Potentiation by the lithium ion of morphogenetic responses to a *Xenopus* inducing factor

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Summary

We have cultured explants of *Xenopus* blastular animal cap tissue from embryos that had received an earlier treatment with LiCl and from their untreated siblings, in various concentrations of XTC-cell-derived mesoderm-inducing factor (XTC-MIF, Smith, 1987; Smith et al. 1988). The pretreatment with lithium that we used transforms later morphogenesis in the whole embryo to give radialized body forms with anterior/dorsal levels of structure grossly over-represented. In addition, animal caps from 'Li+' embryos were allowed to develop without exposure to *in vitro* MIF (Li+ controls) and compared with normal uninduced control explants, and explants were made from normal early blastulae but given various initial treatments with LiCl in culture.

The results confirm that the lithium ion itself will not induce mesoderm in competent, animal cap tissue of *Xenopus*. It does, however, enhance the responsiveness of this tissue to XTC-MIF, in a way that parallels its recently reported effect in the case of another mesoderm inducer of different character, bFGF (Slack et al. 1988).

The effects observed are sufficient to imply that the altered body pattern that follows lithium treatment, in whole embryos, could be caused by modulation of the responses to an unaltered pattern of *in situ* inductive stimuli. We also observe evidence that appreciable inductive signals reach animal pole tissue beyond the limits of mesoderm formation in normal development.

Relatively low concentrations of MIF prevent the development of an epidermis-specific marker in dissociated blastular animal cap cells (Symes et al. 1988). When such experiments are repeated in relation to the lithium pretreatment of embryos, such treatment is seen to have sensitized the cell population, so that the MIF concentration range that assures complete suppression of the marker is reduced.

The results are discussed in relation to induction considered as pattern formation.

Key words: lithium ion, induction, morphogenesis, pattern formation, inducing factors, *Xenopus laevis*.

Introduction

Lithium has long been known to exert profound and systematic effects on morphogenesis. Distortions in the normal maps describing allocation of cells to body parts are seen that are independent of any effects of cytotoxicity, cytostasis or transient arrest of cell movements. These distortions are sufficiently equivalent across embryo types to encourage the belief that if we understood their biochemical causation we should gain a rather fundamental insight into the normal control of development (see review in Horstadius, 1973). The relevant action of lithium in the cell's regulatory economy is not as yet understood, through recent results suggest that it relates to the inositol triphosphate–protein kinase C message transduction pathway (Busa & Gimlich, 1989).

It may transform metabolism throughout the embryo, but affect pattern formation only by affecting the performance of one among the groups of cells that are interacting via signals at early stages. Thus Nieuwkoop (1970), working with the amphibian blastula stage, decided that the ion probably potentiated the interaction whereby endoderm-specified, vegetal pole material induced mesoderm elements from competent animal pole tissue. Although lithium is recorded as being able to cause mesodermal (and neural) differentiation on its own from animal pole tissue *in vitro* ('vegetalization'). Nieuwkoop felt that it was not acting in this way except at near-lethal doses.

Treatment of entire *Xenopus* morulae or early blastulae results in a systematic transformation of the final body pattern (Kao et al. 1986; Kao & Elinson, 1988; Cooke & Smith, 1988; Regen & Steinhardt, 1988). The entire embryo develops as just those regions of the body that are normally made from meridians of the blastula/gastrula far from the position of sperm entry, thus giving a very partial, anterior/dorsal piece of body structure built on a larger-than-normal scale. In addition, the extreme prechordal region is missing from the final pattern of such embryos (Cooke & Smith,
There is now an opportunity to address the question of whether the pattern changes seen after early lithium treatment are understandable as transformations of the system of responses to inductive signals in competent tissue of the animal hemisphere, or as transformations of the vegetally arrayed pattern of the initial inductive stimuli. Molecular candidates have recently been identified for the intercellular signals involved in the induction by the endoderm of a mesodermal rudiment in the marginal zone of amphibian embryos. Their effects upon animal cap tissue as soluble factors in vitro are being characterized (Smith, 1987; Symes & Smith, 1987; Smith et al. 1988; Cooke et al. 1987; Slack et al. 1987; Kimelman & Kirschner, 1987). The polarity and proportioning of normal mesoderm pattern are presumably controlled by a particular spatial array of the in vivo sources of a small set of such initiating signals. The production of normal-sized mesoderm but without axial structural patterns after u.v. irradiation (Scharf & Gerhart, 1980, 1983; Cooke & Smith, 1987) or of hyperdorsal bodies considerably resembling 'lithium' bodies after heavy water (D₂O) treatment (Scharf et al. 1984) are almost certainly due to altered patterns of vegetal signals. The events in the egg's vegetal region during the first cell cycle that correlate with normal pattern formation (Vincent & Gerhart, 1987) are perturbed by these treatments. The timing of the early lithium-sensitive period, though, between 16- and 256-celled stages, would be consistent with lithium modifying either the output of initial inducers or the responses to them.

Clarification of the nature of the lithium transformation at the above level of analysis will be useful in conjunction with a final knowledge of the relevant biochemical point of action in cells. We have therefore tested the effects of earlier treatment with lithium to the whole embryo on the responses of explants of midblastular animal cap tissue to the Xenopus XCT-cell-derivated mesoderm-inducing factor. This is a single molecule, likely to be a member of the β transforming growth factor family (see Rosa et al. 1988) that leads to induction of mesoderm of dorsal axial type at concentrations in the low ng·ml⁻¹ range (Smith et al. 1988). The lithium treatment employed some two hours of development before the cutting out and MIF treatment of explants, is the one that most reliably produces the transformation of normal morphogenesis referred to above. In addition, we have studied the response to XTC-MIF at the single cell level in dissociated blastulae cells from embryos pretreated with lithium and from their control siblings.

The results suggest a potentiation of the responses to the factor by lithium and are in keeping with those of Slack et al. (1988) involving bFGF, which also acts as a mesoderm inducer but which gives less evidence of an in vivo role in induction of axial structure. At the level of patterns differentiated in intact responding tissue, the effects approximate those of a 10-fold concentration increment in the protein. At the single cell level, a sensitization is seen whereby the concentrations of MIF that cause switching off of the expression of an epidermis-specific cytokeratin marker are lowered (Symes et al. 1988). There is also evidence, from the development of lithium pretreated explants, that, in normal development, signals that are an extension of those causing recognizable mesoderm induction are involved in patterning the entire cell population of the animal cap.

The results are discussed in relation to possible mechanisms of patterned induction in intact tissue and in the whole embryo.

Materials and methods

Synchronously fertilized, sibling Xenopus embryos were prepared by standard methods (Cooke & Smith, 1988). Dejellied embryos were kept in 5 % Ficoll (Sigma M, 70000) in 10 % NAM (amphibian saline) until the stage of operations, to ensure a normal distribution of the original egg material around a well-formed blastocoel. Embryos due to develop to the left, fluorescein channel appearances for epidermal cytokeratin to the right. In the completely uninduced normal explant, all viable cells stain for the marker although internally situated cells usually fluoresce at a relatively reduced level. In the 'Li' explant, there is a sharp interface between a surface component of the tissue remaining epidermal and an inner structure in which the marker is turned off. The interface is not always seen, even where there is internal suppression of the marker (not shown). (G) DAPI appearance of a normal explant from the experiment where such explants gave some evidence of 'endogenous' induction. A partially epithelial structure and cell-free vesicles are apparent, though immunofluorescence revealed no muscle cells in such explants. (H) DAPI and (I) immunofluorescent (for muscle) appearance of a Li²⁺ pretreated explant from this experiment. A substantial group of muscle cells is revealed to be present, such cells not having been otherwise observed in explants without in vitro induction in our laboratory. Scale bar, 100 μm.

Fig. 1. Evidence for an induced condition of animal cap tissue from Li⁺-treated embryos. Sections are shown from explanted stage 8+/9 animal caps, allowed to develop in saline without an in vitro MIF treatment until control late 30's stages. (A,B) Feulgen/light green/orange G histology of explants from control and from Li⁺-pretreated sibling blastulae, respectively. (A) The normal completely uninduced appearance where all cells are of epidermal specification (see D), although of abnormal anatomy for this tissue. (B) The more complete differentiation of some of the epidermis to give an epithelium bounding a fluid-filled cavity, though much spongy but non-mesenchymal tissue remains. This is normal for 'Li⁺' animal cap development, but also for the lowest intensity of induced development. (C,D and E,F) DAPI (for nuclei) and immunofluorescent (for an epidermal marker) appearances of control and of Li⁺-pretreated explant development, respectively. DAPI channel appearances of each section are to the left, fluorescein channel appearances for epidermal cytokeratin to the right. In the completely uninduced normal explant, all viable cells stain for the marker although internally situated cells usually fluoresce at a relatively reduced level. In the 'Li' explant, there is a sharp interface between a surface component of the tissue remaining epidermal and an inner structure in which the marker is turned off. The interface is not always seen, even where there is internal suppression of the marker (not shown). (G) DAPI appearance of a normal explant from the experiment where such explants gave some evidence of 'endogenous' induction. A partially epithelial structure and cell-free vesicles are apparent, though immunofluorescence revealed no muscle cells in such explants. (H) DAPI and (I) immunofluorescent (for muscle) appearance of a Li⁺ pretreated explant from this experiment. A substantial group of muscle cells is revealed to be present, such cells not having been otherwise observed in explants without in vitro induction in our laboratory. Scale bar, 100 μm.
dilution in individual wells of a microtitre plate lined with agarose. An explant that is rather larger than the 60° solid angle around the animal pole used by Smith et al. (1985) can be cut out that gives only a low incidence of spontaneous mesoderm formation without in vitro addition of inductive signals. In one experiment, a dialysate of a much more highly purified preparation of XTC-MIF was used (see Cooke et al. 1987), with completely similar results. Culture of explants in
MIF was continuous until healing to give a sphere, when saline was sometimes replaced by 10% NAM to discourage loss of inner cells by 'exogastrulation'.

Explants and combinations were scored for shape change (Symes & Smith, 1987) at control neurula stages (overnight culture at 18°C) and fixed for histological examination during control 33-37 larval stages. The majority of the material was processed in hot (60°C) wax and sectioned at 7 μm for examination after Feulgen, light green, orange G staining (Cooke 1979, 1981). Samples of control material (no MIF added) and of material responding to the lower concentrations of factor were processed in low-melting-point (37°C) PEDS wax for immunocytochemistry on 10 μm sections (Dale et al. 1985). Primary antibodies used were the monoclonals RD35/3A (courtesy of C. C. Wylie), which is specific to epidermis from neurula stages, binding to epidermis-specific cytokeratins, and 12/101 (courtesy of J. Brookes), which is specific to a surface component of somitic muscle cells from tailbud stages onwards.

In experiments examining the response to XTC-MIF in single animal cap explants, relatively small animal pole explants (see Dale et al. 1985) were cut from stage-8 blastulae and accumulated in groups before disaggregation in calcium- and magnesium-free amphibian saline (CMFM) for culture as single cell suspensions, in the presence of various concentrations of the inducing factor. At control stage 19, the suspensions were harvested, dried down onto microscope slides at 25°C and analysed for expression of the epidermal marker by indirect immunofluorescence with the antibody RD35/3A (see Symes et al. 1988).

Results

Intact animal cap tissue
Animal pole explants given the standard whole-embryo lithium treatment described in Materials and methods undergo extensive cytolysis and cytostasis, probably because of the much greater permeability of the 'inner', blastocoelic cell membrane to the ion. Groups of 64- to 128-cell-stage animal blastomeres were therefore explanted and allowed to heal into spheres to varying extents, or placed with 'inner' sides together in pairs, before being exposed to the ion for various lengths of time. Only 2 out of 35 surviving explants, which included many with normal cell number and size as well as some with cytolysis, showed any mesoderm induction. This was of the lowest grade of intensity. This is within the expectation for dissection error on early explants. Thus, in agreement with Slack et al. (1988), we find that LiCl alone will not induce mesoderm in competent Xenopus animal cap tissue.

In four experiments, animal cap explants from stage 8+ were made directly into 67% NAM and into a series of 3-fold dilutions of MIF in the same saline, using lithium pretreated and untreated sibling embryos. The ranges of MIF activity used were between about 0-04 and 80 units ml

One aspect of the results concerns those explants from lithium-treated embryos that received no subsequent MIF treatment in vitro, the 'Li+ controls'. In three of the experiments these differed significantly in appearance from the overall control explants, having a fluid-filled expansion covered with smooth epithelium resembling normal, bilayered epidermis at control larval stages of differentiation (Fig. 1B). This final appearance was preceded at control neurula stages by a slight elongation (maximum dimension near twice the minimum). In the remaining experiment, three of the six true control explants also showed a significant degree of this appearance and one 'Li+ control' explant proved to contain a small group of muscle cells (Fig. 1H–I). We concluded that, in this experiment, it had been particularly difficult to excise explants as described without including some endogenous induced or inducing material.

During the process of dissecting out explants, as the blastular stage advanced, a subtle but increasing difference was noticed between normal and lithium-pre-treated embryos, more marked in some egg batches than in others. The epibolic movements, leading to accumulation of cell layers in a thicker walled marginal zone (Keller, 1980), appeared inhibited in the Li+ blastulae. This resulted in a smaller-appearing blastocoel, set lower in the embryo under a more uniformly thick-walled animal cap (Fig. 2). An immunocytochemical observation is relevant to understanding this phenomenon. Investigation has revealed that expression of the epitope seen by the monoclonal antibody RD35/3A, an epidermis-specific cytokeratin, tends to be turned off to a slight but variable degree internally, even in the spongy 'atypical epidermis' of uninduced animal caps (Fig. 1C,D). In 'Li+ control' explants, however, the marker was frequently turned off more completely, over much more of the internal
tissue (containing quite normal nuclei) that is situated to one side of their subepidermal cavity (Fig. 1E,F). In 'Li⁺ control', but not normal control explants at stages 33–37, a sharp interface often appeared between a surface zone expressing the epidermal marker and the inner tissue where it was unexpressed.

In relation to in vitro MIF responses, lithium pretreatment had a consistent effect in all four experiments, causing explants to form structures similar to those from control embryos that experienced 9- to 12-fold higher concentrations of the inducing factor. We illustrate this in tabular form for two dose-response experiments (Table 1), by a range of histological appearances in Fig. 3, and by the record of explant shapes at control neurula stages (those of greatest MTF-intensities of induction, over at least a hundred-fold difference). We believe that the signal molecule under study is an initiator of a chain of events resulting in the formation of a bubble of bilayered, true epidermis that differentiates as in a normal embryo and overlies internal cells of explants, is accentuated and more complete, and accompanied by a sharp interface between fluorescing surface cells and non-fluorescing deep ones. Bilayered epidermis and the turning off of the marker internally can also occur independently of each other in explants. Animal caps that have been explanted at stages 8/9 from embryos pretreated with lithium at the 64-cell stage regularly develop one or two in vitro MIF (see above). Early blastula animal caps explanted from normal embryos into equivalent lithium treatments show no appearance of induction at above 'false positive' frequency. We conclude that the above-described development requires at least a threshold level of response to the protein factors of the type we have been calling 'mesoderm' inducers'. Lithium itself is not an inducer, but lithium pretreatment (though not post-treatment) of tissue reveals that the entire blastocoel roof receives some level of natural inducers in normal early development.

(1) At the next intensity level in vitro, explants are covered with normal epidermis, have undergone significant shape change away from the spherical, and are

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**Table 1. The responses to XTC-MIF of animal cap explants from normal and from Li⁺-pretreated blastulae**

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For descriptions of response grades, from 0 ('uninduced') through 1 (low threshold) to 4, see text, Results section. For units activity ml see Cooke et al. 1987 and Smith et al. 1988.
filled with a loose, open-textured mesenchyme that does not resemble normal lateral plate mesoderm yet is quite different from the spongy, epidermally specified tissue inside control explants. A small minority of the mesodermal population usually differentiates as somite muscle. Over a considerable further range of intensification of response, further tissue is recruited into somite muscle differentiation, but without the great elongation and segmentation that goes with a coherent, single system of convergent extension (Keller et al. 1985) and without the genesis of neural tissue.

(3) With further increases in intensity, convergent extension and axial elongation occur, and the final form has the majority of its mesodermal population allocated to a large segmenting mass of somite muscle. It may or may not include a partial rod of notochord, but the lower MIF concentration threshold at which notochord occurs is ill-defined (Smith et al. 1988 – the pure factor),
Fig. 3. The potentiation, by Li⁺ pretreatment, of levels of in vitro response to XTC-MIF. Feulgen/light green/orange G-stained sections are shown of explants as in Fig. 1, but after explantation into stated concentrations (units ml⁻¹) of XTC-MIF. Each set of examples after the same in vitro MIF concentration are from one experiment. (A,B) 0-33 units ml⁻¹ normal and Li⁺ pretreated. This was the lowest level responded to by all explants in an experiment in which the normal controls showed zero induction. (A) Epithelialization, as in the 'Li⁺' explant of Fig. 2B, though this is less extensive. (B) Complete epithelialization of remaining epidermis and considerable internal mesenchyme as distinct from spongy undifferentiated epidermis (cf. Fig. 1A,B). (C) Immunofluorescence for muscle on a further 'Li⁺' explant after 0-33 units ml⁻¹ MIF from the same experiment reveals a small tract of uncompacted somite muscle tissue at one position. (D) Normal and (E,F) 'Li⁺' explants, 3-0 units ml⁻¹. The normal response in this case was comparable to the 'Li⁺' response level shown in B, with extensive mesenchyme but less complete epithelialization of the epidermis. The 'Li⁺' response level was particularly strongly enhanced, giving great elongation, massive segmented somite muscle, neural tissue and in one case a partial rod of notochord. (G,H) Normal and (I) 'Li⁺', 27 units ml⁻¹. The normal response here closely resembled the Li⁺-enhanced examples of E,F in shape, tissue composition and the segmented nature of the somite tissue (see oblique section appearance of G). (I) Non-elongated, pot-shaped morphology, massive internal notochord and neural tissue with smaller amounts of unorganized somite, which is an upper-limit response to the inducing factor. (J,K) Two sections through another 'Li⁺' explant after culture in 27 units ml⁻¹ MIF. This shows another version of the radialized 'anterodorsal' morphology that was only obtained in conjunction with lithium in the present study (though see Smith et at. 1988). A massive cement gland within the epidermis is situated across the explant from a small, more axial-looking region, but separated by much uncharacterized internal tissue. Scale bar, 200 μm. ep, epidermally specified tissue; epi, epithelially differentiated epidermis; mes, mesenchyme and mesothelium; m, somite muscle; n, neural tissue; nc, notochord; cg, cement gland.

suggesting that other circumstances affect the possibility of its formation. Neural formations, which are rare at lower response intensities, are regularly seen in the ectoderm of this and the following types of explant development. They are readily identifiable by their great cell density, arranged as pseudostratified epithelium and cell masses as in normal larval CNS, but identity has been checked with immunofluorescence to Xenopus CNS (see Cooke & Smith, 1989). To judge from published descriptions, this occurrence of nervous system with strongly convergently extending, segmenting somite is most typical of response to the class of inducing factor represented by XTC-MIF, as opposed to the heparin binding growth factor-like class.

(4) A pronounced further level of response intensity is in fact accessible with XTC-MIF alone, though in our own experiments it was usually seen only after lithium pretreatment. Explants become smooth-surfaced and compactly 'pot-shaped' by control larval stages, but often after extrusion at neurala stages of a globular or fat rod-shaped body of notochordal tissue. This allocation to notochord, whether retained internally or not, is now one of the larger mesodermal components but with the overall allocation to mesodermal tissues appearing reduced. Muscle is present but not prominent and there is a massive and usually radialized, thick-walled formation of brain tissue in the outer layer. Frequently, a cement gland forms far from the notochord in the epidermis (Smith et al. 1988). The fact that lithium pretreatment will convert a low-grade, mainly mesenchyme-producing response into an axially patterned, somite-producing one, but also converts the latter type of response into the one just described, strengthens the belief that this is an extreme high grade response.

Dissociated cells

The appearances of differentiated explants suggests that after culture in various concentrations of XTC-MIF, differing proportions of the original cells have been diverted from the epidermal into other pathways of development (K. Symes & J. C. Smith, unpublished data). This is consistent with the idea that the character of the non-epidermal states of differentiation found in an explant is a function of the proportion, or the absolute numbers, of cells in the explant that underwent the first switching steps in a multistep process. The concentration of the morphogen may exert its effects, in part, by setting this variable at the early stages of induction. Other models, where the amounts and characters of induced tissue are independent, are of course possible. As a first step in studying these questions, we investigated the effect of the standard lithium pretreatment upon an aspect of the response to MIF that can be seen in populations of single, dispersed animal cap cells. This response, the switching off of expression of an epidermis-specific cytokeratin marker (Symes et al. 1988), is of an all-or-nothing nature within each cell, but occurs in a progressively increasing proportion of cells with exposure to increasing XTC-MIF concentration.
Fig. 4. Shapes of explants from control and Li⁺-treated blastulae, by control neurula stage. Six groups of four explants from one typical experiment are shown. Those in the upper row were from normal sibling blastulae, those in the lower row from siblings pretreated with lithium as described. Those on the left had been cultured in about 1 unit ml⁻¹ MIF activity, those at centre in 9 units ml⁻¹, those on the right at 80 units ml⁻¹. Stippled regions represent internal tissue that has broken through the epidermal surface of explants (usually at their position of original sealing-up) during deformations due to morphogenesis. Note that this is absent in the low threshold response (top left) where most of the surface is wrinkled epidermis. At the level believed to represent radialized anterodorsal (bottom right) tissue there is never strong elongation but the surface is very smooth. A large area of cell columnarization opposite the extruded material is the precursor of a massive radial neural area. Scale bar, 500 μm.

### Table 2. The switching off of epidermal marker expression in dispersed populations of cell by XTC-MIF. The effect of lithium pretreatment

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500 cells per point were screened for expression of the RD35/3a marker. Lithium-pretreated embryos left to develop, in both experiments, showed high incidence and grade of the Li⁺ pattern restriction (Cooke & E. Smith, 1988).

For units/activity ml see Cooke et al. 1987 and Smith et al. 1988.

necessarily expressed in very different quantitative terms, which make the single cell sensitization appear a less dramatic one. But we do not yet understand the causal chain of signals within explants enough to know whether or not the last described effect is, in itself, sufficient to be the sole cause of the tissue-level effect.

### Discussion

The observations suggest that early blastular lithium treatment of whole embryos could transform and restrict body pattern solely by modulation of the responses to inductive signals in the tissue of the animal
hemisphere. Large animal caps explants, at a particular level of normal response intensity to XTC-MIF, indeed resemble endodermless axial larval patterns without head morphogenesis, while their 'Li+' counterparts develop pot-shaped, radialized morphology very reminiscent of the 'Li+' syndrome in whole embryos. There is thus no need to invoke any alteration of the vegetal array of initiating inducer signals themselves to explain the syndrome, though our experiments do not exclude the possibility that such alteration also occurs in the whole 'Li+' embryo.

The overlapping series of results of induction, seen when FGF-type and XTC (TGFβ)-type protein inducers are used with and without lithium potentiation (cf. Slack et al. 1988) may suggest that the intracellular molecular mechanism of the induction is partly general in its effects rather than specific to a receptor/second messenger system acting for one class of the natural inducers only. This would make comprehensible the restriction of pattern to dorsoanterior levels, caused by whole-embryo treatment with Li+ or its injection into ventral blastomeres (Cooke & Smith, 1988; Kao & Elinson, 1988; Busa & Gimlich, 1988), or the rescue of radialized non-axial development that is caused by local injection of the ion (Kao et al. 1986).

The subtly altered blastular anatomy and low-grade ‘induced’ development of isolated animal caps from Li+-pretreated embryos suggest that the whole field of competent tissue, and not just what we recognize as a ‘marginal zone’, is normally invaded by significant levels of inductive stimuli before gastrulation. This is of considerable interest because of evidence that neural induction may not be wholly separate from, or subordinate to, the induction of the mesoderm rudiment as was thought. It may be initiated during the same episode of pattern formation that organizes the mesoderm, by transfer of inductive signals within the plane of the blastular wall. Thus Sharpe et al. (1987) observe that dorsal ‘ectoderm’ is already biased towards response to neuralization by onset of gastrulation, and Kintner & Melton (1987) find molecular evidence for considerable induction of neural tissue even in exogastrulae, where mesoderm has not come to underlie the competent tissue. Keller & Danilchik (1988) and Keller et al. (1985) document the existence of an autonomously behaving, neural-like territory in the marginal zone just distal to the dorsal mesodermal area by the onset of gastrulation movements. Finally Smith et al. (1988) draw attention to the fact that XTC-MIF itself is probably capable of causing neural development without the intervening production of mesodermal cell types, but possibly via a cascade of earlier signals.

Does formation of mesoderm of various characters during induction depend upon the proportion of the cells within a tract of ectoderm that are initially diverted from ectodermal specification, or upon the absolute numbers of cells thus specified, or is it independent of these variables? We and others have already drawn attention to the possibility that a sequence of signals is involved in patterning (Cooke et al. 1987; Gurdon, 1987; Symes et al. 1988). It is therefore interesting that the effect of lithium pretreatment expresses itself in cultures of dispersed competent cells as an increase in the proportion of such cells that make the only response to induction by XTC-MIF that we can observe under these conditions. In intact animal cap tissue, the population of cells ultimately diverted from epidermal development never approaches 100%, but probably does vary with the concentration of MIF initially experienced in vitro. Lithium treatment may augment the ‘level’ of final responses because it augments the proportion of cells making the first response. But it remains possible that the character of later responses in the system, in giving rise to the tissue types, are independently and directly upgraded by the effect of the ion. The exploration of this, and final elucidation of the biochemical mechanism of Li+ potentiation in the responses, must await experiments of kinds that are not yet possible.

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