Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis*

K. SYMES and J. C. SMITH

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

Summary

The first inductive interaction in amphibian development is mesoderm induction, in which an equatorial mesodermal rudiment is induced from the animal hemisphere under the influence of a signal from vegetal pole blastomeres. We have recently discovered that the *Xenopus* XTC cell line secretes a factor which has the properties we would expect of a mesoderm-inducing factor. In this paper, we show that an early response to this factor by isolated *Xenopus* animal pole regions is a change in shape, involving elongation and constriction. We show by several criteria, including general appearance, timing, rate of elongation and the nonrequirement for cell division that these movements resemble the events of gastrulation. We also demonstrate that the movements provide an early, simple and reliable indicator of mesoderm induction and are of use in providing a 'model system' for the study of mesoderm induction and gastrulation. For example, we show that the timing of gastrulation movements does not depend upon the time of receipt of a mesoderm-induction signal, but on an intrinsic gastrulation 'clock' which is present even in those animal pole cells that would not normally require it.

Key words: *Xenopus*, Amphibia, mesoderm induction, cell movement, gastrulation, mesoderm-inducing factors, developmental clocks.

Introduction

Inductive interactions play a major role in the diversification of cell types during vertebrate development (reviewed by Smith, 1987a). The first such interaction in amphibian development occurs around the blastula stage, when an equatorial mesodermal rudiment is induced from the animal hemisphere under the influence of a signal from vegetal pole blastomeres (Nieuwkoop, 1969, 1973; Sudarwati & Nieuwkoop, 1971; Dale, Smith & Slack, 1985; Gurdon, Fairman, Mohun & Brennan, 1985; Sargent, Jamrich & Dawid, 1986). The molecular basis of mesoderm induction is unknown, but we have recently discovered that the *Xenopus* XTC cell line (Pudney, Varma & Leake, 1973) secretes a protein of $M_r$16,000 which has the properties expected of a mesoderm-inducing factor (Smith, 1987b). Thus, isolated animal pole regions exposed to medium conditioned by XTC cells form muscle, notochord and other mesodermal cell types instead of differentiating as epidermis (Smith, 1987b).

One of the earliest responses to XTC-conditioned medium that was noticed during the above study was a dramatic change in shape of the test explants, as if they were attempting to undergo gastrulation movements. In this paper, we investigate these movements further and show by several criteria that they do resemble the events of gastrulation. Thus, the movements begin at the same time sibling host embryos commence convergent extension (Keller, Danilchik, Gimlich & Shih, 1985); the rate of elongation of induced explants is similar to that of isolated dorsal marginal zone regions (Keller et al. 1985); and like the gastrulation movements of whole embryos (Cooke, 1973a,b), the change in shape of induced explants will occur in the absence of cell division.

The observation that animal pole explants can be induced to undergo gastrulation movements will be of importance in coming to understand the cellular and molecular basis of gastrulation (Keller et al. 1985; Keller, 1986). The results are also of interest because the movements mark the earliest known response to mesoderm induction. Until now the earliest marker has been the expression of muscle-specific actin genes, which can only be detected towards the end of gastrulation (Gurdon et al. 1985). The gastrulation
movements of induced animal pole explants occur at least 2 h in advance of this, at the early- to mid-gastrula stage, and we show that they provide a simple and reliable indicator that mesodermal induction has occurred. This early response should simplify the task of coming to understand the intracellular events between receipt of a mesoderm-induction signal and subsequent determination as a mesodermal cell (see Gurdon, 1987).

Finally, the present results allow us to test an idea concerning the timing of gastrulation, that the movements begin a set time after the receipt of a mesoderm-induction signal. We find that this is not the case, for if isolated animal pole regions are exposed to mesoderm-inducing activity at different developmental stages they nevertheless commence gastrulation at about the same time. This indicates that cells in the *Xenopus* embryo contain a gastrulation ‘clock’, which is running even in those animal pole cells that would not normally require it.

**Materials and methods**

**Embryos**

Embryos of *Xenopus laevis* were obtained by artificial fertilization as described by Smith & 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 & Faber (1967).

**XTC-conditioned medium**

Serum-free conditioned medium from the XTC cell line (Pudney et al. 1973) was prepared as described by Smith (1987b). Serum-free conditioned medium from XL cells (Anizet, Huwe, Pays & Picard, 1981), which contains little mesoderm-inducing activity (Smith, 1987b), was used as a negative control in most experiments.

**Operations**

Operations were carried out in Petri dishes coated with 1 % Noble Agar and containing three-quarter-strength NAM. In all experiments, the piece of animal pole responding tissue was a square subtending an average solid angle of about 60° dissected from the centre of the pigmented hemisphere of the embryo. In most experiments, this piece of tissue was transferred, blastocoel-facing surface up, to medium containing mesoderm-inducing activity or a control solution. In some experiments, however, the animal pole tissue was combined with a vegetal pole region as described by Dale et al. (1985) and Gurdon et al. (1985). Combinations and explants were cultured at room temperature (18–22°C).

**Cell division inhibitors**

Four inhibitors of cell division were used in this study: aphidicolin, colchicine, cytochalasin B and mitomycin C. All four were obtained from Sigma and used at 10 μg ml⁻¹.

**Time-lapse micrography**

Still photographs of *Xenopus* animal pole explants were taken with a Zeiss Photomicroscope III and a Plan 2.5/0.08 objective. Epi-illumination was provided by a Schott KL1500 fibre-optic light source. Exposures were made at 20 min intervals by plugging a timed-pulse generator (manufactured at the NIMR) into the appropriate socket on the Photomicroscope. Ilford HP5 film was used.

Video recordings of explants were made with a similar 2.5/0.08 objective mounted on a Leitz Ortholux II microscope. A Hitachi KP120E video camera was used and connected to a Panasonic NV-8050 time-lapse recorder.

**Histology**

Some induced and uninduced explants were allowed to develop for 3 days until control embryos reached stage 40. These explants were fixed and embedded in paraffin wax as described by Smith (1987b) and 10 μm sections were stained by the Feulgen/Light Green/Orange G technique of Cooke (1979).

Explants used for determination of mitotic indices were fixed in Mirsky’s fixative overnight. They were embedded in Historesin (LKB) and sectioned at 2 μm. Every fifth section was mounted on a microscope slide and stained with 10 μg ml⁻¹ 4′,6-diamidino-2-phenylindole-dihydrochloride (DAPI) for 10 min. The sections were examined using a Zeiss Photomicroscope III equipped for epifluorescence.

**SDS–polyacrylamide gel electrophoresis and immunoblotting**

In some experiments, the presence of the myosin heavy chain was used as a marker of muscle formation and thus of mesoderm induction. Specimens were dissolved directly in gel sample buffer as described by Smith (1987b) and run on uniform 6 % mini slab gels (Bio-Rad model 360) using the buffer system of Laemmli (1970). The presence of the myosin heavy chain could be observed after Coomassie blue staining of tracks containing a single induced *Xenopus* animal pole explant. However, for unequivocal identification of this band, the proteins in the gel were usually transferred electrophoretically to nitrocellulose and reacted with a rabbit antiserum raised against adult *Xenopus* myosin heavy chain, exactly as described by Smith (1987b).

**Results**

In most of the experiments described below, heated undiluted XTC-conditioned medium prepared according to Smith (1987b) was used as the source of mesoderm-inducing activity. In response to this, isolated *Xenopus* animal pole regions invariably form muscle and almost always form notochord and neural tissue (Fig. 1); that is, according to the definition of Slack & Forman (1980), Dale et al. (1985) and Smith, Dale & Slack (1985), they form dorsal mesodermal structures. The behaviour of newly induced animal pole explants should therefore be compared with that of the isolated dorsal marginal zone of the *Xenopus* embryo.
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Fig. 1. The effect of XTC-conditioned medium on isolated *Xenopus* animal pole regions. Midblastula (stage 8) *Xenopus* animal pole regions were allowed to develop in heated XTC-conditioned medium (A) or in three-quarter-strength NAM (B) for 3 days at room temperature. The explants were then fixed and sectioned. Explants cultured in XTC-conditioned medium form notochord (not), muscle (mus) and neuroepithelium (neur), cell types normally formed by the dorsal marginal zone. Explants cultured in three-quarters-strength NAM form ‘atypical epidermis’. Bars, 100 µm.

Induced animal pole explants undergo gastrulation-like movements at the appropriate stage

When isolated *Xenopus* midblastula (stage 8) animal pole regions are cultured in a simple balanced salts solution such as NAM (Slack, 1984), or in diluted L15 medium, they tend to curl up into a sphere, enclosing the original blastocoel-facing surface and surrounding it with heavily pigmented superficial cells (Fig. 2). This rounding-up process begins as soon as the animal pole tissue is removed from the donor embryo and is virtually complete within 3 to 4 h. The explants remain spherical for the next 2 or 3 days and eventually differentiate as epidermis: virtually all the cells stain with an antibody to keratin (Dale et al. 1985; Smith et al. 1985) while the outer, and some of the internal, cells react with the epidermis-specific monoclonal antibody 2F7.C7 (Jones & Woodland, 1986).

By contrast, stage 8 animal pole explants cultured in medium conditioned by the XTC cell line differentiate into a variety of cell types including muscle and notochord (Smith, 1987b; Fig. 1). For the first 2–3 h after being dissected from the animal hemisphere of midblastulae such explants are indistinguishable from uninduced controls. However, 4–5 h after dissection, when control embryos are early- to mid-gastrulae, the explants begin to deform. Those internal unpigmented cells that have not yet been covered by superficial cells begin to push away from the body of the explant (Fig. 2B,C); at the same time the pigmented cell layer constricts and elongates (Fig. 2D). When these events are followed by time-lapse video micrography the elongation of the explants is seen to be accompanied by dramatic cell movement and mixing.

We have followed 21 midblastula animal pole explants cultured in XTC-conditioned medium by time-lapse micrography. In each case, the explants began to deform when control embryos commenced convergent extension in the dorsal marginal zone, at stage 10–5–11 (early to midgastrula). The mean rate of extension of the explants was 3.1 µm min⁻¹, which compares remarkably well with the elongation rate of...
isolated dorsal marginal zones, where the noninvoluting marginal zone extends at 3.6 μm min⁻¹ and the involuting marginal zone at 2.6 μm min⁻¹ (Keller et al. 1985).

We have never observed deformation and extension of Xenopus animal pole explants cultured in NAM, diluted L15 medium or in medium conditioned by XL cells, which contains little or no mesoderm-inducing activity (Smith, 1987b). In experiments in which XTC mesoderm-inducing activity is fractionated by gel filtration chromatography (Smith, 1987b) or by other procedures (J. C. Smith & M. Yaqoob, unpublished data), fractions that contained mesoderm-inducing activity also caused animal pole explants to extend (data not shown). Furthermore, the animal pole component of animal–vegetal combinations, prepared as described by Dale et al. (1985) also seem to deform at the midgastrula stage (data not shown, but see fig. 2A, Gurdon & Fairman, 1986) although the movement is less dramatic, perhaps because it is impeded by the vegetal pole cells.

In general appearance (see figs 3, 4 of Keller et al. 1985), and rate and timing of extension (Keller et al. 1985), the movements of induced animal pole explants resemble the gastrulation behaviour of isolated Xenopus dorsal marginal zones. To confirm this conclusion, we have investigated a further aspect of this early response to mesoderm induction: the requirement for cell division.

Gastrulation-like movements of induced animal pole explants do not require cell division

Gurdon & Fairman (1986) have shown that mesoderm induction, as studied in animal–vegetal combinations and assayed by cardiac actin gene activation, does not require cell division. Cooke (1973a, b) has shown that the gastrulation movements of intact embryos will proceed in the absence of cell division. We would predict that the gastrulation-like movements of induced Xenopus animal pole explants should also occur in the absence of cell division.

To investigate this, late blastula (stage 9) animal pole regions were cultured in XTC-conditioned medium or three-quarter-strength NAM in the presence of inhibitors of cell division. Late blastula animal pole regions were used instead of midblastula explants because Cooke (1973a, b) found that the gastrulation of whole embryos treated with mitomycin C or colcemid at the earlier stage was hampered by the small number of cells and large cell size. We used four inhibitors of cell division, all at 10 μg ml⁻¹: aphidicolin, colchicine, cytochalasin B and mitomycin C. The effect of the inhibitors on cell division in animal pole explants was monitored by direct observation. Aphidicolin, colchicine and cytochalasin B acted...
Mesoderm induction in *Xenopus* almost immediately, while mitomycin C inhibited cell division within 1 h.

The result of this experiment depended upon which inhibitor was used and, presumably, on its mode of action. Explants cultured in XTC-conditioned medium and mitomycin C extended almost as well as controls, while explants cultured in mitomycin C alone did not extend (compare Fig. 3A, B with 3C, D). Mitomycin C inhibits DNA synthesis and cell division by binding covalently to DNA and forming cross-links between complementary strands (see Kornberg, 1980). Explants incubated in XTC-conditioned medium and colchicine, which binds to tubulin monomers and prevents their assembly into

![Fig. 3. The effects of cell division inhibitors on the elongation of *Xenopus* animal pole explants. Late blastula (stage 9) *Xenopus* animal pole regions were incubated in three-quarter-strength NAM (A, C, E) or XTC-conditioned medium (B, D, F) containing no inhibitor (A, B) or 10 μg ml⁻¹ mitomycin C (C, D) or 10 μg ml⁻¹ colchicine (E, F). The explants were photographed after 8 h, at 20°C. Notice that explants cultured in mitomycin C (D) elongate almost as well as controls (B) while those cultured in colchicine become significantly deformed (F) compared with uninduced controls (E). Scale bar in F is 200 μm, and applies to all figures.](#)
Table 1. Mitotic indices and cell numbers in induced and uninduced animal pole explants

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Mitotic index</th>
<th>Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninduced</td>
<td>Induced</td>
</tr>
<tr>
<td>0</td>
<td>43.0 ± 39.2</td>
<td>29.2 ± 24.3</td>
</tr>
<tr>
<td>2</td>
<td>15.7 ± 2.2*</td>
<td>21.2 ± 8.9</td>
</tr>
<tr>
<td>4</td>
<td>8.5 ± 1.3</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>6</td>
<td>6.0 ± 1.9</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>4.0 ± 1.5</td>
<td>3.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation except * where only two cases were available and the values are means ± half the range.

Mesoderm induction does not produce an increase in rate of cell division

Although XTC-conditioned medium can induce gastrulation-like movements in *Xenopus* animal pole explants in the absence of cell division, it is possible that under normal circumstances an early 'pleiotypic response' (Hershko, Mamont, Shields & Tomkins, 1971) to induction is an increase in the rate of cell division. To investigate this, explants were cultured in XTC-conditioned medium or in 61% L15 medium and fixed in groups of three at 2h intervals. The explants were embedded in Historesin, sectioned at 2μm and stained with DAPI. Each nucleus on every fifth section was noted and scored as being in mitosis or in interphase. The mean nuclear diameter was measured for each explant and the total number of cells in each explant was calculated using Abercrombie’s (1946) equation together with the modifications introduced by Smith (1979).

The results are shown in Table 1. They indicate that there is no significant increase in the rate of cell division of animal pole explants in response to mesoderm-inducing activity. This is clearest for the later time points, where the mitotic index is low and the cell cycle will have become asynchronous (Newport & Kirschner, 1982). From 4 to 8h the mitotic indices do not differ significantly between induced and uninduced explants and the cell numbers are also similar. At the earlier time points, there is wide variation in the mitotic indices because at stage 8 the cell cycles are only just beginning to lose synchrony; this variation will conceal any real differences in cell division rate at short times after exposure to mesoderm-inducing activity. Such differences are unlikely to have occurred because the cell numbers at later stages are so similar. Furthermore, like Slack, Darlington, Heath & Godsav (1987), who used basic fibroblast growth factor as a mesoderm-inducing factor, we were unable to demonstrate a significant increase of incorporation of [3H]thymidine into animal pole explants in response to XTC-conditioned medium (data not shown).
Gastrulation-like movements provide an early and reliable marker of mesoderm induction

Observation of *Xenopus* animal pole explants cultured in XTC-conditioned medium suggested to us that the gastrulation-like movements might serve as an early marker of mesoderm induction. To examine this possibility, we conducted a blind trial in which one of us (JCS) examined 106 animal pole explants for changes in shape at different times after exposure to XTC-conditioned medium or a control solution. Only one of the 49 explants exposed to a control solution was identified as positive at any time. However, 47 of the 57 explants exposed to XTC-conditioned medium were correctly identified as being induced by control stage 11 and 55 by stage 11-5. After examination, each explant was allowed to continue development until control stage 43. They were then solubilized in gel sample buffer and individually assessed for muscle formation by gel electrophoresis and immunoblotting, as described in the Materials and methods section. None of the explants exposed to a control solution formed muscle, but 56 (98%) of the explants exposed to XTC-conditioned medium showed a clear myosin heavy chain band.

In another experiment, 61 animal pole regions were exposed to mesoderm-inducing activity and 47 to a control solution. The explants were examined at control stage 14, when every one was correctly identified as induced or uninduced.

Gastrulation-like movements commence at the midgastrula stage irrespective of when explants are exposed to mesoderm-inducing activity

According to several criteria, including general appearance, timing, rate of elongation and the non-requirement for cell division, the elongation of induced *Xenopus* animal pole explants resembles the gastrulation movements of isolated dorsal marginal zones. The movements might therefore serve as a model system to approach questions about different aspects of gastrulation in *Xenopus*. One such question concerns the timing of gastrulation, since the nature of the 'clock' which regulates the onset of gastrulation is unknown. Among the candidates that have been rejected are: number of cell divisions, number of DNA replication cycles and the ratio of nuclear to cytoplasmic volume (see review by Satoh, 1985).

One suggestion that has not been explored is that the timing of gastrulation depends upon the time of receipt of a mesoderm-induction signal, for it is the mesodermal cells which provide the motive force for gastrulation. We tested this idea by exposing *Xenopus* animal pole regions to mesoderm-inducing activity at the early blastula stage (stage 7) or the late blastula stage (stage 9). At 20°C the interval between these stages is over 3 h. Thus if the gastrulation movements occur at a fixed time after mesoderm induction there should be a similar difference in the timing of elongation of the explants. If the clock is independent of the timing of mesodermal induction, the interval should be slight.

Eleven experiments have been carried out. In each, a pair of sibling animal pole explants, one exposed to XTC-conditioned medium from stage 7 and one from stage 9, were photographed from control stage 9-5 at 20 min intervals. In every case, the explant exposed to conditioned medium at stage 7 commenced elongation before the one exposed at stage 9. However, the interval was always less than 1 h and sometimes less than 20 min (Fig. 4). This is considerably less than the 3-5 h interval between the two stages in normal development and suggests that the timing of gastrulation does not depend upon the time of mesoderm induction. A conclusion similar to this has also been reached by direct observation of six pairs of explants similarly exposed to XTC-conditioned medium at stage 7 or 9 and by observation of eight time-lapse video recordings.

The slightly earlier elongation of explants exposed to mesoderm-inducing activity at stage 7 compared with stage 9 cannot be explained by the time required for the later explants to transduce the mesoderm-induction signal from cell surface to cytoskeleton. This is because experiments in which highly purified mesoderm-inducing activity is microinjected into the blastocoels of early gastrulae reveal that this time is of the order of 30 min (Cooke, Smith, Smith & Yaqoob, 1987), while the interval between stage 9 and stage 10-5, when elongation commences, is about 4 h. A more likely explanation is that stage-9 animal pole regions are less responsive to induction than stage 7 (Dale et al. 1985; Gurdon et al. 1985) making the elongation less dramatic and more difficult to recognize at its inception. In support of this, a direct comparison of induced explants with synchronous sibling controls shows that the residual interval in timing between early and late treatments represents a delay in the late-treated group rather than an advance in the early group.

Discussion

The results described in this paper show that an early response of *Xenopus* animal pole explants to induction by XTC-conditioned medium is a change in shape, which involves both elongation and constriction (Fig. 2). Since the mesodermal cells of the embryo provide the motive force for gastrulation (Keller, 1986) it seemed likely that the elongation of induced explants was due to gastrulation-like activities of the newly mesodermalized cells. In support of
this, we have shown by several criteria that the behaviour of induced explants does resemble that of the prospective mesoderm and particularly the dorsal marginal zone. These criteria include general appearance, the timing of the movements, the rate of elongation and the nonrequirement for cell division. We shall discuss two aspects of these results.

_Gastrulation movements provide an early marker of mesodermal induction_

Direct observation suggests that the change in shape of induced animal pole explants commences close to control stage 10.5: the early- to mid-gastrula stage. In blind trials, we found that it is possible to identify induced explants with 85% certainty by control stage 11 and with greater than 95% certainty by stage 11.5. This is about 2 h earlier than the expression of the mesoderm-specific α-actin genes frequently used as markers of mesoderm induction (Gurdon, Brennan, Fairman & Mohun, 1984; Sargent et al. 1986). Direct observation of explants is also cheap, simple and rapid and this approach has been a great help in the purification of the mesoderm-inducing activity of XTC-conditioned medium (J. C. Smith & M. Yaqoob, unpublished data).

_Induced animal pole explants as a model for the study of gastrulation_

Gastrulation is a complex process involving epiboly, involution, convergent extension and directed cell migration (Keller et al. 1985; Keller, 1986). To analyse gastrulation, it is necessary to reduce the whole to its component parts and then, if possible, to study each separately. Keller (1986) has shown that the dramatic deformation of the isolated Xenopus dorsal marginal zone is due almost entirely to convergent extension, perhaps the most important part of gastrulation, yet also the least understood. The elongation and constriction of induced Xenopus animal pole explants closely resembles the behaviour of isolated dorsal marginal zones, and is almost certainly due to the same process. Future study of convergent extension in induced animal pole regions rather than in isolated dorsal marginal zones will have the advantages that a homogenous population of cells is under observation and that the cells may be studied from a stage prior to the onset of gastrulation.

The results described in this paper demonstrate that convergent extension in induced animal pole explants does not involve a significant increase in cell division rate and can even occur in the absence of cell division. This is consistent with Keller’s view that this ‘main engine’ of gastrulation functions through cell rearrangement and intercalation rather than, for example, orientated cell division. In future experiments, we intend to examine this further by studying cell mixing and intercalation in induced explants using both direct observation and cell lineage analysis with fluorescein–lysine–dextran (Gimlich & Braun, 1985). We shall also investigate the role of extracellular matrix components such as fibronectin and laminin in convergent extension. Fibronectin has been shown to be present in a fibrillar network in the Xenopus early gastrula (Lee, Hynes & Kirschn, 1984; Nakatsuji, Smolira & Wylie, 1985) and laminin has been detected in urodèle embryos (Nakatsuji, Hashimoto & Hayashi, 1985; Darribère et al. 1986) and will serve as an in vitro substrate for Xenopus mesodermal cell migration (Nakatsuji, 1986).

Our attempts to study the role of cell migration in the extension of induced animal pole explants, using techniques described by Gerhart et al. (1984) and Nakatsuji (1986) were unsuccessful. We had predicted that, like cells isolated from the dorsal marginal zone and unlike cells from the animal pole region, cells from induced animal pole explants should be able to spread and migrate on a fibronectin-coated substrate. They could: but in most experiments so could cells from uninduced explants and cells freshly isolated from the animal pole regions of early gastrulae. This was the typical result observed in over 30 experiments using a range of calcium concentrations and human and bovine fibronectin concentrations. We cannot account for the difference between our results and those of others except to suggest that our disaggregation technique, which avoids the use of chelating agents, is more gentle than other methods and causes least damage to the animal pole cells. We are reluctant to adopt a harsher technique merely to demonstrate what may be a minor quantitative difference between the animal pole cells and the marginal zone cells. If such a difference is indeed minor this would serve to emphasize that cell migration plays a relatively minor role in Xenopus gastrulation (Keller, 1986).
A more successful experiment (Fig. 4) demonstrated that the gastrulation movements of induced animal pole explants tend to occur at a fixed stage of development, rather than at a fixed time after mesoderm induction. The time of mesoderm induction, like the number of cell divisions, number of DNA replication cycles and the ratio of nuclear to cytoplasmic volume (Satoh, 1985), is therefore ruled out as a component of the gastrulation 'clock'. It is noteworthy, however, that the clock is running in cells that normally would not require it – those which in normal development form epidermis (Dale et al. 1985).

These results are similar to those of Gurdon et al. (1985), who demonstrated that the time of muscle-specific actin gene activation in animal–vegetal combinations does not depend upon the stage at which the animal pole region receives an inductive stimulus. However, it is not known whether the clock that regulates the onset of gastrulation is the same clock that regulates actin gene activation, and it is not obvious how this could be tested.

We are grateful to Jonathan Cooke, Jack Price and Michael Sargent for helpful discussions and Tony Magee and Dennis Summerbell for help and advice concerning video micrography. We also thank Emma Smith for help with gel electrophoresis and Heather Streeter and Jillian Charlton for the fibronectin used in the unsuccessful cell spreading experiments. Karen Symes is supported by a MRC studentship.

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(Accepted 9 June 1987)