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Range and stability of cell fate determination in isolated sea urchin blastomeres

BRIAN T. LIVINGSTON and FRED H. WILT

Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

Summary

We have examined the developmental potential of blastomeres isolated from either the animal (mesomeres) or vegetal (macromeres–micromeres) half of 16-cell embryos of the sea urchin *Lytechinus pictus*. We have also examined the effects of two known vegetalizing agents on the development of isolated mesomeres; LiCl treatment and combination with micromeres, the small blastomeres found at the vegetal pole of the 16-cell embryo. The markers for differentiation used were both morphological (invaginations, spicules and pigment cells) and molecular (gut-specific alkaline phosphatase activity, and monoclonal antibodies against antigens specific for gut and oral ectoderm). Embryoids derived from isolated mesomeres expressed markers characteristic of vegetal differentiation only at very low levels. They did express an antigen characteristic of animal development, the oral ectoderm antigen, but with an altered pattern. Isolated macromere–micromere pairs expressed all markers characteristic of vegetal development, but did not express the marker characteristic of animal development. Increasing concentrations of LiCl caused isolated mesomeres to give rise to embryoids with an increasing tendency to express vegetal markers of differentiation, and it was found that expression of different vegetal markers begin to appear at different concentrations of LiCl. LiCl also caused the marker for oral ectoderm to be expressed in a more normal pattern. Combining micromeres with mesomeres also induced mesomere derivatives to differentiate in a vegetal manner. Micromeres were not completely effective in inducing a more normal pattern of expression of the marker for oral ectoderm. The treatment of isolated mesomeres with both LiCl and micromeres produces a synergistic effect resulting in embryoids expressing markers not induced by either treatment alone.

Key words: cell fate, determination, sea urchin, blastomere, lithium chloride.

Introduction

The ability to distinguish animal from vegetal blastomeres at an early stage, combined with the relative ease of experimental manipulations of these blastomeres, made the sea urchin embryo an attractive organism to developmental biologists during the classical era of experimental embryology. Dissections of embryos and eggs have shown that the developmental potentials of the animal and vegetal halves of sea urchins are widely divergent (for reviews, see Horstadius, 1973; Davidson, 1986; Wilt, 1987). Animal halves form ciliated spheres with exaggerated cilia, which sometimes go on to organize themselves into an oral ciliated band. The relative lack of numerous obvious structures has made 'animal' differentiation more difficult to describe. Vegetal halves, on the other hand, form a wide variety of structures, including gut, spicules and pigment cells. They can sometimes go on to form something resembling a normal pluteus, although with a reduced oral lobe. More often, the larvae formed from vegetal halves lack a mouth and stomadeum. This would be expected from the results of modern lineage studies (Cameron *et al.* 1987), which indicate that the macromeres do not contribute to the oral ectoderm. However, the reports (reviewed by Horstadius, 1973) that vegetal halves occasionally do form oral structures suggests that vegetal blastomeres can regulate to form animal structures. It has also been reported that the influence of vegetal blastomeres is necessary for animal blastomeres to give rise to oral structures (Horstadius, 1973). The availability of molecular markers for differentiated oral (Coffman *et al.* 1985; McClay *et al.* 1987) and aboral ectoderm (Lynn *et al.* 1983; Cox *et al.* 1986) now allow a closer examination of the ability of animal blastomeres to self-differentiate, and the ability of vegetal blastomeres to regulate and form oral ectoderm.

Transplantation studies have shown that vegetal blastomeres can change the fate of animal blastomeres (Horstadius, 1973). Most spectacular of these is the ability of micromeres to induce mesomeres to form what looks like a nearly normal pluteus. Again, the criteria for differentiation in these studies was the formation of complex morphological structures. Our understanding of these inductive events would certainly

benefit from the use of the more sensitive molecular markers available for both vegetal- and animal-derived tissues.

LiCl has also been shown to alter the fate of animal blastomeres. It causes a vegetalization of whole embryos (reviewed by Lallier, 1964) and causes isolated animal halves to form structures characteristic of vegetal differentiation; sometimes whole miniature larvae are formed (Von Ubisch, 1929). Recently, it has been shown that LiCl can cause isolated animal blastomeres to express molecular markers specific for tissues derived from vegetal blastomeres in normal embryos (Livingston and Wilt, 1989). The molecular markers for oral and aboral ectoderm mentioned above now make it possible to determine whether LiCl will inhibit the normal differentiation of animal blastomeres as it induces expression of vegetal markers.

The relationship of the vegetalizing influence of LiCl to that of micromeres has been addressed in the literature only in terms of a vegetal gradient (Runnstrom, 1929). What the underlying mechanism of such a gradient might be has not been addressed. The similar effects of two very diverse stimuli on isolated animal blastomeres suggests that there are mechanisms common to both. An understanding of how these two agents act, together and individually, may shed some light on the processes involved in cell determination in sea urchins.

The work described in this paper reinvestigates some of the classical experiments on sea urchin development using modern experimental methods. Our aim is to gain more detailed information on the processes involved in blastomere determination, so that we can define the observed gradient of developmental potential in molecular terms. We have examined the response of isolated mesomeres to increasing dosages of LiCl and found that the response leading to gut formation is graded, and that different vegetal markers of differentiation are induced at different concentrations of LiCl. Using the antigen Ecto V (Coffman *et al.* 1985; McClay *et al.* 1987) as a marker we have shown that isolated mesomeres do express a marker of ectodermal differentiation, although not in the pattern found in whole embryos. Treatment of mesomeres with LiCl causes Ecto V to be expressed in a more restricted pattern. Macromere–micromere pairs give rise to embryoids that express Ecto V in the foregut, but not in ectodermal cells. We have shown that micromeres can induce mesomeres to differentiate in a vegetal manner, both at the morphological and molecular levels, and, finally, we have shown that micomeres and LiCl have a synergistic effect on the differentiation of isolated mesomeres.

Materials and methods

Isolation and culture of blastomeres

Gametes of *L. pictus* were obtained and fertilized by standard methods (Hinegardner, 1967; Lutz and Inoue, 1986; Hall, 1978). Blastomere pairs were obtained using methods similar to those described previously (Livingston and Wilt, 1989). Embryos were placed in calcium-free sea water (CFSW) during the third cleavage division and cultured with stirring to the 16-cell stage. Cultures were then centrifuged and resuspended in CFSW by shaking until the embryos dissociated into pairs of recently divided blastomeres. The animal pairs (mesomeres) and vegetal pairs (macromere–micromere) were then isolated with a micropipette and cultured in sea water at 15°C on agarose-coated plates. LiCl was added immediately after isolation of mesomeres, then removed after 6–7 h of culture. Treatment of isolated mesomeres with LiCl after 7 h in culture had no effect on their differentiation.

Embryoids were scored after three days in culture for invaginations and pigment cells using a dissecting microscope. Spicules were observed after squashing the embryoids with a coverslip using a differential interference contrast microscope.

Recombination of blastomeres

Cultures of *L. pictus* were separated at the 8-cell stage and placed in CFSW. One half of the culture was used to isolate mesomere pairs as described above. Rhodamine B isothiocyanate (Sigma) was added to the other half as described by Ettensohn and McClay (1986). At the 16-cell stage, the stained culture was centrifuged and resuspended in CFSW until it was a single cell suspension. Micromeres were then removed from this culture using a micropipette. Small depressions were made in sea water agarose plates, and a pair of mesomeres were combined with a single stained micromere in each depression. After 6–7 h culture it was possible to determine if the two types of blastomeres had adhered. Those that had adhered were transferred to fresh sea water and cultured for three days.

Antibody staining

Fixation of embryoids and antibody staining was done using methods described by Ettensohn and McClay (1988), with the following modifications. The embryoids were fixed in siliconized depression slides, then transferred to poly-L-lysine (Sigma)-coated coverslips. Incubations and washes were then all done in drops on the coverslips, which were kept in a moist chamber. Fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (Sigma) were used to visualize the location of the primary antibody. Ecto V and Endo 1 antibodies were generously provided by David McClay.

Results

Response to increasing dosage of LiCl

The details of the differentiative response of mesomeres to LiCl were investigated. Mesomeres pairs were isolated (Livingston and Wilt, 1989, Fig. 1), treated with various concentrations of LiCl for 6–7 h, then cultured in sea water. After 3 days in culture, the resulting embryoids were scored for survival and for invaginations and spicules, both of which are characteristic of vegetal differentiation. The number of mesomere pairs that survived the three days in culture was also determined for each concentration of LiCl used. The results are shown in Fig. 2. The percentage of mesomere pairs that survived to give rise to embryoids remained relatively constant at between 70–80 % for concentrations of LiCl ranging from 0 to [illegible] mM. Concentrations above this level were toxic.

Untreated mesomeres displayed very low levels of invaginations (<5 %) and spicules (1.6 %) and they did

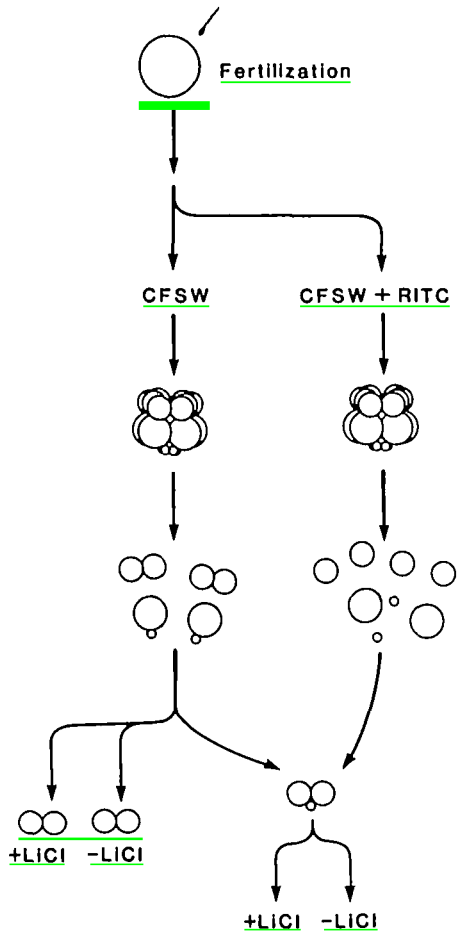

Fig. 1. Isolation and recombination of blastomeres. A scheme of how mesomeres and RITC-stained micromeres are obtained is shown; details are discussed in the text.

not localize an oral field antigen (Ecto V) or display staining for a gut enzyme (alkaline phosphatase) or gut antigen (Endo 1).

Mesomere pairs give rise to some embryoids that exhibit invaginations at the lowest LiCl concentration used (10 mM) and the percentage of embryoids containing invaginated cells increases rapidly with increasing concentrations of LiCl. At a concentration of 40 mM LiCl, greater than 90% of the embryoids in culture exhibit invaginations. This response to LiCl then levels off as the concentration is increased, until 100% of the embryoids exhibit invaginations. An invagination is defined as a contiguous adherent group of cells that buckle into the blastocoel cavity. The majority of these invaginations did not elongate, and the extent of elongation as a function of LiCl concentration was not determined.

A higher concentration of LiCl (20 mM) is necessary to induce mesomere pairs to produce embryoids that elaborate skeletal spicules. The proportion of embryoids with spicules then remained at a constant level. This level of appearance (25%) of spicules by LiCl is lower than that seen in another species, *S. purpuratus* (Livingston and Wilt, 1989), which attains close to 50% with spicules. Less than 1% of the

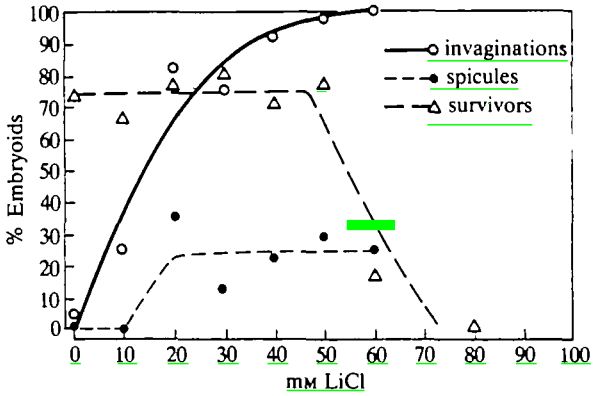

Fig. 2. Response of isolated blastomeres to increasing dosages of LiCl. Data points show the proportion of three-day-old embryoids at each given concentration of LiCl that exhibited invaginations, spicules and the proportion that survived three days in culture. The numbers of embryoids examined for the different cultures at each concentration were as follows: 0=558 (invaginations), 186 (spicules); 10 mM=42 (invaginations), 38 (spicules); 20 mM=44 (invaginations), 40 (spicules); 30 mM=77 (invaginations), 61 (spicules); 40 mM=375 (invaginations), 117 (spicules); 50 mM=58 (invaginations), 51 (spicules); 60 mM=21 (invaginations), 18 (spicules).

embryoids that developed from isolated mesomeres, either untreated or treated with LiCl, produced cells containing echinochrome pigment.

Although cultures from different females showed some variation in their response to any given level of LiCl concentration, in all individual experiments, increasing the dosage of LiCl increased the percentage of embryoids exhibiting the vegetal structures described (Table 1). Similar experiments have been performed using *S. purpuratus*, and the dose–response curves have proven to be remarkably similar, although shifted toward lower LiCl concentrations (data not shown). LiCl was not an effective stimulus if one waited until after the seventh cleavage division to expose the mesomeres to the ion. Prior to that time, increasing time of exposure to LiCl caused an increase in vegetal differentiation of mesomeres.

Expression of Ecto V

We have shown previously that mesomeres isolated from *S. purpuratus* and treated with LiCl develop into embryoids that exhibit molecular markers of vegetal differentiation (Livingston and Wilt, 1989). This result is also found with *L. pictus*, using two markers for the gut lineage, alkaline phosphatase activity (Gustafson and Hasselberg, 1950; Pfohl, 1975; Evola-Maltese, 1957) and the gut-specific differentiation antigen, Endo 1 (Wessel and McClay, 1985). Invaginations of any size induced in mesomere derivatives by LiCl express both of these markers (Table 1).

We next investigated the expression in isolated mesomeres of a marker that is normally expressed in oral ectodermal cells, which are derivatives of animal blastomeres. The monoclonal antibody, Ecto V, (Coffman *et al.* 1985; McClay *et al.* 1987) recognizes an antigen that

Table 1. *Summary of markers examined in blastomere cultures*

	Total no.	% Alk. Phos.	% Endo 1	% Spicules	% General Ecto V	% Broadly localized Ecto V	% Strictly localized Ecto V	% Pigment
Mesomeres	520	5	5	1.6	97	3	0	0.6
Meso+LiCl	367	92	92	22	73	22	5	0.3
Meso+Mic	162	40	40	39	90	10	0	3
Meso+Mic+LiCl	135	97	97	48	36	6	58	48
Mac/Mic	288	98	98	50	35	0	65	90

The first column shows the total number of each type of embryoid examined. The following columns show the percentage of these embryoids that show the character designated at the top. All embryoids were examined for invaginations and pigment cells, and representative samples (50–200) were examined for spicules, enzyme and antibody staining. Assays for alkaline phosphatase were performed as described in Livingston and Wilt, 1989.

is first expressed in a non-localized pattern in blastula. During gastrulation, the antigen gradually becomes localized to the oral ectoderm (derived from mesomeres) and the foregut (derived from macromeres). We examined Ecto V expression in isolated mesomeres, isolated macromere–micromeres and in isolated mesomeres treated with LiCl (Fig. 3). We found that Ecto V is expressed in embryoids derived from isolated mesomeres (Fig. 3A; Table 1). The expression begins during the second day in culture and is not localized. The expression is more intense after three days in culture, and remains distributed over the entire surface of the embryoid.

Treatment of mesomeres with LiCl does not inhibit the expression of Ecto V; remarkably, embryoids derived from LiCl-treated mesomeres express Ecto V at levels apparently similar to untreated mesomeres, and in many of the cases examined showed a shift in expression of this antigen to surface cells located away from the site of the invaginations (Fig. 3B), a pattern not unlike that found in normal embryos. The invaginated cells did not express Ecto V, though the appearance of an invagination is necessary but not sufficient for the partial localization of Ecto V in the surface epithelium. In the majority of the cases of LiCl-treated mesomeres that show localized expression, a broad range of surface cells, slightly shifted away from the site of invagination, expressed Ecto V (Fig. 3B; Table 1).

In one culture, the LiCl-treated mesomeres gave rise to highly vegetalized embryoids consisting mostly of exogastrulae. In these cases, both the tip of the gut and a small patch of ectoderm were labeled with Ecto V (Fig. 3E). This is the same pattern seen in whole embryos that form exogastrulae (D. McClay, personal communication). Embryoids derived from macromere–micromere pairs also expressed Ecto V, in a localized pattern (Fig. 3C; Table 1). Many of these embryoids were compact, with large invaginations and small blastocoeles, which made it difficult to determine which tissue was expressing the antigen. Focusing through the embryoid showed that in these cases the Ecto V expression observed was located in cells in the interior

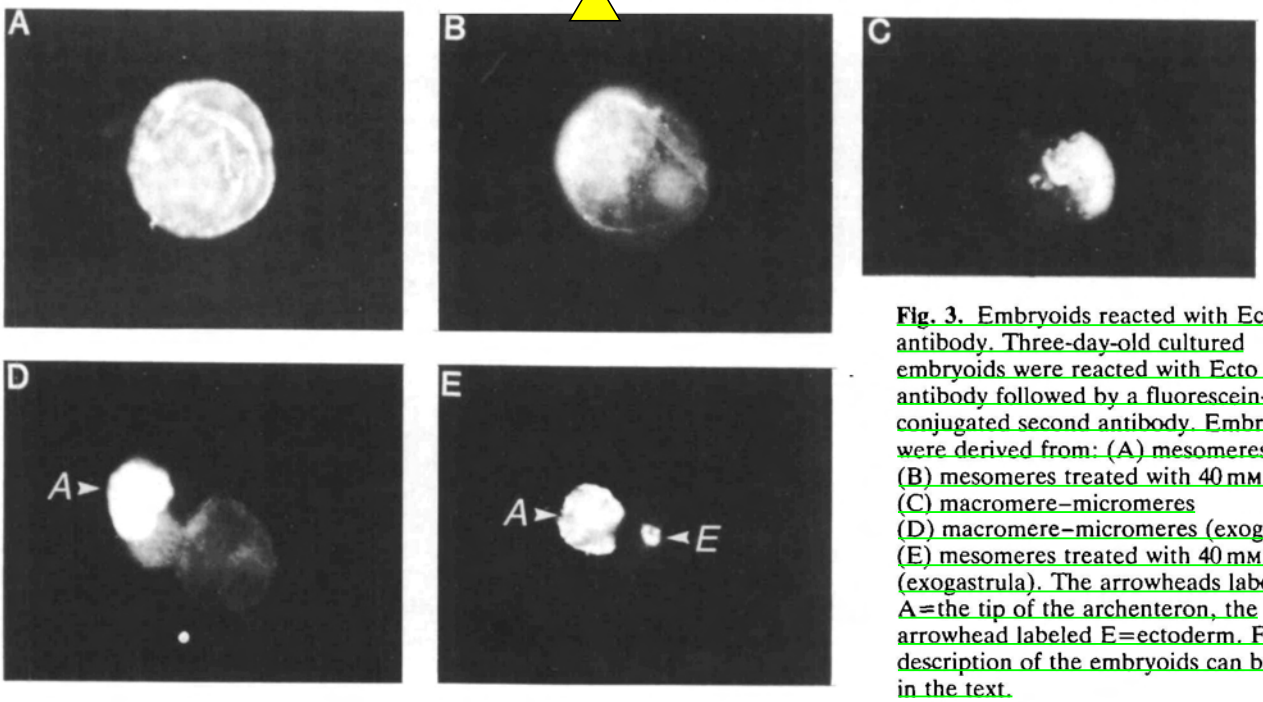

Fig. 3. Embryoids reacted with Ecto V antibody. Three-day-old cultured embryoids were reacted with Ecto V antibody followed by a fluorescein-conjugated second antibody. Embryoids were derived from: (A) mesomeres (B) mesomeres treated with 40 mM LiCl (C) macromere–micromeres (D) macromere–micromeres (exogastrula) (E) mesomeres treated with 40 mM LiCl (exogastrula). The arrowheads labeled A=the tip of the archenteron, the arrowhead labeled E=ectoderm. Further description of the embryoids can be found in the text.

and the surrounding layer of surface cells was unlabeled. When embryoids derived from these vegetal blastomeres occasionally formed exogastrulae, only the tip of the everted gut was labeled (Fig. 3D). We conclude that mesomeres may autonomously express the Ecto V antigen but its appropriate localization is deficient; macromeres also express Ecto V but do not display the surface ectodermal localization characteristic of the stomadeum.

Inductive effects of micromeres on isolated mesomeres

The effects of combining a micromere with a mesomere pair were examined using the methods diagrammed in Fig. 1. Embryoids resulting from combinations of mesomeres and RITC-stained micromeres were examined for the appearance of the morphological markers of vegetal differentiation: invaginations, spicules and pigment cells (Fig. 4). 40 % of the embryoids contained invaginated cells, and the cells in the invaginations were not stained with rhodamine, indicating a mesomere origin. The invaginations induced by micromeres stained positively for alkaline phosphatase activity, an enzyme specific for the gut of sea urchin embryos (data not shown) and for Endo 1, an antigen specific for the mid and hind gut (Fig. 5C). 39 % of the embryoids contained skeletal spicules, which were always surrounded by cells stained with rhodamine (Fig. 5A,B). These results indicate that micromeres may differentiate to form spicules in this permissive environment and, more importantly, that micromeres induce mesomeres to form an endodermal gut. Only 3 % of the embryoids exhibited cells containing pigment (Fig. 6A,B). The embryoids derived from mesomere–micromere combinations also stained positively for the oral field antigen Ecto V (Table 1). In the majority of these cases the antigen was distributed uniformly over the surface of the embryoid (Fig. 5F, upper embryo).

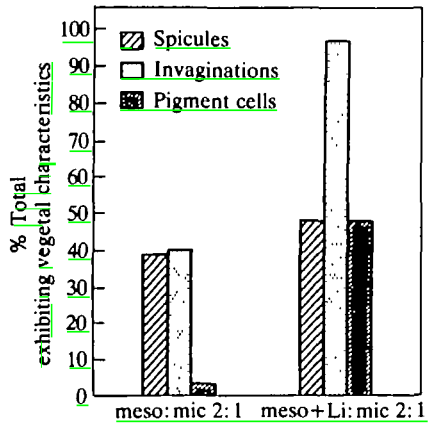

Fig. 4. Quantitative summary of blastomere recombinations. Bar graphs show the proportion of embryoids derived from mesomere–micromeres (2:1) and mesomere–micromeres (2:1) treated with 40 mM LiCl that exhibit invaginations, spicules or pigment cells. The number of embryoids examined for each characteristic is as follows: meso–mic (2:1): invaginations=159, spicules=145, pigment=162, meso+LiCl–mic (2:1): invaginations=147, spicules=105, pigment=135.

However, in 10 % of the cases, there was a slight shift of expression away from the point of invagination (Fig. 5F, lower embryo).

Effect of LiCl on mesomere–micromere combinations

Mesomere–micromere combinations were treated with LiCl for 6–7 h, cultured for three days in sea water, and their differentiation evaluated just as described for untreated mesomere–micromere combinations. Some results are shown in Fig. 4 and summarized in Table 1. 97 % of the embryoids contained invaginations, and none of the invaginations had cells that were stained with rhodamine. 48 % of such embryoids contained spicules, of which all but two were surrounded by cells stained with rhodamine. The most striking feature of these cultures was that 48 % of the embryoids contained pigment cells, and the pigment cells were not stained with rhodamine, indicating that they were derived from mesomeres (Fig. 6C–E). Pigment cells normally arise from the veg 2 lateral tier of macromeres, far removed from the animal–vegetal border. As a control for the ability to detect rhodamine-stained pigment cells, rhodamine-stained macromeres were combined with mesomeres and cultured for three days. The resulting embryos contained pigment cells, and these cells were positive for rhodamine staining (data not shown).

The presence of molecular markers of differentiation was also examined (see Table 1). All embryoids tested (97 % exhibited invaginations) were positive for alkaline phosphatase activity and the presence of Endo 1 (data not shown). They were also positive for the presence of Ecto V. However, the pattern of expression was strikingly different from the other experimental situations examined. 58 % of the embryoids showed a tightly localized patch of surface cells expressing the antigen (Fig. 6H), while 6 % showed a broader band of localized superficial expression. This proportion of embryoids showing localized expression is very close to that seen in embryoids derived from macromere–micromere pairs (Table 1).

Discussion

Newly available tools now allow us to investigate the effects of LiCl on sea urchin blastomeres in a more quantitative manner (Livingston and Wilt, 1989). Using isolated mesomeres, we have determined the dose–response curve for the effects of LiCl on isolated animal blastomeres. First, the formation of gut-like structures in response to LiCl is not an all or none 'switch', but is instead a graded response and, second, different vegetal structures are induced to appear at different thresholds of LiCl concentrations. Since embryoids descended from a single pair of LiCl-treated mesomeres can form an organized structure with both invaginations and spicules, it is clear that not all cells derived from a single mesomere pair respond alike to the treatment. This could mean that there are interactions between cells within an embryoid that modulate the response to LiCl.

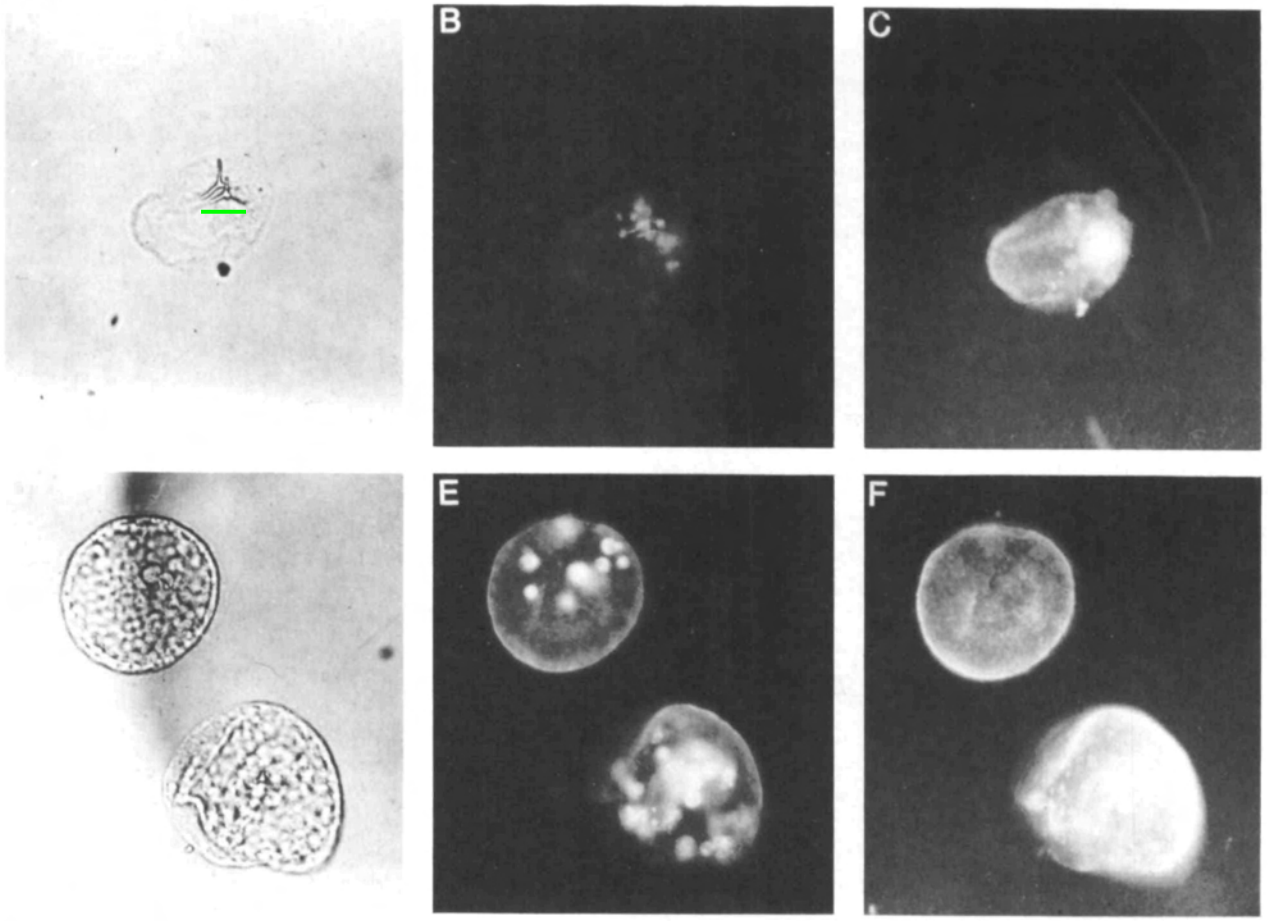

Fig. 5. Embryoids derived from mesomeres combined with rhodamine-labeled micromeres, reacted with Endo 1 and Ecto V antibodies. Embryoids were reacted with primary and fluorescein-labeled second antibody and visualized using fluorescent microscopy. A, B and C show the same embryoid. D, E and F show the same embryoid. (A) Embryoid viewed in light field; (B) embryoid showing rhodamine-stained cells (micromere derived); (C) embryoid showing Endo 1 antibody staining; (D) embryoids in light field; (E) embryoids showing rhodamine stained cells; (F) embryoids showing Ecto V staining.

Though the four different mesomere pairs originating from the animal half of a single embryo may have different potentials and responses (Cameron *et al.* 1989), the basal expression of the characters examined does not reveal such a bias.

Animal halves of sea urchins embryos have been described as being in a rather developmentally arrested state (Horstadius, 1973). As a first step in investigating the differentiation of isolated blastomeres using molecular markers of animal differentiation, we have examined the expression of the antigen Ecto V in our cultures. This antigen is normally expressed in oral ectoderm, descended from mesomeres, and, in the foregut, descended from macromeres. Although the embryoids in the cultures are only 1/8 the normal size of plutei and lack the normal easel shape of these larvae, we would still expect any cells on the surface of these embryoids that have differentiated into oral ectoderm to express this antigen. If these embryoids have organized their ectoderm into more than one type of cell, we might expect to see only a subset of the ectoderm expressing Ecto V. Similarly, we would expect invaginated cells that have differentiated into foregut to express this antigen.

What was found is that isolated mesomeres were capable of expressing Ecto V. This is direct evidence that isolated mesomeres are capable of some differentiation. The pattern of expression was generalized, indicating that either all of the cells of the embryoids had differentiated into oral ectoderm, or that expression of the antigen had begun, as in normal blastula, in a generalized pattern, and that in isolated mesomeres the signals necessary for localization of the antigen are lacking. We favor the latter possibility, since localization of Ecto V expression is possible in these embryoids (see below). However, the use of more markers, for both oral and aboral ectoderm, is necessary to clearly understand the capabilities of mesomeres to differentiate autonomously.

Isolated mesomeres treated with LiCl also expressed Ecto V, showing that, at least in this case, LiCl does not inhibit the expression of an animal marker. Also, in a significant number of cases, Ecto V was expressed in a somewhat more normal pattern in embryoids derived

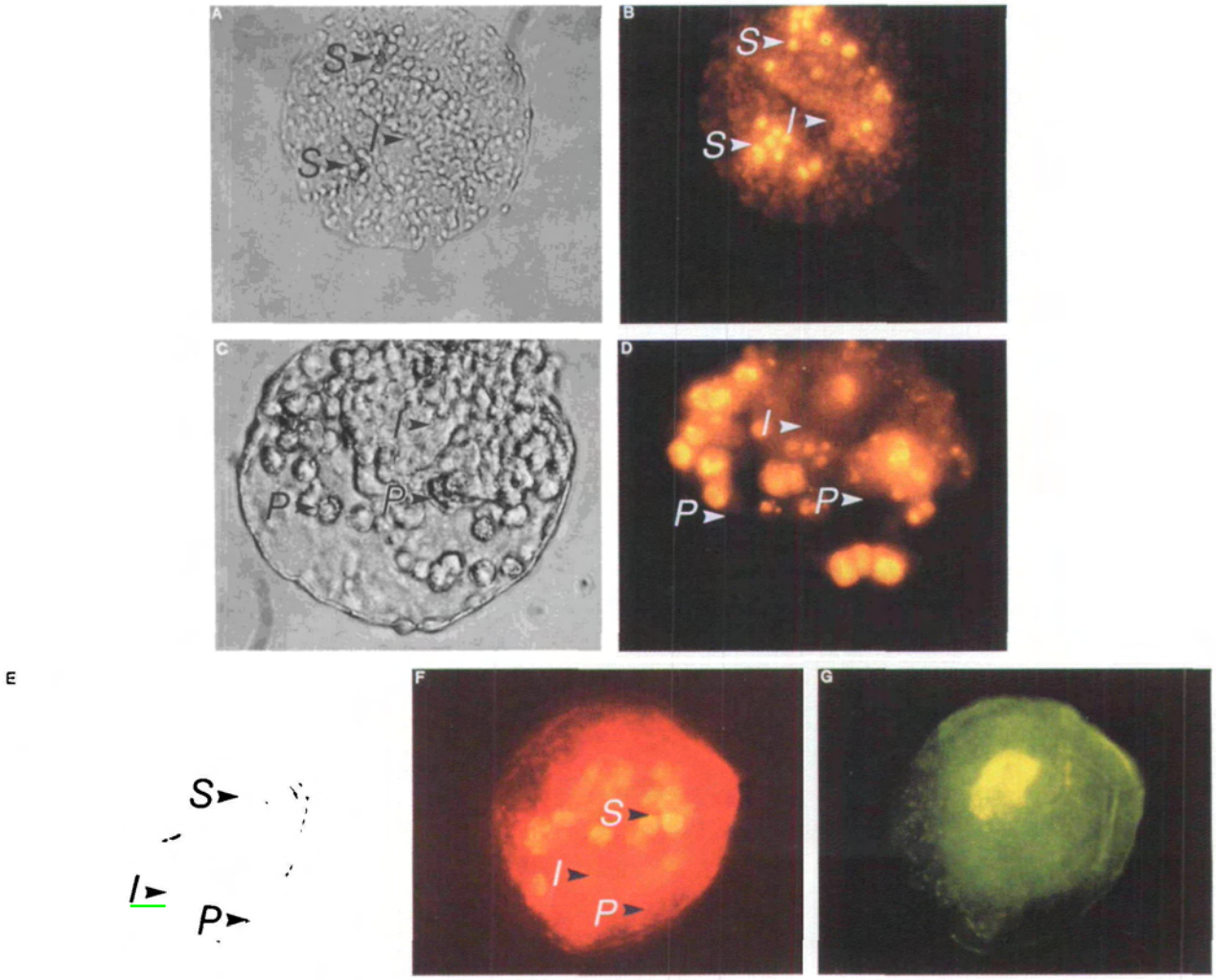

Fig. 6. Combined effect of LiCl and rhodamine-labeled micromeres on mesomere differentiation. A and B show an embryoid derived from a mesomere–micromere (2:1) combination viewed in light field (A) and visualized for rhodamine staining (B). (C) (light field) and (D) (RITC stain) show a mesomere+LiCl–micromere (2:1) combination. E, F and G show a mesomere+LiCl–micromere (2:1) combination in light field (E) and visualized for rhodamine (F) and Ecto V staining (G), respectively. S, spicule; I, invagination; P, pigment cell.

from LiCl-treated mesomeres. In these cases, the embryoids had invaginations, and the expression of Ecto V was shifted away from the point of invagination, although it was still broadly distributed over the surface of the embryoid. It is probable that induction of an invagination helps to establish or maintain an oral–aboral axis. However, the presence of an invagination alone did not seem to be sufficient to cause a shift in Ecto V expression, and the localization of Ecto V caused by LiCl was in most cases a broad distribution, rather than a tight localization.

Isolated macromere–micromere pairs formed embryoids that exhibited strict localization of Ecto V expression to the foregut, as would be expected. Ecto V expression was not seen in localized patches of surface cells, which indicates that derivatives of macromeres did not alter their normal fate and differentiate as oral ectoderm. The majority of the invaginations that moved to the interior in embryoids derived from LiCl-treated mesomeres did not show expression of Ecto V, although they did express Endo 1, a marker of mid and hind gut differentiation. The only embryoids derived from LiCl-treated mesomeres that expressed Ecto V in the gut were those that exogastrulated or formed complete guts. This is consistent with the idea that there are different extents of induction of mesomeres by LiCl, with structures normally derived from less vegetal blastomeres, such as mid and hind gut, being more easily induced than structures normally derived from blastomeres closer to the vegetal pole, such as the foregut.

Experiments in which micromeres were added to mesomere pairs has confirmed earlier work (Horstadius, 1973) indicating that micromeres can induce mesomeres to form invaginations. In addition, we have shown that these invaginations are differentiated at the molecular level, exhibiting markers appropriate for a normal gut. The proportion of embryoids derived from mesomere–micromere combinations that formed invaginations is lower than that seen with the optimal dose of LiCl, although the ratio of the two blastomeres was the same as that seen in normal embryos. This could be due to the fact that micromeres are initially localized to one side of the embryoid, and their effect depends on cell contact or a diffusible substance. LiCl, on the other hand, uniformly surrounds all blastomeres during treatment. Although all of the embryoids contained rhodamine-stained cells, indicating that the micromeres had divided and that the descendants were still present, the added micromeres formed spicules in 39 % of the embryoids (Table 1). Isolated micromeres from *L. pictus* do not form spicules in culture, in contrast to micromeres isolated from *Strongylocentrotus purpuratus*, *Arbacia* and *Hemicentrotus*, so *L. pictus* micromeres may be less robust in spicule formation under these experimental conditions.

The combined effect of LiCl and micromeres on mesomeres is not strictly an induction of vegetal differentiation, because LiCl and micromeres do not inhibit expression of Ecto V in mesomere-derived 'ectodermal' cells, but rather cause it to be expressed in a more normal pattern. This suggests that LiCl and micromeres do not cause mesomeres to go from an animal state of determination to a more vegetal one, but instead expand the ability of mesomeres to form a more diverse range of tissue types. This, combined with some interaction among the cells within an embryoid, allows them to arrange themselves into something resembling a normal embryo.

The responsiveness of mesomeres to outside 'inductive' influences is formally similar to the response of animal cap cells of amphibia (see Gurdon, 1987; Jacobson and Sater, 1988, for reviews). In amphibia, prospective endoderm located at the vegetal pole induces contiguous animal cap cells to form mesodermal tissues. In the sea urchin, the animal cap cells may be induced to form a variety of tissues, especially gut, an endodermal derivative, and to some extent spicules and pigment cells (mesodermal). Interestingly, the induction of endodermal tissue (gut) can be carried out by cells considered to be of mesodermal origin (micromeres). Further experiments are necessary to determine what role the inductive relationships in sea urchin play in pattern formation in normal development.

All of the evidence presented suggests that determination in these blastomeres involves a network of interacting regulatory pathways, rather than sole reliance on a simple gradient of morphogens. At least some of the effectors would have to be present in both active and inactive forms (Davidson, 1989), and the proportion of these two forms in a blastomere could determine its fate. The most attractive model to date would involve positive and negative signals at the cell surface which are transmitted into the cell *via* second messengers. These could then trigger a cascade of events, such as phosphorylation, which would then determine the final state of the cell. It is our hope that further work using this system will shed more light on the mechanisms involved.

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References

Cameron, R. A., Fraser, S. E., Britten, R. J. and Davidson, E. H. (1989). The oral-aboral axis of a sea urchin embryo is specified by first cleavage. *Development* **106**, 641–647.

Cameron, R. A., Hough-Evans, B. R., Britten, R. J. and Davidson, E. H. (1987). Lineage and fate of each blastomere of the eight cell sea urchin embryo. *Genes and Development* **1**, 75–84.

Coffman, J. A., Nelson, S. and McClay, D. R. (1985). A cell surface protein that identifies the ventral surface of the ectoderm of sea urchin gastrulae. *J. Cell Biol.* **101**, 469a.

Cox, K. H., Angerer, L. M., Lee, J. J., Davidson, E. H. and Angerer, R. D. (1986). Cell lineage specific programs of expression of multiple actin genes during sea urchin embryogenesis. *J. molec. Biol.* **188**, 159–172.

DAVIDSON, E. H. (1986). *Gene Activity in Early Development*. 3rd edn. New York: Academic Press.

DAVIDSON, E. H. (1989). Lineage specific gene expression and the regulative capacities of the sea urchin embryo: a proposed mechanism. *Development* **105**, 421–445.

ETTENSOHN, C. A. AND MCCLAY, D. R. (1986). The regulation of primary mesenchyme cell migration in the sea urchin embryo: transplantations of cells and latex beads. *Development* **117**, 380–391.

ETTENSOHN, C. A. AND MCCLAY, D. R. (1988). Cell lineage conversion in the sea urchin embryo. *Devl Biol.* **125**, 396–409.

EVOLA-MALTESE, C. (1957). Histochemical localization of alkaline phosphatase activity in the sea urchin embryo. *Acta Embryol. Morphol. exp.* **1**, 99–104.

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

GUSTAFSON, T. AND HASSELBERG, I. (1950). Alkaline phosphatase activity in developing sea urchin. *Expl Cell Res.* **1**, 371–375.

HALL, H. G. (1978). Hardening of the sea urchin fertilization envelope by peroxidase-catalyzed phenolic coupling of tyrosine. *Cell* **15**, 343–355.

HINEGARDNER, R. (1967). In *Methods in Developmental Biology*, (eds. Wilt, F. H. and Wessels, N. K.) Crowell, New York, pp. 139–155.

HORSTADIUS, S. (1973). *Experimental Embryology of Echinoderms*. Oxford: Clarendon Press.

JACOBSON, A. G. AND SATER, A. K. (1988). Features of embryonic induction. *Development* **104**, 341–359.

LALLIER, R. (1964). Biochemical aspects of animalization and vegetalization in the sea urchin embryo. *Adv. Morphogen* **3**, 147–196.

LIVINGSTON, B. T. AND WILT, F. H. (1989). Lithium evokes expression of vegetal specific molecules in the animal blastomeres of sea urchin embryos. *Proc. nat. Acad. Sci.* **86**, 3669–3673.

LUTZ, D. A. AND INOUE, S. (1986). In *Methods Cell Biol.* **27**, 90–109.

LYNN, D. A., ANGERER, L. M., BRUSKIN, A. M., KLEIN, W. H. AND ANGERER, R. C. (1983). Localization of a family of mRNAs in a single cell type and its precursors in sea urchin embryos. *Proc. nat. Acad. Sci. U.S.A.* **80**, 2656–2660.

MCCLAY, D. R., COFFMAN, J. A. AND WESSEL, G. M. (1987). Cell recognition during sea urchin gastrulation: expression of molecules associated with morphogenesis. In *Molecular Approaches to Developmental Biology*. (R. Firtel and E. Davidson, eds.) A. R. Liss Inc. New York. pp. 231–241.

PFOHL, R. J. (1975). Alkaline phosphatase of sea urchin embryos: chromatographic and electrophoretic characterization. *Devl Biol.* **44**, 333–345.

RUNNSTROM, J. (1929). Uber Selbstifferenzierung und Induktion bei dem Seeigelkeim. *Wilhelm. Roux. Arch. EntwMech. Org.* **117**, 123–145.

VON UBISCH, L. (1929). Uber die Determination der larvalen Organe under der Imaginalanlage bei Seeigelen. *Wilhelm Roux Arch. EntwMech. Org.* **117**, 81–122.

WESSEL, G. M. AND MCCLAY, D. R. (1985). Sequential expression of germ-layer specific molecules in the sea urchin embryo. *Devl Biol.* **111**, 451–463.

WILT, F. H. (1987). Determination and morphogenesis in the sea urchin embryo. *Development* **100**, 559–575.

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