Mesoderm induction and the control of gastrulation in *Xenopus laevis*: the roles of fibronectin and integrins

J. C. SMITH¹, K. SYMES¹†, R. O. HYNES²,³ and D. DeSIMONE³*

¹Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
²Howard Hughes Medical Institute and ³Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

* Present address: University of Virginia, Health Sciences Center, Department of Anatomy and Cell Biology, Box 439, School of Medicine, Charlottesville, VA 22908, USA
† Present address: Department of Cell and Molecular Biology, 385 LSA, University of California, Berkeley, CA 94720, USA

Summary

Exposure of isolated *Xenopus* animal pole ectoderm to the XTC mesoderm-inducing factor (XTC-MIF) causes the tissue to undergo gastrulation-like movements. In this paper, we take advantage of this observation to investigate the control of various aspects of gastrulation in *Xenopus*.

Blastomeres derived from induced animal pole regions are able, like marginal zone cells, but unlike control animal pole blastomeres, to spread and migrate on a fibronectin-coated surface. Dispersed animal pole cells are also able to respond to XTC-MIF in this way; this is one of the few mesoderm-specific responses to induction that has been observed in single cells.

The ability of induced animal pole cells to spread on fibronectin is abolished by the peptide GRGDSP. However, the elongation of intact explants is unaffected by this peptide. This may indicate that fibronectin-mediated cell migration is not required for convergent extension.

We have investigated the molecular basis of XTC-MIF-induced gastrulation-like movements by measuring rates of synthesis of fibronectin and of the integrin β₁ chain in induced and control explants. No significant differences were observed, and this suggests that gastrulation is not initiated simply by control of synthesis of these molecules. In future work, we intend to investigate synthesis of other integrin subunits and to examine possible post-translational modifications to fibronectin and the integrins.

Key words: gastrulation, fibronectin, integrins, mesoderm induction, mesoderm-inducing factors, XTC-MIF, amphibian embryo, *Xenopus laevis*.

Introduction

Two lines of evidence indicate that fibronectin plays an important role in amphibian gastrulation. First, fibronectin is localised to the roof of the blastocoel, the surface on which presumptive mesodermal cells migrate (Boucaut and Darribère, 1983; Lee et al. 1984; Nakatsuji et al. 1985a). Second, if antibodies to fibronectin or its receptors, integrins, are microinjected into the blastocoels of amphibian embryos, gastrulation is inhibited (Boucaut et al. 1984a; Darribère et al. 1988). Similar results are obtained by injecting synthetic peptides corresponding to a major cell-binding site of the fibronectin molecule (Boucaut et al. 1984b).

Although this work demonstrates that fibronectin and its receptors are required for gastrulation to proceed, it does not address the question of how gastrulation is initiated and controlled. The simplest suggestion is that gastrulation is triggered by the synthesis of these molecules, and this idea is supported by studies of the temporal expression of fibronectin and integrins. The rate of synthesis of fibronectin increases dramatically at the mid-blastula transition (MBT; see Newport and Kirschner, 1982), and the protein is first detectable immunocytochemically about three hours later, at the beginning of gastrulation (Boucaut and Darribère, 1983; Lee et al. 1984). This increase in fibronectin synthesis does not require RNA synthesis, and must involve activation of maternal message. The integrins consist of α and β subunits (see Hynes, 1987). Analysis of *Xenopus* β₁ subunit mRNA shows that expression begins around the gastrula stage (DeSimone and Hynes, 1988); information is not yet available about the α subunits.

These observations do not, of course, prove that gastrulation is controlled by synthesis of fibronectin and its receptor. The similarity in timing may be coincidental, and indeed transcription of many genes first occurs...
at the MBT or soon afterwards (see, for example, Sargent and Dawid, 1983; Kreig and Melton, 1985). In this paper, we test the functions of fibronectin and integrins more directly by taking advantage of the recent observation that the XTC-mesoderm-inducing factor (XTC-MIF; see Smith, 1987; Rosa et al. 1988; Smith et al. 1988; Dawid et al. 1989; Smith, 1989) induces gastrulation-like movements in isolated animal pole regions (Symes and Smith, 1987; Cooke and Smith, 1989). This observation makes it possible to manipulate gastrulation and thus ask how its various aspects are controlled.

Our results indicate that animal pole blastomeres exposed to XTC-MIF, whether as intact explants or as isolated cells, are able to spread and migrate on a fibronectin-coated substrate. In this respect, they resemble prospective mesodermal cells, but differ from uninduced animal pole cells (Nakatsuji, 1986). However, acquisition of the ability to spread on fibronectin is not accompanied by an increase in synthesis of fibronectin or of the integrin β1 chain. We conclude from this that initiation of gastrulation is not controlled through synthesis of these proteins, although it is possible that existing molecules are redistributed within cells or undergo post-translational modification.

The ability of both XTC-MIF-induced animal pole blastomeres and of presumptive mesodermal cells to spread on fibronectin is abolished by the peptide GRGDSP, which contains the fibronectin cell attachment site (Pierschbacher and Ruoslahti, 1984; Yamada and Kennedy, 1984). However, the elongation of induced animal pole explants is unaffected by this peptide. This suggests that the ‘convergent extension’ movements of gastrulation (see Keller, 1986; Keller and Danilchik, 1988; Keller and Tibbetts, 1989) do not depend on interactions involving RGD sites in fibronectin or other matrix molecules.

Materials and methods

Embryos

Embryos of *Xenopus laevis* were obtained by artificial fertilization as described by Smith and Slack (1983). They were chemically dejellied using 2% cysteine hydrochloride (pH 7.8–8.1), washed and transferred to Petri dishes coated with 1% Noble agar and containing 10% normal amphibian medium (NAM; Slack, 1984). The embryos were staged according to Nieuwkoop and Faber (1967).

**XTC-mesoderm-inducing factor**

XTC-MIF was partially purified from heated XTC-cell-conditioned medium by DEAE–Sepharose chromatography followed by phenyl Sepharose chromatography, as described by Cooke et al. (1987) and Smith et al. (1988). One unit of mesoderm-inducing activity is defined as the minimum quantity that must be present in 1 ml medium for induction to occur. The sample of partially purified XTC-MIF used in these experiments contained approximately 7.7×10^3 units mg^{-1} protein.

**Preparation of cell substrates**

Tissue culture dishes (35 mm diameter, Nunc), or the wells of multiwell or microtitre plates (Nunc), were treated at room temperature for 4–18 h with 20–100 μg ml^{-1} bovine or rat plasma fibronectin, kindly supplied by Dr Heather Streeter (NIMR) or Mr Terry Butters and Dr Colin Hughes (NIMR). The surfaces were then rinsed and ‘blocked’ with NAM containing 0.5% bovine serum albumin (BSA) for 20 min.

**Embryo dissections**

Mid-blastula *Xenopus* embryos (stages 8–9) were dissected into animal, marginal zone, and vegetal pole regions using electrolytically sharpened tungsten needles.

**Cell spreading assays**

Animal pole or dorsal marginal zone regions were transferred to calcium- and magnesium-free medium (CMFM: 100 mM-NaCl, 5 mM-KCl, 1 mM-NaHCO_3, 2.5 mM-sodium phosphate, pH 7.5 and 0.25% (w/v) gentamycin sulphate; Sargent et al. 1986). The outer layer of cells, which is difficult to dissociate, was discarded and the inner layer was disaggregated into a single-cell suspension. These cells were seeded onto fibronectin-coated surfaces in NAM containing 0.5% BSA and, where appropriate, XTC-MIF, and they were scored 1–2 h later.

Cells could be divided into three classes (see Fig. 1 for examples). Non-adherent cells are spherical and move about the dish when it is disturbed. Adherent cells cannot easily be dislodged by disturbing the dish, but like non-adherent cells they are roughly spherical. They do not flatten but frequently undergo ‘circus movements’, during which cytoplasmic protrusions move slowly around the circumference of the cell. Spread cells adhere to the substrate, flatten, and send out processes. They can sometimes be seen to migrate over the fibronectin-coated substrate (see Fig. 4). Spread cells are clearly distinct from adherent cells.

Cultures were scored independently by two observers (J.C.S. and K.S.), one of whom was ignorant of the coding of the experiment. The two assessments did not differ significantly.

**Antibodies and inhibitory peptides**

These experiments used a rabbit antiserum raised against *Xenopus* plasma fibronectin (Heasman et al. 1981) and a rabbit antiserum raised against a 39-amino acid peptide corresponding to a COOH-terminal portion of vertebrate integrin β1 subunits (Mancantonio and Hynes, 1988). The synthetic peptides GRGDSP and GRGESP were synthesized on an Applied Biosystems Peptide Synthesizer and purified by HPLC by Gene Yee, to whom we are very grateful.

**Metabolic labelling of embryonic tissue and immunoprecipitation**

Dissected pieces of mid-blastula *Xenopus* embryos were incubated in NAM, with or without XTC-MIF as appropriate, in the presence of 0.3–0.9 mCi ml^{-1} [35S]methionine at room temperature (19–22°C). At the end of the incubation period, the tissues were rinsed three or four times in NAM and frozen on dry ice in a minimum volume of fluid.

Samples for immunoprecipitation using the anti-fibronectin antibody were homogenized in 2 M-urea as described by Lee et al. (1984). Samples for analysis using the anti-integrin antibody were homogenized in 0.1 M-NaCl, 1% Triton X-100, 1 mM-PMSE, 0.22 trypsin inhibitor unit (TIU) ml^{-1} aprotilin, and 20 mM-Tris, pH 7.6. In both cases, aliquots were then removed to determine total radioactivity incorporated into acid-insoluble material, and the extracts were diluted five-fold with ‘immunoprecipitation buffer’ (0.1 M-KCl, 5 mM-MgCl_2, 1% Triton X-100, 1% sodium deoxycholate, 2 mM-PMSE,
0.22 TIU ml\(^{-1}\) aprotinin, and 0.1 \(\mu\text{-Tris, pH 8.2}\) containing 0.4 \% pre-immune serum. After 15 min at 4°C, a 10 \% volume of Protein A–Sepharose slurry was added, and after a further 15 min the mixtures were centrifuged and the supernatants divided equally between two or three fresh tubes. To one such tube immune serum was added to 0.4 \% , and to another pre-immune serum was added to the same concentration. As a further control for integrin immunoprecipitations, samples contained 0.4 \% immune serum plus 100 \(\mu\text{g}\) ml\(^{-1}\) of the original peptide antigen as competitor.

These tubes were incubated at 4°C for 1 h with gentle rocking, and then a 20 \% volume of Protein A–Sepharose slurry was added and the incubation repeated. Finally the tubes were centrifuged and the pellets washed four times with immunoprecipitation buffer before solubilizing proteins in 50 \(\mu\text{l}\) of gel sample buffer (Laemmli, 1970).

Samples were analysed by polyacrylamide gel electrophoresis using the buffer system of Laemmli (1970) with a 7 \% separating gel to detect integrins and a 5 \% separating gel to detect fibronectin. Fluorography was carried out according to Bonner and Laskey (1974). Sample loading was normalised according to total incorporation of radioactivity into acid-precipitable material.

**Results**

As in previous work, treatment of stage 8–9 *Xenopus* animal pole regions with 5–20 units ml\(^{-1}\) XTC-MIF resulted in dramatic elongation of the explants, and eventually the formation of dorsal mesodermal cell types, including notochord and muscle (data not shown, but see Smith, 1987; Cooke et al. 1987; Symes and Smith, 1987; Smith et al. 1988).

**Blastomeres from induced ectoderm can spread on fibronectin**

We were first interested to discover whether the cells of animal pole explants exposed to XTC-MIF could spread and migrate on fibronectin-coated surfaces. Accordingly, explants were exposed to 20 units ml\(^{-1}\) XTC-MIF or a control solution from stage 8 to stage 10 in NAM containing 10 \% of the normal divalent cation concentration to prevent them ‘rounding up’. The explants were then placed, blastocoel-facing surface down, onto surfaces coated with 50 \(\mu\text{g}\) ml\(^{-1}\) fibronectin, and observed at intervals. Dorsal marginal zone explants were dissected from early gastrula embryos and cultured in the same way, essentially as described by Shi et al. (1989).

As expected, cells from uninduced animal pole regions did not adhere to the fibronectin-coated substrate, while some blastomeres from the dorsal marginal zone regions adhered and began to migrate away from the body of the explant (data not shown). Cells from explants exposed to XTC-MIF behaved like dorsal marginal zone blastomeres and like them adhered to the substrate, spread and began to migrate. In some cases virtually all of the cells seemed to stick to the fibronectin-coated surface.

To obtain a more quantitative analysis, induced and uninduced explants and dorsal marginal zone regions were disaggregated into single cell suspensions at stage 10 and the blastomeres were seeded onto substrates coated with 20, 50 or 100 \(\mu\text{g}\) ml\(^{-1}\) fibronectin. Three different concentrations of fibronectin were used to allow for possible variation in spreading behaviour of blastomeres from different egg batches. After 1–2 h the proportions of non-adherent, adherent and spread cells were determined (see Materials and methods). The proportions of adherent and non-adherent cells varied between different experiments, depending both on egg batch and on the concentration of fibronectin used (see Winklbauer, 1988). However, when the proportion of spread cells to unspread (non-adherent plus adherent) cells was considered, the results obtained with all three concentrations of fibronectin were similar and consistent from experiment to experiment. Figs 1 and 2 show data obtained with 50 \(\mu\text{g}\) ml\(^{-1}\) fibronectin, in which 37 \% of cells from the dorsal marginal zone region spread on fibronectin, as compared with only 1 \% from uninduced animal pole explants. Treatment of animal pole explants with XTC-MIF resulted in 45 \% of the cells becoming spread after disaggregation.

In preliminary work, mentioned in Symes and Smith (1987), we found that uninduced animal pole blastomeres were able to spread on fibronectin-coated surfaces almost as well as marginal zone cells. This may have been due to use of a glass, rather than a plastic, substrate, or to inadequate ‘blocking’ of free substrate with bovine serum albumin (see Winklbauer, 1988).

**Single cells can respond to XTC-MIF and spread on fibronectin**

We next tested whether dispersed animal pole cells could respond to XTC-MIF by spreading on fibronectin. Dissected animal pole regions were disaggregated into single cell suspensions at stage 9 (late blastula stage) and cultured in 20 units ml\(^{-1}\) XTC-MIF or a control solution for 1 h, until control embryos reached stage 10, the beginning of gastrulation. Dorsal marginal zone regions were then dissected from early gastrulae and disaggregated, and the three populations of cells were seeded onto substrates coated with 20, 50, or 100 \(\mu\text{g}\) ml\(^{-1}\) fibronectin, as before. The results were similar with all three concentrations of fibronectin; Fig. 3 shows data obtained with 100 \(\mu\text{g}\) ml\(^{-1}\). Over 60 \% of blastomeres from the dorsal marginal zone spread on the fibronectin-coated surface, whereas none from uninduced animal pole regions did so. However, over 95 \% of cells exposed to XTC-MIF while in a dispersed state spread on the substrate. This result indicates first that single cells are able to respond to XTC-MIF by spreading on a fibronectin-coated surface. Second, the data show that virtually all the deep cells of the animal pole are able to respond to XTC-MIF by spreading on fibronectin, and that the smaller percentage of spread cells observed when intact explants were exposed to the factor (Fig. 2) is probably due to inaccessibility of the inner cells.

Continuous observation of cells that had been exposed to XTC-MIF showed further that they could migrate on the fibronectin-coated surface (Fig. 4).
Spreading of induced cells on fibronectin is inhibited by the peptide GRGDSP

To discover whether the spreading of induced animal pole blastomeres depends on a specific interaction between the cells and fibronectin, we used the synthetic peptide GRGDSP, which corresponds with the primary fibronectin cell attachment site (Pierschbacher and Ruoslahti, 1984; Yamada and Kennedy, 1984). Control cultures contained the peptide GRGESP, in which Asp is replaced by Glu. Animal pole blastomeres were disaggregated from Xenopus embryos at the late blastula stage and treated with XTC-MIF in the presence of different concentrations of peptide. The proportions of spread cells were determined 1.5 h later. MIF-induced spreading was completely inhibited by 5 mM-GRGDSP, whereas the same concentration of GRGESP reduced the proportion of spread cells by only 46%. The same experiment showed that spreading of dorsal marginal zone cells is completely inhibited by 2 mM- and 5 mM-GRGDSP, as has been reported for prospective meso-

Fig. 1. Individual cells from XTC-MIF-treated *Xenopus* animal pole regions, like those from the dorsal marginal zone, adhere to a fibronectin-coated surface; those of uninduced animal pole regions do not. (A) The dorsal marginal zone region of a *Xenopus* early gastrula was disaggregated into a single cell suspension and seeded onto tissue-culture plastic coated with 50 μg ml−1 fibronectin. Many of the cells attach to the substrate, flatten, and send out processes. (B) Animal pole regions exposed to XTC-MIF from the mid-blastula to the early gastrula stage were disaggregated into a single cell suspension which was seeded onto a similar fibronectin-coated substrate. Many of the cells have spread. The arrow indicates a spread cell, and the arrow-head a non-adherent cell. (C) Cells disaggregated from uninduced animal pole regions do not spread on a fibronectin-coated surface. The arrow indicates an adherent cell. Scale bar in C is 50 μm, and also applies to A and B.

Fig. 2. Blastomeres derived from the dorsal marginal zone and from animal pole regions treated with XTC-MIF spread on a fibronectin-coated substrate; cells from uninduced animal pole regions do not. Blastomeres were disaggregated from dorsal marginal zone regions (DMZ), uninduced animal pole regions (AP) or animal pole regions that had been treated with XTC-MIF (AP-MIF). They were seeded onto tissue-culture plastic which had been treated with 50 μg ml−1 fibronectin, and the results were scored after 2 h as described in the Materials and methods. An average of 171 cells was counted for each treatment. Similar results were obtained with cells seeded onto 100 μg ml−1 and 20 μg ml−1 fibronectin. For 100 μg ml−1, the proportions of cells derived from DMZ, AP, and AP-MIF regions that spread on the substrate were, respectively, 43%, 3% and 34% (average of 200 cells per point). For 20 μg ml−1, the proportions were 27%, 0.4% and 40% (average of 206 cells per point).
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Fig. 3. Single animal pole blastomeres treated with XTC-MIF are able to spread on a fibronectin-coated substrate. Blastomeres were disaggregated from animal pole regions at the late blastula stage and cultured in a control solution (AP) or XTC-MIF (AP-MIF) until the early gastrula stage. The cells were then seeded onto tissue-culture plastic which had been treated with 100 μg ml⁻¹ fibronectin, along with cells from the dorsal marginal zone of normal embryos (DMZ). The results were scored after 2 h as described in the Materials and methods. An average of 286 cells was counted for each treatment. Similar results were obtained with cells seeded onto 50 μg ml⁻¹ and 20 μg ml⁻¹ fibronectin. For 50 μg ml⁻¹, the proportions of DMZ, AP, and AP-MIF cells that spread on the substrate were, respectively, 25 %, 1 % and 67 % (average of 287 cells per point). For 20 μg ml⁻¹, the proportions were 19 %, 0 % and 84 % (average of 206 cells per point).

derm from the urodele Pleurodeles waltl (Shi et al. 1989). The slight inhibition of spreading observed with the peptide GRGESP has been noted in other studies (Dufour et al. 1988) and may reflect a lower affinity interaction of this peptide with the integrin receptor binding site (Hautanen et al. 1989).

The peptide GRGDSP does not inhibit elongation of induced animal pole explants

The ability of induced animal pole blastomeres to spread on fibronectin reflects the role of cell migration in amphibian gastrulation (see Keller, 1986). However, an equally important aspect of gastrulation, particularly in Xenopus, is convergent extension, which is driven more by cell rearrangement and intercalation than by cell migration (Keller, 1986), and which is probably responsible for the elongation of induced animal pole explants (Symes and Smith, 1987). We have investigated whether fibronectin-mediated interactions are

Fig. 4. Disaggregated animal pole blastomeres treated with XTC-MIF migrate on a fibronectin-coated substrate. A and B show animal pole blastomeres prepared as described in the legend to Fig. 3. They were photographed at intervals of 10 min, beginning at the equivalent of stage 11; the figure in the top right hand corner of each frame represents the time in minutes. Scale bar in the final frame of B is 30 μm, and applies to all frames.
required for convergent extension by incubating explants in the peptides GRGDSP and GRGESP in the presence of XTC-MIF or a control solution. The results of two experiments using a total of 160 explants show that even 10 mM-GRGDSP has no effect whatsoever on convergent extension of induced explants (Fig. 5). Further incubation of such explants also showed that the peptides had no effect on mesodermal cell differentiation (data not shown). These results suggest that the RGD cell attachment site is required for migration of prospective mesodermal cells, but is not necessary for convergent extension.

**Induced ectoderm does not synthesize fibronectin or the integrin β1 chain at an increased rate**

The molecular basis of the ability of single induced animal pole blastomeres to spread on fibronectin, and of a population of such cells to cooperate in convergent extension movements, is unknown. To investigate this we have studied rates of fibronectin and integrin β1 chain synthesis in induced explants compared with uninduced tissue.

To estimate the rate of synthesis of fibronectin, induced and uninduced animal pole regions, marginal zone regions and vegetal pole regions were incubated in [35S]methionine from stage 9 (late blastula) to stage 12–13 (late gastrula). Fibronectin synthesis was determined by immunoprecipitation and results from one of two similar experiments are shown in Fig. 6A. There is no enhancement of fibronectin synthesis in induced animal pole regions compared with uninduced explants, and this is consistent with the observation that animal pole regions synthesize fibronectin at a similar rate to the marginal zone. It was noteworthy, however, that in two out of three experiments the vegetal pole region was seen to synthesize significantly more fibronectin as a fraction of its total protein synthesis than the other regions (see Fig. 6A). This is currently under investigation. Overall, these results indicate that the ability of induced animal pole blastomeres to spread on fibronectin is not due to an increased rate of synthesis of fibronectin itself.

A more likely possibility is that the ability of induced cells to spread on fibronectin is due to an increase in the expression of fibronectin receptors. We therefore investigated the rate of synthesis of the integrin β1 chain. Induced and uninduced animal pole regions, marginal zone regions, and vegetal pole regions were incubated in [35S]methionine from stage 9 (late blastula) to stage 12–13 (late gastrula). Integrin β chain synthesis was determined by immunoprecipitation. Five successful experiments were carried out. In most, there was little enhancement of the rate of synthesis of integrin β1 chain in induced explants compared with uninduced explants; the largest increase, shown in Fig. 6B, was 2.8-fold. To discover whether the slight increase observed in some experiments reflected a brief period of a more marked enhancement of synthesis, two time-course experiments were conducted, with integrin synthesis being measured from stages 9–10, 10–11, 11–12 and 12–13. No such ‘burst’ of synthesis was observed (data not shown). In contrast to the measurements of fibronectin synthesis, there was no enhancement of integrin β1 chain synthesis in the vegetal hemisphere of the embryo, but some experiments revealed a higher rate of synthesis in the marginal zone than in the animal pole region (Fig. 6B).

These results indicate that there can only be a very slight increase in synthesis of the integrin β1 chain in response to mesoderm induction. It is not clear whether this would be sufficient to allow animal pole blastomeres to spread on fibronectin. Another possibility, however, is that changes in integrin α subunit synthesis might occur in response to XTC-MIF. Fig. 6B reveals clear differences in α subunit synthesis between animal and vegetal pole regions, and it is possible that more subtle differences occur between induced and uninduced animal pole regions.
Fig. 6. XTC-MIF does not cause a stimulation of fibronectin or integrin \( \beta_1 \) chain synthesis in Xenopus animal pole regions. More fibronectin is synthesized in the vegetal hemisphere of the embryo. Animal pole regions, in the presence or absence of XTC-MIF, marginal zone regions, and vegetal pole regions of Xenopus embryos were cultured in \([35S]\) methionine from the mid blastula stage to the late gastrula stage. Extracts were then subjected to immunoprecipitation using an anti-fibronectin antibody, with normal rabbit serum (NRS) as a control (A), or using an antibody directed against the integrin \( \beta_1 \) chain, with pre-immune serum as a control (B). As an additional control in B, immunoprecipitations were also carried out in the presence of the original peptide antigen. In both A and B, gel loading was normalized according to counts per minute incorporated into total acid-insoluble material.

(A) Lanes 1, 3 and 5 show levels of fibronectin synthesis in animal pole, marginal zone, and vegetal pole regions respectively. Lanes 2, 4 and 6 show NRS controls for lanes 1, 3 and 5 respectively. Lanes 7 and 8 compare fibronectin synthesis in control (lane 7) and XTC-MIF-treated (lane 8) animal pole explants respectively. Arrow indicates fibronectin. (B) Lanes 1, 4, 7 and 10 show levels of integrin \( \beta_1 \) chain synthesis in animal pole regions treated with XTC-MIF, control animal pole regions, marginal zone regions, and vegetal pole regions respectively. Lanes 2, 5, 8 and 11 show pre-immune serum controls for lanes 1, 4, 7 and 10 respectively. Lanes 3, 6, 9 and 12 show controls in which the original peptide antigen was included in the immunoprecipitation reaction. Arrow indicates integrin \( \beta_1 \) chain. The 2.8-fold increase in synthesis of the integrin \( \beta_1 \) chain observed in animal pole explants treated with XTC-MIF (compare lanes 1 and 4) is the largest we have observed in a total of 7 experiments. Bracket indicates integrin \( \alpha \) chains that co-precipitate with the \( \beta \) chain. Note that these differ in the animal pole and vegetal pole regions.

Discussion

This paper describes two principal results. First, we show that Xenopus animal pole blastomeres exposed to XTC-MIF acquire the ability to spread and migrate on fibronectin. This ability is abolished by incubating cells in a peptide corresponding to the fibronectin cell binding site, but this peptide does not inhibit the convergent extension movements of intact XTC-MIF-treated explants. Convergent extension may instead depend on active local cell mixing (Keller and Hardin, 1987; Keller and Tibbetts, 1989; our unpublished observations). In the second part of the paper, we demonstrate that the ability of induced blastomeres to spread on fibronectin is not accompanied by an increase in the rate of synthesis of fibronectin itself, or by a significant increase in the rate of synthesis of one component of potential fibronectin receptors, the integrin \( \beta_1 \) chain. We discuss these two points separately.

Animal pole cells exposed to XTC-MIF spread and migrate on fibronectin

In the first part of this paper, we show that Xenopus animal pole blastomeres exposed to XTC-MIF acquire the ability to spread and migrate on a fibronectin-coated surface. This spreading is completely inhibited by the synthetic peptide GRGDSP, but less so by the peptide GRGESP. Prospective mesodermal cells of the marginal zone also have the ability to spread and migrate on fibronectin, but uninduced animal pole cells do not (Nakatsuji, 1986; see also Komazaki, 1988). Interestingly, Winklbauer (1988) has recently shown that endodermal cells of Xenopus also adhere strongly to fibronectin; it is not possible, therefore, to decide directly from these data whether XTC-MIF induces animal pole cells to become mesoderm or to become endoderm.

The role of fibronectin-mediated cell adhesion and migration in amphibian gastrulation has been discussed by Keller (1986) and Keller and Tibbetts (1989), who suggest that the mesoderm of Xenopus can be divided into two subpopulations. It is only the early-involuting mesoderm, which differentiates into head, heart, blood islands and lateral mesoderm, that migrates and spreads on the blastocoel roof. The later-involuting mesoderm, that differentiates into notochord and somite, does not migrate but undergoes convergent extension, which causes a dramatic elongation and narrowing of the mesoderm through active cell rearrangement and intercalation. Keller (1986) suggests that one role of migration may be to orientate the early convergent
extension movements, preventing the formation of an external excrescence; migration does not seem to be required, however, for the convergent extension movements themselves, or for constriction of the blastopore or the organization of the mesodermal mantle (see Schechtman, 1942; Keller, 1984; Keller et al. 1985).

Our data indicate that blastomeres of animal pole regions exposed to XTC-MIF are able to participate both in migration (Fig. 4) and in convergent extension (Fig. 5), and Keller’s conclusion that cell migration is not required for convergent extension is supported by our observation that elongation of XTC-MIF-treated animal pole explants is not inhibited by the synthetic peptide GRGDSP (Fig. 5). Unequivocal interpretation of this experiment is difficult because it is not possible to be certain that the peptide gained access to all the cells in the treated animal pole regions. Nevertheless, the fact that explants treated with GRGDSP were indistinguishable from explants treated with GRGESP and from untreated controls argues that fibronectin-mediated adhesive interactions are not required for convergent extension of induced animal pole regions. In support of this, it is significant that microinjection of RGD-containing peptides into the blastocoels of *Xenopus* embryos has little effect (J.C.S., unpublished observations), whereas this treatment completely blocks gastrulation in the urodele *Pleurodeles* (Boucaut et al. 1984b). This may reflect the fact that in urodeles mesodermal cells tend to migrate more as individuals after involution, while in *Xenopus* it is a cohesive population of cells that undergoes convergent extension movements (Keller, 1986).

The ability of XTC-MIF-treated animal pole blastomeres to spread and migrate on fibronectin is significant because it occurs at the level of single cells, and, as Gurdon (1987) has emphasized, this should simplify the molecular analysis of induction. Until recently the only mesoderm-specific response that has been observed in single cells has been the suppression of epidermal differentiation (Symes et al. 1988); positive responses, such as muscle formation, have required cells to be present in groups (Gurdon, 1988; Symes et al. 1988), or at least to undergo several cell divisions (Godsave and Slack, 1989). For this reason it is of great importance to understand the molecular basis of the adhesion of induced cells to fibronectin and discover how this is linked with the activation of ‘immediate early’ genes such as Mix. 1 (Rosa, 1989).

_Mesoderan induction is not accompanied by an increase in synthesis of fibronectin or the integrin β₁ chain_

The data presented here show that XTC-MIF does not significantly affect rates of synthesis of fibronectin or of the integrin β₁ chain (Fig. 6). We have not examined levels of mRNA for these proteins, but it seems likely that these are not affected by XTC-MIF either; this is currently under investigation. Therefore, one can conclude that the biological effects of XTC-MIF are not mediated by control of overall synthesis of fibronectin or of the integrin β₁ chain. However, several other possible mechanisms whereby XTC-MIF could affect fibronectin-mediated adhesion and/or migration must be considered.

In the case of fibronectin, it is possible that XTC-MIF differentially affects the expression of the variously spliced forms. Fibronectin synthesis occurs from both maternal and embryonic transcripts, the latter appearing at around the time of gastrulation. (Lee et al. 1984; DeSimone, Norton and Hynes, in preparation). The maternal mRNA is already spliced and the predominant form contains all the alternatively spliced segments (DeSimone, Norton and Hynes, in preparation). Barron some sort of selective translation induced by XTC-MIF which, though possible, seems improbable, it would appear that expression of different forms of fibronectin from the maternal pool is an unlikely point of regulation. However, the activation of fibronectin transcription, which occurs shortly before gastrulation, raises the possibility that XTC-MIF may regulate splicing of these transcripts. This requires investigation, especially since XTC-MIF shares many properties with transforming growth factor β (TGF-β; Smith et al. 1988) and indeed TGF-β has also been shown to induce elongation with subsequent mesoderm formation in *Xenopus* animal pole explants (Rosa et al. 1988). TGF-β has been reported to affect splicing of fibronectin RNA in cultured cells (Balza et al. 1988), and fibronectin splicing is also altered during wound healing (ffrench-Constant et al. 1989) where TGF-β is thought to play a role (Mustoe et al. 1987).

Another potential level of regulation of fibronectin expression is at post-translational steps including export and assembly into extracellular matrix. TGF-β promotes the latter process in cultured mammalian and avian cells (Ignotz and Massagué, 1986, 1987) and XTC-MIF could do so as well.

However, the results presented in Figs 1–3 demonstrate that animal pole cells treated with XTC-MIF show increased adhesion and spreading on exogenous fibronectin and it seems more likely that this effect is mediated through alterations in fibronectin receptors.

The fact that synthesis of the integrin β₁ chain is not greatly affected by XTC-MIF may not be surprising. TGF-β treatment of cultured mammalian and avian cells elevates the surface level of several integrins including fibronectin receptors (Ignotz and Massagué, 1987; Roberts et al. 1988; Heino et al. 1989). However, much of this effect arises by increases in synthesis of α subunits, while the rate of synthesis of the β₁ subunit changes only a little and rises only relatively late after TGF-β treatment (Roberts et al. 1988). Furthermore, cells transformed by oncogenic viruses show reduced fibronectin receptor function and alterations in α subunit expression (Plantefaber and Hynes, 1989) while expression of the integrin β₁ chain at both mRNA and protein levels is largely unaffected (Norton and Hynes, 1987; Plantefaber and Hynes, 1989). There are preliminary indications that the pattern of α subunits varies throughout the embryo (Fig. 6B) and it is possible that this occurs in response to XTC-MIF or other inducing factors. Alterations in the pattern of integrin subunit
expression could affect both the ability of cells to adhere to and migrate on extracellular matrix and/or the assembly of matrix itself. However, further investigation of these interesting possibilities will require specific reagents, which are currently not available for amphibians.

In summary, our experiments appear to rule out overall synthesis of fibronectin and the integrin β chain as points of regulation by XTC-MIF but more subtle effects of this factor on the expression of fibronectin and integrins remain possible. Furthermore, effects of XTC-MIF on other matrix proteins such as laminin, which is known to be present in the amphibian embryo (Darribère et al. 1986; Nakatsuji et al. 1985b), also need examination.

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