm induction and origins of the embryonic axis

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Mesoderm-inducing factors and the control of gastrulation

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Summary

One of the reasons that we know so little about the control of vertebrate gastrulation is that there are very few systems available in which the process can be studied in vitro. In this paper, we suggest that one suitable system might be provided by the use of mesoderm-inducing factors. In amphibian embryos such as *Xenopus laevis*, gastrulation is driven by cells of the mesoderm, and the mesoderm itself arises through an inductive interaction in which cells of the vegetal hemisphere of the embryo emit a signal which acts on overlying equatorial cells. Several factors have recently been discovered that modify the pattern of mesodermal differentiation or induce mesoderm from presumptive ectoderm. Some of these mesoderm-inducing factors will also elicit gastrulation movements, which provides a powerful model system for the study of gastrulation, because a population of cells that would not normally undertake the process can be induced to do so. In this paper, we use mesoderm-inducing factors to attempt to answer four questions. How do cells know when to gastrulate? How do cells know what kind of gastrulation movement to undertake? What is the cellular basis of gastrulation? What is the molecular basis of gastrulation?

Key words: *Xenopus*, gastrulation, mesoderm induction, activin, BMP-4, FGF, Wnt, integrins.

Introduction

While descriptions of cell behaviour during vertebrate gastrulation have become exquisitely detailed and accurate (see chapters by Bortier, Ho, Keller, Lawson and Stern), an understanding of the process at the levels of cell and molecular biology is lacking. In this article, we ask to what extent this problem might be approached by the use of mesoderm-inducing factors. In amphibian embryos such as *Xenopus laevis*, gastrulation is driven by cells of the mesoderm, and the mesoderm itself arises through an inductive interaction in which cells of the vegetal hemisphere of the embryo emit a signal that acts on overlying equatorial cells (Fig. 1; see review by Smith, 1989). In the last five years, several factors that induce mesoderm from presumptive ectoderm, or which modify the pattern of mesodermal differentiation, have been discovered. These include members of the TGFb superfamily such as activins A and B and bone morphogenetic protein 4 (BMP-4), members of the fibroblast growth factor family such as bFGF or eFGF, and members of the Wnt family such as Xwnt-8 (see reviews by Smith, 1989, 1992; Whitman and Melton, 1989; Dawid and Sargent, 1990; and papers by Smith and Harland, 1991; Sokol et al., 1991; Chakrabarti et al., 1992; Köster et al., 1991; Dale et al., 1992; Jones et al., 1992).

The types of mesoderm induced by each factor, and their effects in combination with each other, differ. Thus activin induces a range of mesodermal cell types, from organizer to tail, according to its concentration (Green et al., 1990; Green and Smith, 1990); BMP-4 induces posterior/ventral mesoderm (Köster et al., 1991; Dale et al., 1992; Jones et al., 1992) and is capable of 'ventralizing' the response to activin (Dale et al., 1992; Jones et al., 1992); FGF also induces posterior/ventral mesoderm but has little ventralizing activity when applied in combination with activin (Kimelman and Kirschner, 1987; Slack et al., 1987; Green et al., 1990; Cooke, 1989). Members of the Wnt family do not induce mesoderm from presumptive ectoderm, but animal caps derived from embryos that have received injections of Xwnt-8 RNA respond to bFGF by forming dorsal, rather than ventral, mesoderm (Christian et al., 1992). Injection of Wnt RNA is also capable of inducing complete secondary axes in *Xenopus* embryos and of rescuing embryos that have been made completely ventral by ultra-violet light irradiation of their vegetal hemispheres before first cleavage (Smith and Harland, 1991; Sokol et al., 1991).

Previously, we have shown that mesoderm-inducing factors cause ectodermal tissue to undergo gastrulation movements (Symes and Smith 1987; Cooke and Smith, 1989; Smith et al., 1990). In this paper, we take advantage of this observation to attempt to answer four questions. How do cells know when to gastrulate? How do cells know what kind of gastrulation movement to undertake? What is the cellular basis of gastrulation? What is the molecular basis of gastrulation? We begin with a brief description of normal gastrulation, based on the work of Keller (1991; and this volume), which highlights the processes that we seek to understand.

Xenopus gastrulation

Gastrulation in *Xenopus* converts the radially symmetrical

blastula into a three-layered structure with anteroposterior and dorsoventral axes. The first external sign of gastrulation is visible about 10 hours after fertilization, when the blastoporal pigment line forms on the dorsal side of the embryo. The pigment line arises because bottle cells in this region undergo apical constriction and, as this happens, they displace the deep mesoderm associated with them inwards and upwards, thus initiating involution. Although bottle cell formation and involution begin on the dorsal side of the embryo, the movements then spread laterally and ventrally, reaching the ventral side of the embryo about 2 hours later.

Once involution has begun, gastrulating cells exhibit three distinct types of behaviour. The first mesoderm to involute undergoes *cell migration*, using the fibrillar matrix on the inner surface of the blastocoel roof as a substratum. On the dorsal side of the embryo, these cells eventually contribute to the head mesoderm (see Winklbauer and Selchow, 1992). Cell migration, however, contributes little to the total force required for gastrulation; if the blastocoel roof, the substratum for migration, is removed, gastrulation proceeds relatively normally (Keller et al., 1985). The main driving force for gastrulation derives from later-involuting cells which, on the dorsal side of the embryo, go on to form notochord and somite. These cells undergo 'convergent extension', during which the marginal zone constricts to form a smaller ring (*convergence*) and lengthens along the anteroposterior axis (*extension*). The greatest degree of convergent extension occurs in this dorsalmost section of the marginal zone; lateral and ventral regions converge to similar extents, but the degree of extension decreases in progressively more ventral regions, such that cells in the ventral midline can be said only to converge (Keller and Danilchik, 1988).

Convergence and extension are driven by cell intercalation (see Keller, 1991). Convergence occurs predominantly through radial intercalation, while extension is the result of both radial and mediolateral intercalation. These processes can be studied in isolated explants of the marginal zone region, where, in contrast to the ventral marginal zone, the dorsal marginal zone undergoes dramatic elongation. A detailed description of cell intercalation during convergent extension is given in the chapter by Keller.

In what follows, we discuss to what extent the use of mesoderm-inducing factors might bring about an understanding of these different aspects of gastrulation.

The timing of gastrulation

The timing of gastrulation in normal *Xenopus* development is remarkably precise; members of a batch of embryos fertilized at the same time will form a dorsal blastopore lip, after about 10 hours of development, within 20 minutes of each other (see review by Cooke and Smith, 1990). The nature of the timer responsible for this synchrony is unknown. One obvious suggestion, that it is timed by reference to the mid-blastula transition (MBT), is not correct, because if the timing of the MBT is changed, in axolotl, newt, sturgeon or *Drosophila* embryos, by changing the nuclear to cytoplasmic ratio, gastrulation still starts at the normal time (see Cooke and Smith, 1990; Yasuda and Schubiger, 1992). Other potential timers have also been ruled out; it is not, for example, simply a question of counting numbers of cell cycles, because gastrulation will proceed on time if cell division is inhibited at the late blastula stage (Cooke, 1973). Furthermore, the 'pseudogastrulation' movements displayed by some unfertilized amphibian eggs begin at the same time, and take the same time, as the normal movements in their fertilized siblings. The 'timer' that controls these movements must be cytoplasmic and independent of transcription, because they will occur in enucleated oocytes (see review by Satoh, 1982), and this is consistent with results in *Caenorhabditis elegans*, which show that gastrulation movements start at the normal time in the presence of α-amanitin (results of L. Edgar, cited by Yasuda and Schubiger, 1992). Unfortunately, in spite of all this circumstantial, albeit interesting, evidence, there is no idea as to the molecular basis of the gastrulation clock.

Is it important that gastrulation is so accurately timed a process? In normal development, gastrulation begins at dorsal lip of the blastopore and movements spread around to the ventral side. Surprisingly, this aspect of the timing of gastrulation does not seem to be essential. If the dorsoventral timing of gastrulation is reversed, by applying a temperature gradient across the embryo, development is normal and resulting embryos are indistinguishable from controls (Black, 1989). Under conditions where the metabolic rate is uniform throughout the embryo, however, there is evidence that the proper timing of gastrulation *is* important in setting up the correct spatial pattern of gene expression and differentiation.

Ultraviolet irradiation of the vegetal hemisphere of the fertilized *Xenopus* egg results in embryos that lack anterior and dorsal structures (see chapter by Gerhart, this volume, and Cooke and Smith, 1987). Such embryos begin gastrulation movements later than controls, with extreme 'ventralized' cases beginning gastrulation at the time of appearance of the ventral blastopore lip in synchronous normal embryos. By contrast, embryos treated with LiCl at the 32-cell stage develop as extreme anterior-dorsal body patterns. Gastrulation movements in these embryos begin on time, but the blastopore lip appears synchronously around the entire marginal zone, rather than spreading from the dorsal to the ventral side. It is difficult to answer the question of whether these abnormal temporal patterns of gastrulation are an early manifestation of the ventralized and dorsalized phenotypes of the embryos or whether they are the cause of them. One experiment, however, is consistent with the suggestion that the total time that cells spend gastrulating influences their anteroposterior pattern of differentiation. Treatment of gastrulating embryos with polysulphonated compounds such as trypan blue or sodium suramin arrest convergent extension movements, and the resulting tadpoles have body axes that are truncated at different anteroposterior levels depending on the time of application of the compound; the earlier the treatment, the more posterior the body axis that develops, whereas if treatment is delayed until the end of gastrulation, the embryos that develop are normal (Gerhart et al., 1989). These results are consistent with the suggestion that the shorter the time that cells spend gastrulating, whether because they start late or because their

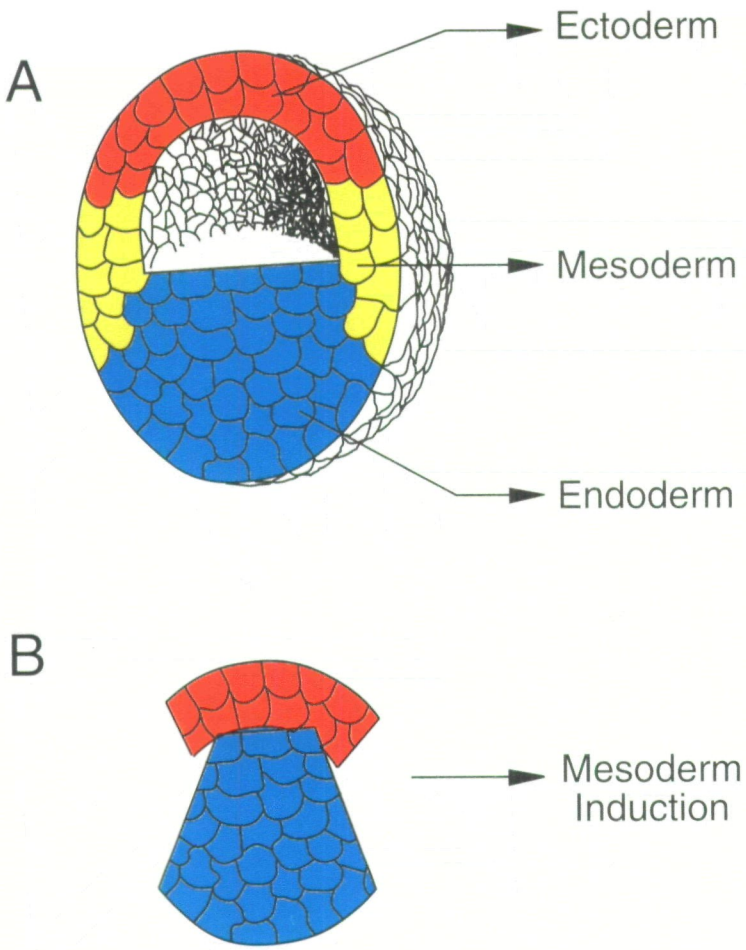

Fig. 1. Mesoderm induction occurs when a signal from cells of the vegetal hemisphere act on overlying equatorial cells. (A) In normal development ectoderm derives from cells of the animal cap (red), mesoderm from the equatorial region (yellow), and endoderm from the vegetal pole (blue). (B) Mesoderm induction can be demonstrated by apposing presumptive ectoderm with presumptive endoderm; some of the ectodermal cells are induced to form mesodermal cell types such as muscle and notochord.

movements are interrupted by sodium suramin, the more ventral the cell types they form.

Mesoderm-inducing factors and the timing of gastrulation

What can mesoderm-inducing factors tell us about the timing of gastrulation? Treatment of animal pole regions with activin A causes the tissue to undergo a dramatic elongation, very similar to the elongation of isolated dorsal marginal zone regions (Symes and Smith, 1987; see Keller et al., 1985 for the behaviour of dorsal marginal zones). Additional evidence that the elongation represents gastrulation movements comes from the observation that the rates of elongation of the induced animal cap explants and the dorsal marginal zone regions are similar and, most significantly, that they begin at the same time. The time of onset of the gastrulation-like movements in induced animal caps is independent of the stage at which the cells are exposed to activin (Symes and Smith, 1987), and this rules out another suggestion for the timing of gastrulation, that it occurs a certain time after mesoderm induction.

It is harder to judge the time of onset of gastrulation-like movements in response to FGF, because, consistent with the suggestion that FGF induces ventral-type mesoderm (Green et al., 1990), the gastrulation movements seen in response to this factor are less dramatic. This makes it more difficult to decide when they begin. An alternative approach to this problem was introduced by Cooke and Smith (1989), who microinjected mesoderm-inducing factors into the blastocoels of host embryos. This causes the cells of the roof of the blastocoel to undergo a transformation in behaviour that resembles the transformation seen in the mesoderm of the marginal zone. By fixing and dissecting embryos at different times after injection, it is relatively simple to judge the time of onset of gastrulation-like movements. When activin was injected into the blastocoel, irrespective of the time of injection (over a 3.5 hour range), or the concentration of factor (over a 250-fold range), this transformation began at the same time, shortly after the onset of gastrulation at the dorsal side of the marginal zone. This result confirms that of Symes and Smith (1987), but also indicates that concentration of inducing factor is also irrelevant to the timing of gastrulation. When FGF is injected into the blastocoel, the time of the transition in cell behaviour is also independent of the time of injection and of the concentration of factor. However, this time is approximately 1.5 hours later than that seen in response to activin, and corresponds more to the time of appearance of the ventral lip of the blastopore (Cooke and Smith, 1989). It is possible, therefore, that at least one aspect of the control of the timing of gastrulation is influenced by the nature of the factor(s) that induce the mesoderm, the tissue that drives gastrulation.

What do these experiments with mesoderm-inducing factors tell us about the timing of gastrulation? Firstly, they shed no light on the nature of the cytoplasmic clock, save to indicate that the clock is present and running even in cells of the animal pole of the embryo, which would not normally need to refer to it. They do, however, indicate that gastrulation is not timed by reference to the time of mesoderm induction nor to the concentration of mesoderm-inducing factor. This latter point is of interest because different concentrations of activin *do* induce different cell types (Green et al., 1990; Green and Smith, 1990) with higher concentrations inducing progressively more dorsoanterior tissue types. If, therefore, the duration of gastrulation does determine anteroposterior position in the embryo, one prediction would be that gastrulation movements in response to low concentrations of activin stop before those seen in response to higher concentrations. This has not been investigated directly, although lower concentrations of activin do cause less elongation than higher ones (unpublished observations). It is not known, however, whether this is due to a slower rate of elongation or to an earlier cessation. Overall, unless clues come from other organisms such as *Drosophila*, it seems likely that the best route to understanding the nature of the gastrulation 'clock' will be to work backwards from the molecular changes that drive the process. These might include, for example, changes in expression of cell adhesion molecules (see below).

Specification of cellular behaviour during gastrulation

At least three distinct types of cellular behaviour drive gastrulation: cell migration, convergence and extension. Different types of behaviour are followed by cells in different regions of the embryo at different times. How do cells 'know' which type of behaviour they should undertake? We have studied the responses made by animal pole tissue to different mesoderm-inducing factors, alone and in combination, to discover how, in principle, cell behaviour might be specified. We compare the gastrulation-like responses made by the cells with the types of tissue induced by each factor or factors.

Convergent extension

Our experiments have not yet allowed us to study convergence and extension separately, so here we refer only to convergent extension. The cells that undertake the most vigorous convergent extension are those of the dorsal marginal zone, which go on to form head mesoderm and notochord, and which express genes such as *goosecoid* (Cho et al., 1991). Notochord, and *goosecoid* expression, are induced from animal pole tissue by activin (Green et., 1990; Cho et al., 1991) and, similarly, activin induces dramatic convergent-extension-like movements from this tissue (Symes and Smith, 1987). As discussed above, these movements also resemble those of convergent extension in that they begin at about the same time that convergent extension begins in the normal embryo. By contrast, FGF does not induce dramatic elongation from animal pole regions, although it does elicit a more subtle change in shape (Slack et al., 1987), which might be interpreted as being due to convergence in the absence of extension. This is, of course, consistent with the proposed role of FGF as an inducer of ventral/posterior mesoderm (Green et al., 1990; Amaya et al., 1991).

One way in which the extent, if not the nature, of these movements might be modified in different regions of the embryo is through different concentrations of inducing factor; lower concentrations of activin, for example, cause

less elongation than higher ones (see above). Another possibility is that additional factors modify the putative activin and FGF signals that operate in the embryo. Recently, two such additional signals have been discovered. mRNA for the *Xenopus* homologue of bone morphogenetic protein 4 (XBMP-4) is expressed at low levels maternally, and the gene is then strongly activated throughout the embryo at the mid-blastula transition (Köster et al., 1991; Dale et al., 1992). Injection of mRNA for XBMP-4 into the fertilized egg of *Xenopus* causes the resulting embryo to become severely 'ventralized', a phenotype that may be due to the abilities of XBMP-4 to induce strong expression of *Xhox3* (see Ruiz i Altaba and Melton, 1989), and to override the effect of activin, causing animal caps incubated even in high concentrations of activin to differentiate as ventral cell types (Dale et al., 1992; Jones et al., 1992). This ventralization of the response to activin is also observed at earlier stages, during gastrulation, where the activin-induced elongation of animal pole regions is strongly inhibited by XBMP-4 (Fig. 2). This effect may be important in regulating the extent and duration of convergent extension, but without detailed knowledge of the spatial and temporal distribution of XBMP-4 protein, it is not possible to speculate further.

Another factor that can influence convergent extension is Xwnt-8, a member of the Wnt family of growth factors. Injection of mRNA encoding int-1, Xwnt-8 or wingless protein into the ventral side of *Xenopus* embryos results in duplication of the embryonic axis, while injection into the dorsal side has little effect (McMahon and Moon, 1989; Smith and Harland, 1991; Sokol et al., 1991; Christian et al., 1991; Chakrabarti et al., 1992; reviewed by Smith, 1992). In this respect, the effect of Xwnt-8 is the opposite of that of XBMP-4, in that it 'dorsalizes' ventral mesoderm; consistent with this, if animal caps derived from embryos that have received injections of Xwnt-8 RNA are treated with FGF, they undergo dramatic elongation, as if they had been treated with activin (Christian et al., 1991). Again, the significance of this observation in the absence of knowledge about the spatial distribution of Xwnt-8 protein is not clear, but it does define another route by which gastrulation might be regulated. Finally, as the transcriptional responses to different inducing factors are elucidated, it may become possible to manipulate gastrulation movements by

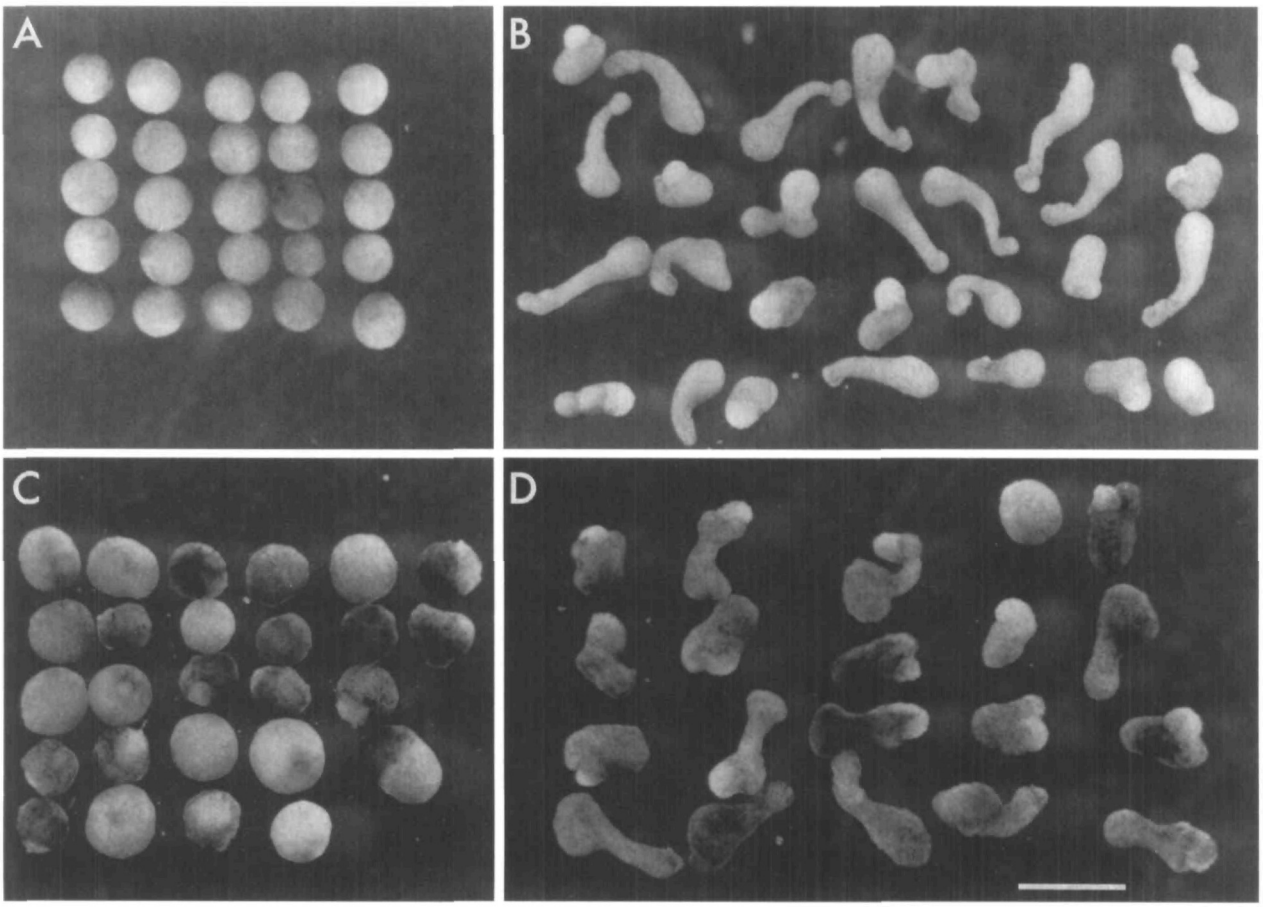

Fig. 2. BMP-4 inhibits elongation in activin-treated animal cap explants. Animal poles were excised at stage 8 and cultured in the presence (B, C, D) or absence (A) of activin until stage 18. In embryos that had been injected with 1. 5 ng of XBMP-4 RNA at the one cell stage, activin-induced elongation was severely inhibited (C), but was unaffected by the injection of antisense RNA (D). These experiments were carried out in collaboration with Dr Leslie Dale (Birmingham University, UK; see Dale et al., 1992) with whose kind permission this figure is included.

overexpression of intracellular responses to induction rather than through the extracellular signals (see below).

Cell spreading and migration

The other type of cell behaviour observed during gastrulation is cell migration. In the intact *Xenopus* embryo, this is most easily studied in the earliest-involuting cells, which go on to form head mesoderm. These cells do not undergo convergent extension and may be important in ensuring that exogastrulation does not occur (see above). In vitro, cell spreading may be studied by dissociating prospective mesodermal cells and seeding them onto fibronectin (FN)-coated tissue-culture plates. Mesodermal cells from all regions of the marginal zone spread and migrate on this substratum, as will prospective endodermal cells, but cells derived from the animal pole region do not adhere, and remain as loosely attached spheres (Nakatsuji, 1986; Winklbauer, 1988). Prospective ectodermal cells can, however, be induced to spread and migrate on fibronectin if they are treated with activin (Smith et al., 1990; see Fig. 3). This is a rapid response, and one that can be induced in single cells; it does not, therefore, require a 'community effect' (Gurdon, 1988).

Recently, we have gone on to investigate whether other mesoderm-inducing factors also cause animal pole cells to spread and migrate on fibronectin. Our first experiments used bFGF, in response to which factor animal pole cells attach strongly to the FN substratum, but do not spread or migrate. This was slightly surprising, because fibroblast growth factor has been suggested to induce mesoderm of ventral character (Slack et al., 1987; Green et al., 1990), and cells derived from the ventral marginal zone of the early gastrula spread on FN in a manner very similar to that of cells from the dorsal marginal zone (J. E. H., unpublished observations). It remains possible that other factors induce cell spreading in the ventral marginal zone, and we are now investigating this by treating cells simultaneously with FGF and activin, and by observing the behaviour of cells derived from animal pole regions that have received injections of *Xwnt-8* or XBMP-4 mRNA and are cultured in the presence, or absence, of activin and FGF. Preliminary results indicate that FGF inhibits activin-induced spreading on FN, and it is possible that this provides a way of regulating cell migration during gastrulation.

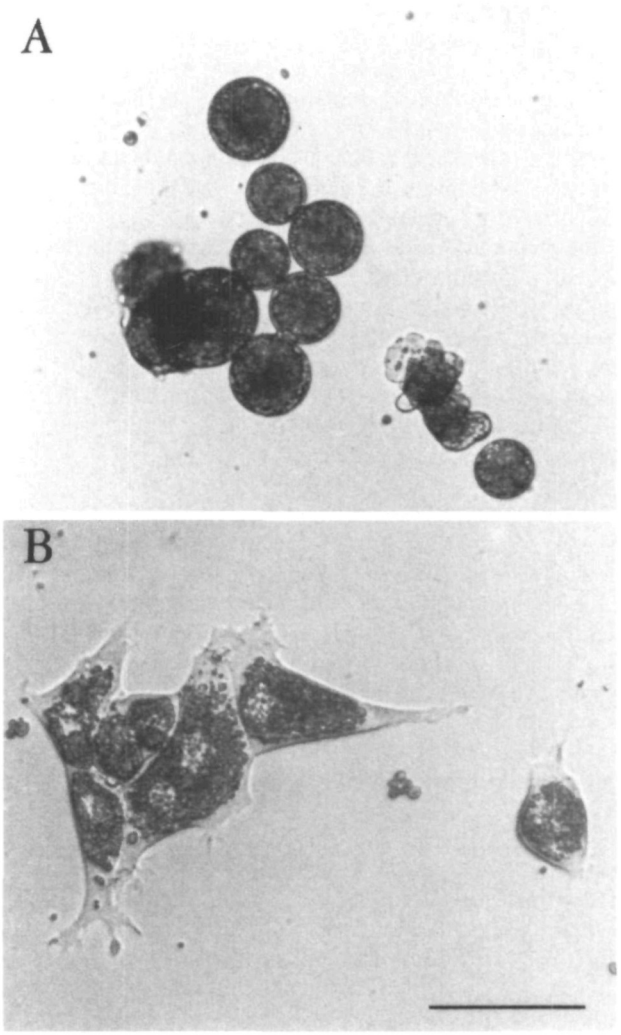

Fig. 3. Mesoderm induction causes cells to spread and migrate on fibronectin. Uninduced animal cap cells do not spread on a fibronectin-coated substratum (A), whereas those treated with activin do (B). Scale bar is 25 μm.

The cellular basis of gastrulation

Accurate and detailed descriptions of cell behaviour during gastrulation have been made by Keller and colleagues (see chapter by Keller in this volume), and it might appear that studying the gastrulation-like movements seen in response to mesoderm-inducing factors would have little to add to these. As with the other aspects of gastrulation discussed in this paper, however, the advantage of studying gastrulation in response to defined factors is that the situation is simplified: a narrower range of cell behaviours will occur, and the existence of one type of behaviour will not confuse analysis of another.

Convergent extension

Keller has suggested that the dramatic extension produced by convergent extension movements can be achieved through relatively short-range cell intercalation. We have investigated this question in activin-induced convergent extension of animal caps by microinjecting a lineage tracer into *Xenopus* embryos at the two-cell stage such that half the embryo becomes labelled (DeSimone et al., 1991). Such embryos were allowed to develop to the mid-blastula stage, when animal pole regions were dissected and cultured in the presence or absence of activin. Histological analysis of such caps immediately after dissection showed that little mingling of labelled and unlabelled cells had occurred during early development, in agreement with the results of Wetts and Fraser (1989). We therefore went on to examine cell mixing in explants allowed to develop until the equivalent of stage 12. 5, by which time animal caps exposed to activin had undergone considerable elongation, while controls remained spherical.

In both induced and uninduced explants, blastomeres mixed with each other only to a very limited extent. In both,

a few individual cells could be found away from the main group, but overall the impression, in agreement with Keller, is that convergent extension does not involve long-range cell migration but a short-range, and perhaps directed, exchange of neighbours. Keller and Hardin (1987) suggested that such exchange of neighbours might occur through 'jostling' of adjacent cells, and this idea is supported by the appearance of induced and uninduced cells in the scanning electron microscope. At the equivalent of the early gastrula stage, induced and uninduced explants appear similar, with rounded cells at the surface of the explant that give the impression of being motile. By the late gastrula stage, however, the external surface of uninduced animal caps is very smooth, with the cells forming a flat epithelial-like sheet; the induced explants, by contrast, still have a motile appearance.

Cell spreading and migration

We have made little progress in the analysis of the cellular activities involved in spreading and migration, although it seems likely that these will involve major changes in the cytoskeleton and in proteins associated with the cytoskeleton. Rather, we have concentrated on changes in expression of cell adhesion molecules such as the integrins.

The molecular basis of gastrulation

To understand the molecular basis of gastrulation, it is necessary to discover the sequence of events between the receipt of a mesoderm-inducing signal such as activin and the onset of gastrulation behaviour. One way of going about this is to follow the events in the order in which they occur. This approach is being followed already in an effort to understand the patterning of the *Xenopus* mesoderm, and receptors both for FGF and activin have been demonstrated in the early embryo (Gillespie et al., 1989; Musci et al., 1990; Friesel and Dawid, 1991; Amaya et al., 1991; Kondo et al., 1991; Mathews et al., 1992), progress has been made in understanding second messenger pathways (Maslanski et al., 1992), and several mesoderm-specific genes such as *Xhox3* (Ruiz i Altaba and Melton, 1989) Xenopus *Brachyury* (Smith et al., 1991) and *goosecoid* (Cho et al., 1991) have been found. The next step in this approach is to ask whether overexpression of, for example, *Brachyury* can lead to mesoderm-like movements in animal pole cells, and such work is in progress (V. T. Cunliffe, J. E. H. and J. C. S., unpublished work).

The other approach is complementary to that outlined above. A very rapid response to mesoderm induction is the acquisition of the ability of cells to spread and migrate on FN. If the molecular events responsible for this change in behaviour can be elucidated, we can in principle work backwards from them towards the initial inductive event. One class of molecules likely to be involved is the integrin family. Indeed, there is already good evidence that FN-integrin interactions play an important role in amphibian gastrulation. This comes from work on *Pleurodeles*, which has shown that involution and migration are blocked by injection of anti-fibronectin (FN) antibodies into the blastocoel of the embryo (Boucaut et al., 1984), by intrablastocoelic injection of peptides corresponding to the cell binding site of FN (Boucaut et al., 1985) and by intracellular injection of an antibody targeted to the cytoplasmic domain of integrin β_1 (Darribère et al., 1990). Intrablastocoelic injection of antibodies raised against the extracellular domain of integrin β_1 also inhibits gastrulation by blocking formation of the matrix over which the cells migrate. Thus it is likely that the migration element of gastrulation is, at least in *Pleurodeles*, dependent on the interaction of FN with integrin β_1. Recently, we have investigated whether the same is true in *Xenopus*, and, if so, whether mesoderm-inducing factors such as activin influence the expression of integrins in *Xenopus*.

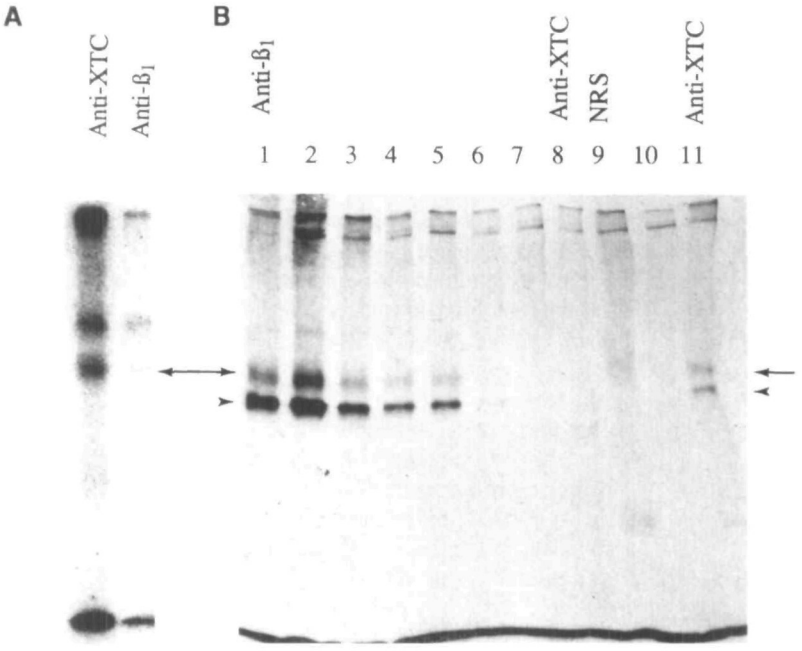

Fig. 4. An antiserum raised against XTC cells recognizes only integrin β_1. Proteins immunoprecipitated with an anti-XTC cell antiserum (Anti-XTC) or an anti-integrin β_1 antiserum (Anti-β_1) from surface- (A) or metabolically labelled (B) neurula extracts show the same electrophoretic mobility in SDS gels. The mature form (arrows) appears on the surface but the immature form (arrowheads) does not. Further evidence that the two sera recognize the same molecule comes from preclearing experiments (B). Sequential precipitations (lanes 1-7) with the anti-β_1 serum from metabolically labelled neurula extracts can remove all the target molecule from this solution; subsequent immunoprecipitation with the anti-XTC cell serum yields nothing (lane 8). Preclearing precipitations with normal rabbit serum (precipitations 1 and 7 are shown here; lanes 9 and 10) do not remove the target molecule for the anti-XTC serum (lane 11).

Our initial experiments used an anti-integrin β_1 antiserum (Marcantonio and Hynes, 1988) and a fibronectin affinity column to identify FN-binding proteins on the cell surfaces of mid-gastrula stage *Xenopus* embryos (Howard et al., 1992). This work indicated that the major fibronectin-binding protein on the surface of *Xenopus* gastrula cells contains integrin β_1, and we therefore went on to analyze the expression of this molecule during gastrula and later stages; previous studies had demonstrated the presence of integrin β_1 mRNA and protein up to and during blastula stages (DeSimone and Hynes, 1988; Smith et al., 1990; Gawantka et al., 1992). Immunoprecipitation from surface-labelled gastrula extracts revealed very little integrin β_1, as previously reported (Krotoski and Bronner-Fraser, 1990; Gawantka et al., 1992), although expression was much higher by neurula stages. This expression pattern was confirmed by immunoprecipitation of integrin β_1 from metabolically labelled cells, which revealed a 105×10^3 M_r polypeptide at all stages, representing the precursor form of integrin β_1, while mature species of M_r $116\text{-}120\times10^3$ were only clearly detectable after neurulation. Immunocytochemical studies performed in our laboratory and elsewhere (Krotoski and Bronner-Fraser, 1990) reflect this pattern of synthesis, with low levels of expression of integrin β_1 - so low as to be undetectable with many antisera - until notochord and somite formation.

The low levels of expression of the mature functional form of integrin β_1 cast some doubt on its role in gastrulation in *Xenopus*. The anti-integrin β_1 antiserum used for the expression studies was raised against a C-terminal peptide (Marcantonio and Hynes, 1988), but intracellular microinjection of this antibody did not disrupt function (J. E. H., unpublished observations). We therefore raised antisera against the extracellular domain of the mature molecule using XTC cells, which we knew to express integrin β_1 at high levels, as an immunogen. The resulting sera were indeed targeted to the external portion of integrin β_1 (Fig. 4), and were used to demonstrate that the spreading of newly induced mesodermal cells is dependent on integrin β_1 (Fig. 5; Howard et al., 1992). This is true not only for activin-induced animal cap cells but also for cells excised from the dorsal marginal zone, providing us with further evidence that activin closely mimics the action of the endogenous inducer. Furthermore, as in *Pleurodeles*, gastrulation is disrupted if this anti-integrin β_1 serum is injected into the blastocoel, apparently because the matrix of the blastocoel roof is disrupted (Fig. 6; Howard et al., 1992). These data do not contradict the work of Keller et al. (1985), which shows that gastrulation in *Xenopus* can occur even if the blastocoel roof is removed; rather, they suggest that an overlying matrix is required only in the very early stages of involution and that the coordinated force of convergent extension is then sufficient to complete the process in the absence of further directional cues. In *Pleurodeles*, involuting cells migrate more independently, convergent extension is therefore necessarily less significant, and cells require continued directional cues.

These results indicate that integrin β_1 is involved in gastrulation in *Xenopus*, and to understand gastrulation it will be necessary to understand how the function of integrin β_1 is regulated. One approach to this question is to use meso-

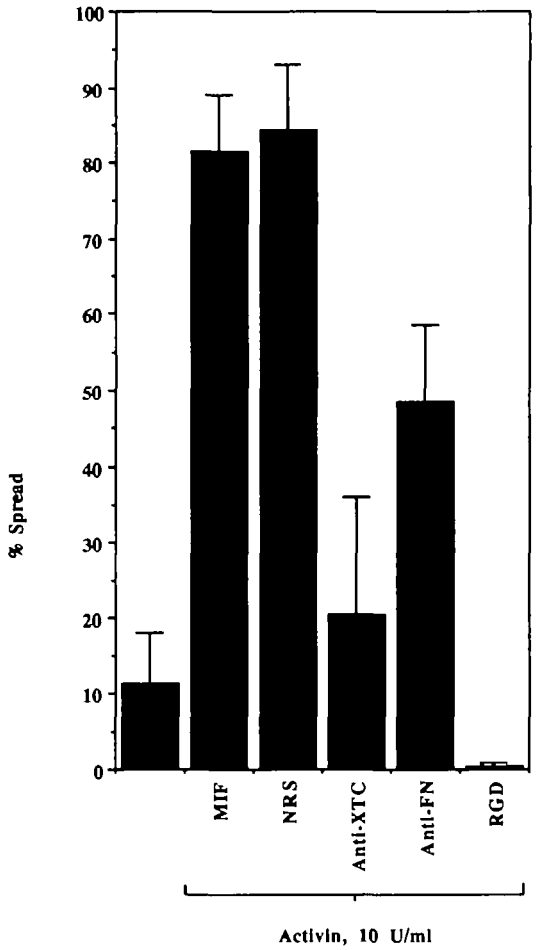

Fig. 5. Anti-XTC antisera block the spreading of activin-induced *Xenopus* blastomeres. Cells were treated with activin, or left untreated, and plated in the presence of the indicated antisera. The percentages of cells that had spread were assessed 1-2 hours later. The treatments are: –, no activin; remaining cases were all treated with activin; MIF, no antiserum; NRS, normal rabbit serum; Anti-XTC, Anti-XTC antiserum; Anti-FN, anti-fibronectin; RGD, 5 mM GRGDTP.

derm-inducing factors such as activin, which, as we describe above, causes animal pole cells to undergo both convergent extension and cell migration, the latter being dependent on integrin β_1. An understanding of how the function of this molecule is regulated by activin in this context could therefore provide insight into the intracellular events which initiate gastrulation. In our attempts to investigate this, we have, unfortunately, been unable to show any increase in expression of integrin β1 in response to activin, either at the level of rate of synthesis (Fig. 7; Smith et al., 1990) or of cell-surface expression (J. E. H., unpublished observation). This suggests that the mechanism underlying this very rapid response is a more subtle modulation of integrin function. This might involve a change in lipid environment (Conforti et al., 1990; Hermanowski-Vosatka et al., 1992), an altered interaction with the cytoskeleton, or a different dimerization partner (Dedhar, 1990). This last modification cannot be studied until α-specific antisera become available in *Xenopus*. In future work, we intend to address these problems.

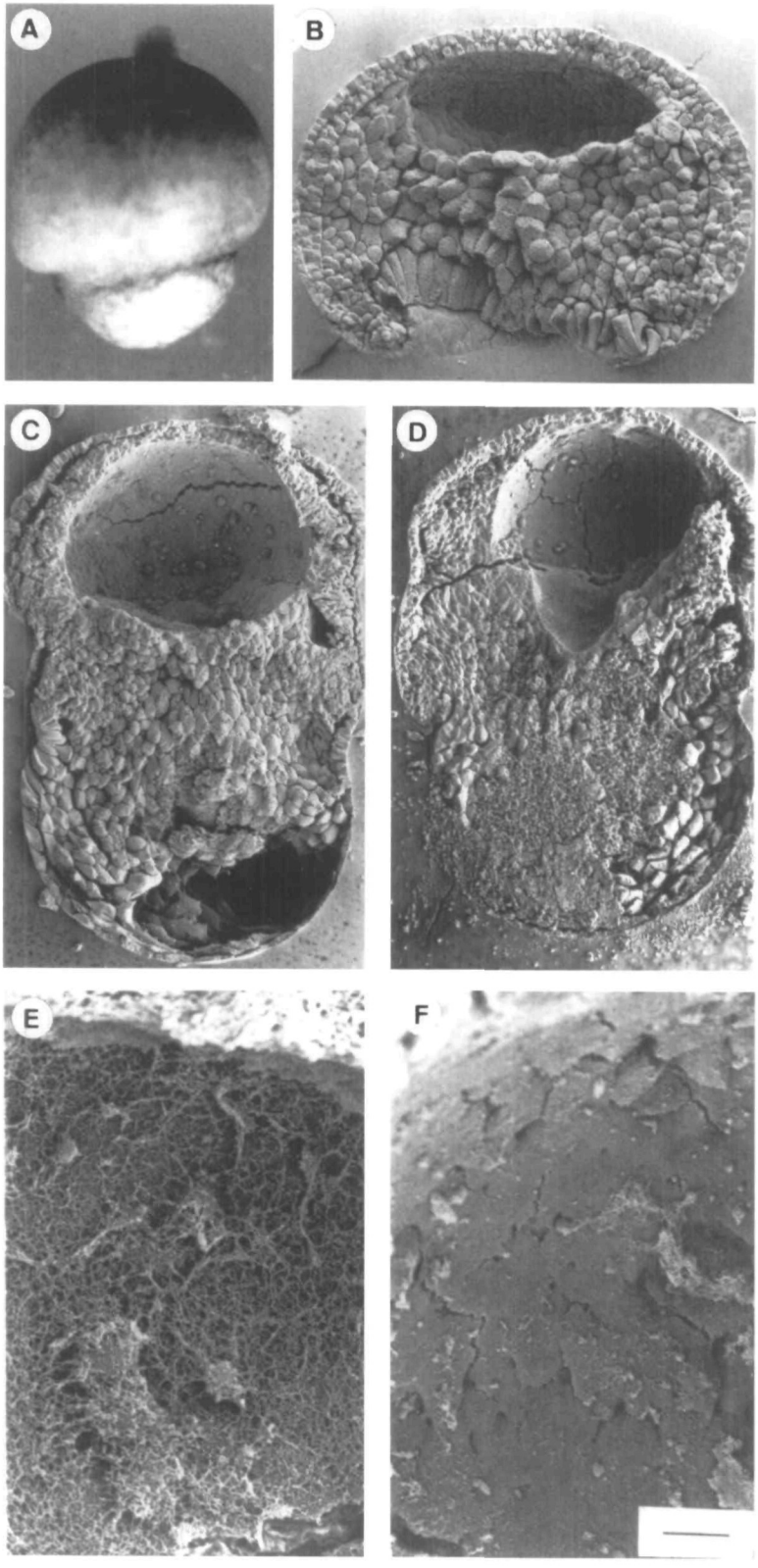

Fig. 6. Fibronectin-integrin interactions are required for gastrulation to occur in *Xenopus*. Involution in untreated embryos (B) normally begins at stage 10 and continues until the ingressing cells on the dorsal side (left in all pictures) have passed the centre of the blastocoel roof. Injection of anti-fibronectin (A,C,E) and anti-XTC cell (D, F) antisera into the blastocoel of stage 9 embryos severely restricts involution of mesodermal cells. In the presence of anti-FN antibodies in the blastocoel, the blastopore lip forms normally (A; stage 10. 5 embryo), but involution does not occur (A, C). In these scanning electron micrographs, the embryos were fixed at stage 10. 5 (B) or 12 (C-F) and cleaved as in Hirst and Howard (1992). Scanning electron micrographs of the blastocoel roof of embryos receiving injections of anti-XTC antiserum suggest that this disruption is caused by loss of the extracellular matrix (ECM) (F). In embryos injected with anti-fibronectin antibody the ECM is intact (E), and disruption is assumed to result from an inability of involuting cells to interact with FN. Bar represents 200 μm (B, C, D), 20 μm (E, F), 300 μm (A). Micrographs were kindly produced by Liz Hirst.

Conclusions

Gastrulation is a fundamental problem in developmental biology and, to arrive at an understanding of the process, it will be necessary to combine the techniques of experimental embryology, cell biology and molecular biology. In this paper, we have described the use of mesoderm-inducing factors such as activin A in the study of gastrulation. The ability of factors such as these to induce naive cells to undergo gastrulation movements provides a powerful tool

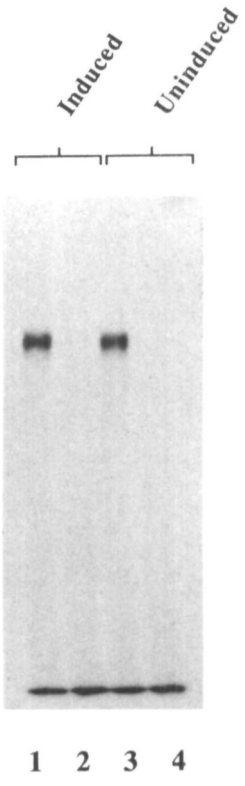

Fig. 7. Synthesis of integrin β_1 is not significantly affected by activin treatment. Animal pole explants were incubated in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of activin, together with [^{35}S]methionine. Levels of integrin β_1 synthesized during the labelling period were assessed by immunoprecipitation with the anti integrin β_1 antiserum (lanes 1 and 3) or normal rabbit serum (lanes 2 and 4).

for coming to understand what makes cells behave in particular ways at particular times.

References

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. *Cell* **66**, 257-270.

Black, S. D. (1989). Experimental reversal of the normal dorsal-ventral timing of blastopore formation does not reverse axis polarity in *Xenopus laevis* embryos. *Dev. Biol.* **134**, 376-381.

Boucaut, J. -C., Darribère, T., Boulekbache, H. and Thiery, J. -P. (1984). Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos. *Nature* **307**, 364-367.

Boucaut, J. -C., Darribère, T., Li, S. D., Boulekbache, H., Yamada, K. M., and Thiery, J. -P. (1985). Evidence for the role of fibronectin in amphibian gastrulation. *J. Embryol. Exp. Morph.* **89 Supplement**, 211-227.

Chakrabarti, A., Matthews, G., Colman, A. and Dale, L. (1992). Secretory and inductive properties of *Drosophila wingless* (Dwnt-1) protein in *Xenopus* oocytes and embryos. *Development*, in press.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the Xenopus homeobox gene *goosecoid*. *Cell* **67**, 1111-1120.

Christian, J. L., Olsen, D. J. and Moon, R. T. (1992). *Xwnt-8* modifies the character of mesoderm induced by bFGF in isolated *Xenopus* ectoderm. *EMBO J.* **11**, 33-41.

Conforti, G., Zanetti, A., Pasquali, R. I., Quaglino, D. J., Neyroz, P. and Dejana, E. (1990). Modulation of vitronectin receptor binding by membrane lipid composition. *J Biol. Chem.* **265**, 4011-4019.

Cooke, J. (1973). Morphogenesis and regulation in spite of continued mitotic inhibition in *Xenopus* embryos. *Nature* **242**, 55-57

Cooke, J. (1989). Mesoderm-inducing factors and Spemann's organiser phenomenon in amphibian development. *Development* **107**, 229-241.

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

Cooke, J. and Smith, J. C. (1989). Gastrulation and larval pattern in *Xenopus* after blastocoelic injection of a *Xenopus*-derived inducing factor: experiments testing models for the normal organization of mesoderm. *Dev. Biol.* **131**, 383-400.

Cooke, J. and Smith, J. C. (1990). Measurement of developmental time by cells of early embryos. *Cell* **60**, 891-894.

Dale, L., Howes, G., Price, B. M. J. and Smith, J. C. (1992). Bone morphogenetic protein 4: a ventralizing factor in early *Xenopus* development. *Development* **115**, in press.

Darribère, T., Guida, K., Larjava, H., Johnson, K. E., Yamada, K. M., Thiery, J. -P. and Boucaut, J. -C. (1990). In vivo analyses of integrin β_1 subunit function in fibronectin matrix assembly. *J. Cell Biol.* **110**, 1813-1823.

Dawid, I. B. and Sargent, T. D. (1990). The role of growth factors in embryonic induction in amphibians. *Curr. Top. Dev. Biol.* **24**, 31-55.

Dedhar, S. (1990). Integrins and tumor invasion. *BioEssays* **12**, 583-590.

DeSimone, D. W. and Hynes, R. O. (1988). Xenopus laevis integrins. Structural conservation and evolutionary divergence of integrin beta subunits. *J Biol. Chem.* **263**, 5333-5340.

DeSimone, D. W., Smith, J. C., Howard, J. E., Ransom, D. G. and Symes, K. (1991). The expression of fibronectins and integrins during mesodermal induction and gastrulation in *Xenopus*. In *Gastrulation: Movements, Patterns and Molecules* (ed. R. Keller, W Clark and F. Griffin), pp185-198.

Friesel, R. and Dawid, I. B. (1991). cDNA cloning and developmental expression of fibroblast growth factor receptors from *Xenopus laevis*. *Mol. Cell Biol.* **11**, 2481-2488.

Gawantka, V., Ellinger-Ziegelbauer, H. and Hausen, P. (1992). β_1-integrin is a maternal protein that is inserted into all newly formed plasma membranes during early *Xenopus* embryogenesis. *Development* **115**, 595-606.

Gerhart, J. C., Danilchik, M., Doniach, T., Roberts, S., Rowning, B. and Stewart, R. (1989). Cortical rotation of the *Xenopus egg*: consequences for the anteroposterior pattern of embryonic dorsal development. *Development* **1989 Supplement**, 37-51.

Gillespie, L. L., Paterno, G. D. and Slack, J. M. W. (1989). Analysis of competence: receptors for fibroblast growth factor in early *Xenopus* embryos. *Development* **106**, 203-208.

Green, J. B. A., Howes, G., Symes, K., Cooke, J. and Smith, J. C. (1990). The biological effects of XTC-MIF: quantitative comparison with *Xenopus* bFGF. *Development* **108**, 173-183.

Green, J. B. A. and Smith, J. C. (1990). Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* **347**, 391-394.

Gurdon, J. B. (1988). A community effect in animal development. *Nature* **336**, 772-774.

Hermanowski-Vosatka, A., Van Strijp, J. A. G., Swiggard, W. J. and Wright, S. D. (1992). Integrin modulating factor-1: A lipid that alters the function of leukocyte integrins. *Cell* **68**, 341-342.

Hirst, E. M. A., and Howard, J. E. (1992). A simple technique to control the position and orientation of dry fracture planes for studying surfaces hidden within bulk tissue by scanning electron microscopy. *J. Microscopy*. In press.

Howard, J. E., Hirst, E. M. and Smith, J. C. (1992). Are b_1 integrins involved in *Xenopus* gastrulation? *Mech. Dev.* In press.

Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V. E. and Hogan, B. M. L. (1992). DVR-4 (Bone Morphogenetic Protein-4) as a posterior-ventralizing factor in Xenopus mesoderm induction. *Development* **115**, 639-648.

Keller, R. (1991). Early embryonic development of Xenopus laevis. *Methods in Cell Biology* **36**, 61-113.

Keller, R. E. and Danilchik, M. (1988). Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. *Development* **103**, 193-210.

Keller, R. E., Danilchik, M., Gimlich, R. and Shih, J. (1985). The function of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. Exp. Morph.* **89 Supplement**, 185-209.

Keller, R. E. and Hardin, J. (1987). Cell behaviour during active cell rearrangement: evidence and speculations *J. Cell Sci Suppl.* **8**, 369-393.

Kimelman, D. and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGF-beta and the identification of an mRNA coding for FGF in the early Xenopus embryo. *Cell* **51**, 869-877.

Kondo, M., Tashiro, K., Fujii, G., Asano, M., Miyoshi, R., Yamada, R., Muramatsu, M. and Shiokawa, K. (1991). Activin receptor mRNA is

expressed early in *Xenopus* embryogenesis and the level of the expression affects the body axis formation *Biochem. Biophys Res. Comm.* **181**, 684-690.

Köster, M., Plessow, S., Clement, J. H., Lorenz, A., Tiedemann, H. and Knöchel, W. (1991). Bone morphogenetic protein 4 (BMP-4), a member of the TGF-b family, in early embryos of *Xenopus laevis*: analysis of mesoderm-inducing activity. *Mech. Dev.* **33**, 191-199.

Krotoski, D. and Bronner-Fraser, M. (1990). Distribution of integrins and their ligands in the trunk of *Xenopus laevis* during neural crest cell migration. *J. Exp. Zool.* **253**, 139-150.

McMahon, A. P. and Moon, R. T. (1989). Ectopic expression of the proto-oncogene *int-1* in Xenopus embryos leads to duplication of the embryonic axis. *Cell* **58**, 1075-1084.

Marcantonio, E. E. and Hynes, R. O. (1988). Antibodies to the conserved cytoplasmic domain of the integrin β_1 subunit react with proteins in vertebrates, invertebrates and fungi. *J. Cell Biol.* **106**, 1765-1772.

Maslanski, J. A., Leshko, L. and Busa, W. B. (1992). Lithium-sensitive production of inositol phosphates during amphibian embryonic mesoderm induction. *Science* **256**, 243-245.

Mathews, L. S., Vale, W. W. and Kintner, C. R. (1992). Cloning of a second type of activin receptor and functional characterization in *Xenopus* embryos. *Science* **255**, 1702-1705.

Musci, T. J., Amaya, E. and Kirschner, M. W. (1990) Regulation of the fibroblast growth factor receptor in early *Xenopus* embryos *Proc Natn. Acad. Sci. USA* **87**, 8365-8369.

Nakatsuji, N. (1986). Presumptive mesoderm cells from *Xenopus laevis* gastrulae attach to and migrate on substrata coated with fibronectin or laminin. *J. Cell Sci.* **86**, 109-118.

Ruiz i Altaba, A. and Melton, D. A. (1989). Interaction between peptide growth factors and homeobox genes in the establishment of antero-posterior polarity in frog embryos. *Nature* **341**, 33-38.

Satoh, N. (1982). Timing mechanisms in early embryonic development *Differentiation* **22**, 156-163.

Slack, J. M. W., Darlington, B. G., Heath, J. K. and Godsave, S. F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature* **326**, 197-200

Smith, J. C. (1989). Mesoderm induction and mesoderm-inducing factors in early amphibian development. *Development* **105**, 665-677.

Smith, J. C. (1992). A *wnt*er's tale. *Current Biology* **2**, 177-179.

Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a Xenopus homolog of *Brachyury (T)* is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.

Smith, J. C., Symes, K., Hynes, R. O. and DeSimone, D. (1990). Mesoderm induction and the control of gastrulation in *Xenopus laevis*: the roles of fibronectin and integrins. *Development* **108**, 229-238.

Smith, W. C. and Harland, R. M. (1991). Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing center. *Cell* **67**, 753-765.

Sokol, S., Christian, J. L., Moon, R. T. and Melton, D. A. (1991). Injected Wnt RNA induces a complete body axis in Xenopus embryos. *Cell* **67**, 741-752.

Symes, K. and Smith, J. C. (1987). Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis*. *Development* **101**, 339-349.

Wetts, R. and Fraser, S. E. (1989). Slow intermixing of cells during *Xenopus* embryogenesis contributes to the consistency of the fate map. *Development* **105**, 9-15.

Whitman, M. and Melton, D. A. (1989). Growth factors in early embryogenesis. *Ann. Rev. Cell Biol.* **5**, 93-117.

Winklbauer, R. (1988). Differential interaction of *Xenopus* embryonic cells with fibronectin *in vitro*. *Dev. Biol.* **130**, 175-183.

Winklbauer, R. (1990). Mesodermal cell migration during *Xenopus* gastrulation. *Dev. Biol.* **142**, 155-168.

Winklbauer, R. and Selchow, A. (1992). Motile behavior and protrusive activity of migratory mesoderm cells from the Xenopus gastrula. *Dev. Biol.* **150**, 335-351.

Yasuda, G. K. and Schubiger, G. (1992). Temporal regulation in the early embryo: is MBT too good to be true? *Trends in Genetics* **8**, 124-127.