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Early gene expression along the animal-vegetal axis in sea urchin embryoids and grafted embryos

Christian Ghiglione, Francoise Emily-Fenouil, Patrick Chang and Christian Gache*

URA 671 CNRS, Université Paris VI, Station Marine, 06230 Villefranche-sur-Mer, France

*Author for correspondence (e-mail: gache@ccrv.obs-vlfr.fr)

SUMMARY

The *HE* gene is the earliest strictly zygotic gene activated during sea urchin embryogenesis. It is transiently expressed in a radially symmetrical domain covering the animal-most two-thirds of the blastula. The border of this domain, which is orthogonal to the primordial animal-vegetal axis, is shifted towards the animal pole in Li^+-treated embryos. Exogenous micromeres implanted at the animal pole of whole embryos, animal or vegetal halves do not modify the extent and localization of the *HE* expression domain. In grafted embryos or animal halves, the Li^+ effect is not affected by the presence of ectopic micromeres at the animal pole. A Li^+-induced shift of the border, similar to that seen in whole embryos, occurs in embryoids developing from animal halves isolated from 8-cell stage embryos or dissected from unfertilised eggs. Therefore, the spatial restriction of the *HE* gene is not controlled by the inductive cascade emanating from the micromeres and the patterning along the AV-axis revealed by Li^+ does not require interactions between cells from the animal and vegetal halves. This suggests that maternal primary patterning in the sea urchin embryo is not limited to a small vegetal center but extends along the entire AV axis.

Key words: AV axis, lithium, embryoids, micromeres, sea urchin, zygotic gene, *Paracentrotus lividus*

INTRODUCTION

As in many species, the egg, embryo and early larvae of the sea urchin appear to be rotationally symmetrical around the animal-vegetal axis (AV axis). The oral-aboral orientation, while established early, is morphologically visible only after gastrulation.

Classical studies have demonstrated the importance of the primordial AV axis for the organization of the embryo (Boveri, 1901; Hörstadius, 1973; Davidson, 1986, 1989). Cleavage planes are invariably oriented with respect to the AV axis, the first two being parallel to and the third perpendicular to it. At 4th cleavage, the first unequal division produces 4 micromeres at the vegetal pole and, much later, gastrulation occurs by invagination from the vegetal pole. As shown by the fate map at the 64-cell stage, groups of cells having distinct fates form horizontal layers, perpendicular to the AV axis. When isolated, these cell layers also have different fates and react differently to apposition of cells from other layers. Meridional halves (or quarters) produced by dissociation of the first 2 (or 4) blastomeres, formed by meridional cleavage, as well as halves obtained by cutting the egg parallel to the AV axis, give rise to nearly normal embryos of reduced size (a half or a quarter). In contrast, animal and vegetal halves obtained from early embryos or by cutting eggs along their equatorial plane, perpendicular to the AV axis, have very different fates. The animal half becomes a permanent blastula which has an enlarged apical tuft of stereocilia, does not produce spicules and does not gastrulate. The vegetal half develops into a larva whose defects in ectodermal structures (i.e., lack of mouth and reduced oral lobe), range from mild to severe, depending on species and egg batches (Hörstadius, 1973; Maruyama et al., 1985). The AV axis is thus defined before fertilization and provides positional information to determine cell fate.

Cell fate is not, however, rigidly set up, as shown by incubating cleavage embryos with animalizing and vegetalizing agents (Lallier, 1975). These provoke alteration of larval morphology, which in several cases, results from change in cell fate. Li^+ has long been known to produce vegetalization, which is a switch from ectoderm to endoderm fate (Herbst, 1892; Nocente-McGrath et al., 1991). The series of blastomere manipulations carried out by Hörstadius (reviewed by Hörstadius, 1973) also demonstrated that the potency of most of the early blastomeres was greater than their prospective fate and that cell fate could be modified by interactions between cells. Although the inductive power of the micromeres was clearly recognized, the results were globally interpreted in the framework of the double gradient hypothesis (Runnström, 1928, 1929). As pointed out by Wilt (1987) and Davidson (1989) a simple mosaic interpretation is insufficient to explain conditional cell fate and resorting to poorly defined interactions between the 2 gradients is inadequate. Davidson has reinterpreted Hörstadius' results and integrated recent data on cell lineage and spatial patterns of gene expression into a model for cell lineage specification (Davidson, 1989). This model considers specification both by some localized determinants

and by cell interactions, with a strong emphasis on the latter. As a limited example, localized information along the AV axis would be first restricted to a small vegetal center maternally defined, and patterning along the entire AV axis would occur stepwise by a series of cell interactions initiated by the micromeres and thus beginning at the 16-cell stage.

In addition to the inductive capacity of the micromeres, the Davidson model incorporates data on several genes which are expressed in late embryos within territories corresponding to domains defined by lineage and tissue structure (Angerer and Davidson, 1984; Cameron and Davidson, 1991). We have isolated 2 genes, *HE* and *BP10*, whose behavior is quite different from all other genes known (Lepage and Gache, 1990; Lepage et al., 1992a,b; Ghiglione et al., 1993). *HE* and *BP10* are strictly zygotic genes, only transiently active between the 8-cell stage and the prehatching blastula stage, and dissociation experiments suggest they are autonomously activated. They are both spatially restricted to the animal-most two-thirds of the blastula, an area which corresponds approximately to the presumptive ectoderm. The border of this expression domain, which is perpendicular to the AV axis, is shifted towards the animal pole in embryos treated with Li^+. This suggests that the *HE* and *BP10* genes read the positional information which contributes to specify cell fate along the AV axis. *HE* and *BP10* are the earliest spatially restricted genes in the sea urchin embryo and their control appears to be strongly dependent on the initial AV polarity of the oocyte. Therefore, they facilitate a reassessment of some aspects of gene control in the early blastomeres.

Here we study how the spatial expression of the *HE* gene is affected by micromere implantation in whole embryos and by Li^+ in isolated animal halves, in order to estimate better the influence of potentially inductive cells implanted at ectopic positions and the extent of maternal organization along the AV axis.

MATERIALS AND METHODS

Spawning and fertilization of eggs from *Paracentrotus lividus*, fertilization envelope removal and embryo culture have been described previously (Lepage and Gache, 1989, 1990). Separation of animal and vegetal halves was carried out using a glass needle on 8-cell stage embryos settled at the bottom of Petri dishes coated with agarose in Ca^{2+}-free artificial sea water. The embryoids were raised in small Petri dishes in Millipore filtered sea water containing antibiotics.

Bisection of unfertilized eggs along the equatorial plane was carried out as described by Maruyama et al. (1985) except that orientation of the eggs was done using the subequatorial pigmented band present in some egg batches of *P. lividus* (Boveri, 1901; Schroeder, 1980).

Micromere transplantations were performed according to the method of Ransick and Davidson (1993) using the hyaline extraction medium of Fink and McClay (1985) to dissociate and graft micromere quartets (communicated by A. Ransick and E. Davidson).

Treatment with LiCl began either 30 minutes after fertilization or within a few minutes after embryo bissection, as indicated, and using in all cases a final concentration of 30 mM.

Immunolabeling was carried out exactly as described by Lepage et al. (1992a) using an affinity-purified anti-HE primary antibody and an anti-rabbit Ig phosphatase-conjugated secondary antibody (Lepage et al., 1992b).

The number (*n*) of embryos or embryoids used in each experiment is indicated in the text. In all cases, the morphology and labeling pattern reported were displayed by over 80% of the embryos observed, except where otherwise indicated.

RESULTS

Orientation of 4- and 8-cell stage embryos

To orient the 8-cell stage embryo and separate animal from vegetal halves by manual dissection with 100% yield, we devised a very simple method. This method does not require preliminary visualization of the jelly canal, reference to the subequatorial pigment band found only in some egg batches from *P. lividus*, or partial random dissociation of embryos followed by sorting out dissociated quarters. Embryos devoid of their fertilization envelopes were raised in suspension culture until the 4-cell stage, then transferred to Ca^{2+}-free sea water and allowed to settle (Fig. 1). Embryos were kept still until they underwent the 3rd division. Due to their shape, the 4-cell stage embryos can only stand with the AV axis in the vertical position and at the 8-cell stage, the equatorial 3rd cleavage, which separates the animal and vegetal halves, is thus horizontal. Rotating an embryo by 90° in any direction results in positioning the equatorial plane in a vertical position, parallel to the axis of rotation, and the two halves can be easily

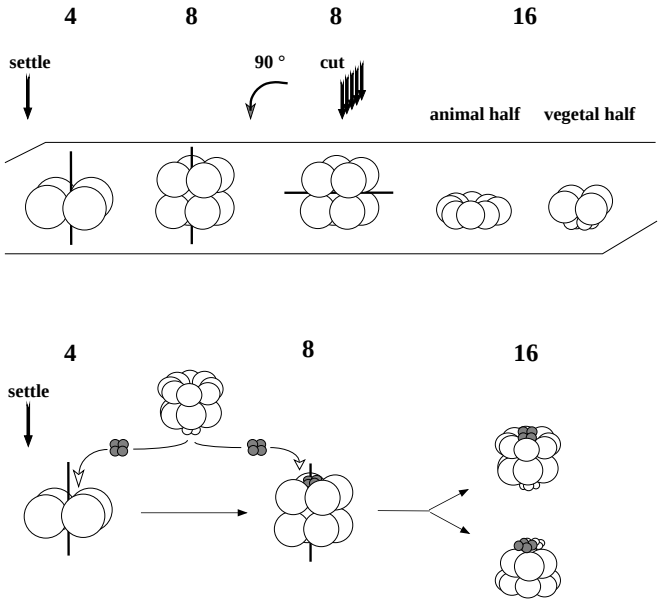

Fig. 1. Orientation of 4-cell and 8-cell stage embryos for separation of animal and vegetal halves (upper) and micromere transplantation (lower). The thick line represents the AV axis. The first 2 cleavage planes are orthogonal to each other and contain the AV axis. The 3rd cleavage plane is perpendicular to the AV axis and separates the embryo into 2 equatorial halves. Separation of animal and vegetal halves: 4-cell stage embryos suspended in Ca^{2+}-free sea water were allowed to settle at the bottom of agarose-coated Petri dishes and were left undisturbed until completion of 3rd cleavage. The embryos were individually rotated by 90° and cut along the equatorial plane with a glass needle. Animal and vegetal halves were identified and sorted out after 4th cleavage. Micromere transplantation: grafting of micromeres at the top of 4-cell stage and 8-cell stage embryos oriented as above, ensures that implantation occurs exclusively at either the animal or the vegetal pole.

separated along this plane. The animal and vegetal halves were identified unambiguously after the 4th cleavage which is radial and equal in the animal half and equatorial and unequal in the vegetal half.

The same method allows implantation of micromeres at one of the poles of 4- or 8-cell stage embryos. Micromeres from 16-cell stage donor embryos were implanted at the upper side of 4- cell stage or 8-cell stage recipient embryos settled as indicated above. Therefore, grafting occurred at either the animal pole or at the vegetal pole, the 2 types of embryos being easily identified and sorted-out at the 16-cell stage.

HE expression in whole embryos and embryoids grafted with micromeres

The micromeres have a well documented inductive capacity which plays a key role in cell specification along the AV axis (Davidson, 1989). As discussed below, our dissociation experiments (Ghiglione et al., 1993) do not completely exclude that this patterning process contributes to the normal control of the *HE* gene and does not prove that micromeres cannot interfere with it. Thus it was of interest to ask if, in whole embryos, the micromeres could directly influence the expression of the *HE* gene when present at ectopic positions within the normal *HE* domain of expression or in excess number at their normal position. This should give indications on the relationship between inductive events initiated by the micromeres and Li^+-sensitive pathways, and allow testing the plasticity of *HE* expression through its response to a non-physiological signal.

In order to transplant micromeres from a donor embryo to a recipient embryo we followed the method described by Ransick and Davidson (1993), the embryos being oriented as described above to ensure grafting at one of the two poles (Fig. 1). At the pre-hatching blastula stage the implanted embryos have a normal morphology, whether the micromeres were implanted at the animal or at the vegetal pole. The grafted micromeres and their descendants can be seen as a small fluorescent patch, if cells from the donor embryo have been labeled with RITC (Fig. 2A). Embryos receiving micromeres at the vegetal pole develop normally, at least to the gastrula stage, and the expression domain of the *HE* gene is indistinguishable from that of control embryos (not shown). The outcome is quite different when micromeres were implanted at the animal pole as expected from the experiments of Hörstadius (1973) and Ransick and Davidson (1993). At the mid-gastrula stage, all the embryos presented 2 sets of triradiate spicules which formed at opposite sides of the embryo (Fig. 2B). The supernumerary pair was obviously produced by primary mesenchyme cells ingressing from the animal pole where exogeneous micromeres were implanted. As discussed by Hörstadius (1973) and Ransick and Davidson (1993), the existence of an ectopic center for spicule formation indicates that cells from the original animal pole have been respecified. In all cases, at least one archenteron was present. In some cases, a second reduced invagination, often limited to a blastopore, could be seen at the opposite pole of the embryo. In about 30% of the cases (n=12), two archenterons of nearly equal size invaginated and fused together before reaching the blastula wall (Fig. 2C). This confirms that ectoderm cells have been respecified into vegetal plate cells as already described and discussed for the *S. purpuratus* embryo (Ransick and Davidson, 1993). The expression of the *HE* gene in grafted embryos was revealed by immunolabeling at the blastula stage, that is, before any of the morphological changes induced by the grafted cells could be observed. The same result was obtained in all cases (n=50). A typical labelled embryo is shown in Fig. 2D,E. When seen from the vegetal pole, the vegetal most part of the *HE* expression domain appears as a narrow regular ring of labeled cells (Fig. 2D). This is exactly what is observed in normal embryos. Cells in the animal half are uniformly labeled except for a small patch precisely localized at the animal pole and whose size is equivalent to the size of the micromeres and their descendants (Fig. 2E and compare with A). This indicates that all animal blastomeres from the recipient embryo express the *HE* gene while the foreign cells do not. So the pattern of *HE* expression in the recipient embryo has not been affected by the presence

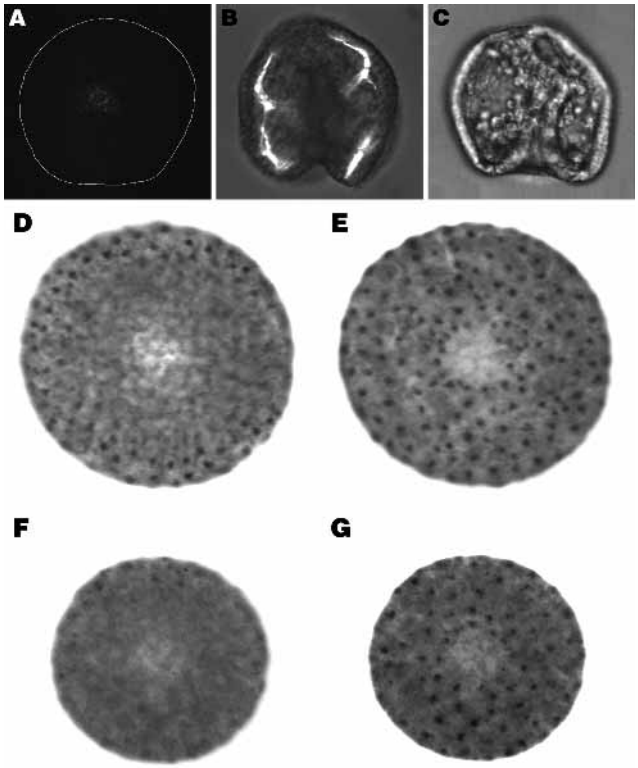

Fig. 2. *HE* gene expression in whole embryos and vegetal halves implanted with micromeres. In all cases micromeres were implanted at the 4- or 8-cell stage and the recipient embryo or embryoid developed to the indicated stage. The immunolabelled HE protein which is concentrated before secretion appears as a single stained dot in each cell expressing the *HE* gene. (A) Grafted embryo at the blastula stage (RITC filter). Micromeres from the donor embryo were labelled with RITC prior to transplantation. The embryo image is outlined by a thin white line and viewed approximately from the animal pole. (B) Grafted embryo at the gastrula stage (bright-field image and polarized light). Side view with the animal pole near the top. (C) Grafted embryo at the gastrula stage (DIC). Side view with the animal pole near the top. (D,E) Polar views from the vegetal (D) and animal (E) poles of the same grafted embryo at the blastula stage (bright-field image). The embryo was immunolabeled for the hatching enzyme at the same stage as the embryo seen in A. (F,G) Polar views from the vegetal (F) and opposite (G) poles of the same blastula stage embryoid which developed from a vegetal half separated at the 8-cell stage and grafted with micromeres opposite to the vegetal pole.

of the grafted micromeres and the induced respecification of the ectodermal cells surrounding them. While it is not possible to detect *HE* expression and follow cell fate on the same embryo, our conclusion is supported by the very high yield of the transplantation experiments as seen by morphological criteria and the consistency of the labelling experiments.

The effect of Li^+ on whole embryos suggests that there is a gradient of sensitivity to Li^+ along the AV axis (Ghiglione et al., 1993). Cells closer to the animal pole are less sensitive to Li^+ and could be also less sensitive to the micromere influence. Micromeres were therefore implanted at the top of vegetal halves separated at the 8-cell stage (*n*=40). Thus, foreign micromeres were on the AV axis, opposite the endogenous micromeres and in direct contact with all cells from the vegetal half at the time of implantation and surrounded by cells descending from the veg 2 layer at the blastula stage. *HE* expression in a typical grafted embryo is shown in Fig. 2F,G. The *HE* domain is restricted to the animal-most half as expected, except for foreign cells in the polar region opposite the original vegetal pole. Thus the micromeres appear to be unable to repress *HE* expression in cells derived from the veg 1 layer as well as those derived from the animal half.

HE expression in grafted embryos treated with Li^+

Li^+ has been described as potentiating the inductive capacity of the micromeres. We therefore investigated the effect of Li^+ in embryos with micromere grafts, i.e., having micromeres and their descendants at both poles. Both donor and recipient embryos were continuously treated with 30 mM Li^+ beginning 30 minutes after fertilization. A typical labelled blastula developing from such embryoids (*n*=48) is shown in Fig. 3. The expression domain was strongly reduced, and the residual expression territory appears as a narrow belt surrounding a small clear area. Thus the original domain border was shifted as expected towards the animal pole, and the domain limit created by implantation of micromeres was unaffected by Li^+. This may also be the case in animal halves since the same pattern was observed when micromeres were grafted to provide orientation in Li^+-treated animal halves (see below and Fig. 5D). In the aforementioned experiments, the micromeres were exposed to Li^+ only after grafting. Thus, in no case did Li^+ appear to modulate micromere activity upon *HE* gene expression.

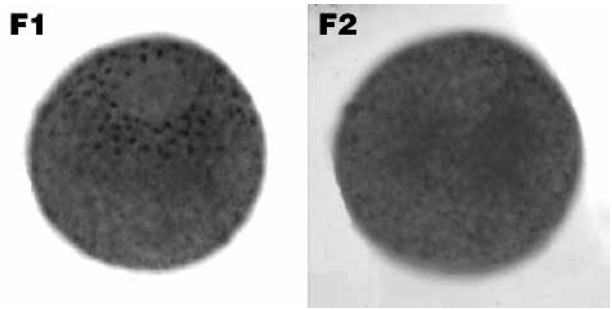

Fig. 3. *HE* gene expression in host embryos grafted with micromeres and treated with LiCl. Micromeres were implanted at the animal pole of whole embryos as in Fig. 5 but the donor and recipient embryos were continuously treated with 30 mM LiCl, beginning 30 minutes after fertilization. Photograph pairs Fl and F2 show the two sides of the same embryoid.

HE spatial pattem in animal and vegetal halves isolated from 8-cell stage embryos

The possibility to isolate and raise embryo fragments provides an alternative approach to analyse the expression of the *HE* gene and its possible alteration by Li^+.

Animal halves isolated from 8-cell stage embryos eventually develop into ciliated spheres with a well formed blastocoel and a locally thickened wall (Fig. 4D). Vegetal halves give rise to small plutei-like larvae often with some defects (Fig. 4F). These results are in agreement with previous reports (Hörstadius, 1973; Maruyama et al., 1985). At an earlier stage, both animal and vegetal halves form blastulae which have no sign of polarization and are morphologically indistinguishable from control blastulae excepting their reduced size and cell number (Fig. 4A,C,E).

In unperturbed embryos the expression domain of the *HE* gene extends over the animal side of the embryo, including all cells from the animal hemisphere and about one third of the cells from the vegetal hemisphere, in the subequatorial area adjacent to the animal half (Fig. 4A). In 100% of the blastulae produced from animal halves (*n*=55), all cells are labelled (Figs

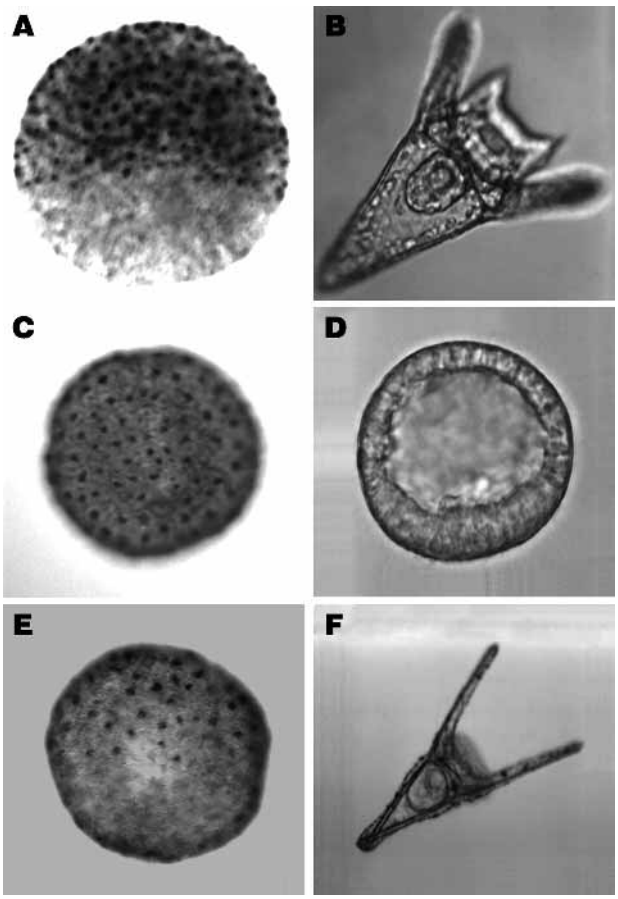

Fig. 4. Development of complete embryos and embryo halves. (A,B) Whole embryos, (C,D) animal half embryoids, (E,F) vegetal half embryoids. Left column, blastulae immunolabeled about 10 hours after fertilization. Right column, larvae 48 hours after fertilization.

4C and 5A) indicating that the expression domain of the *HE* gene encompasses all of the embryoid cells. In the vegetal half blastulae, only a fraction of the cells are labelled (Fig. 4E). As already described (Lepage et al. 1992b), there is some variability in the position of the expression boundary in the vegetal part of the normal embryo. This is reflected in vegetal half blastulae where in more than 80% of the cases (n=51), the boundary was within the expected range. The partition of the *HE* domain between the isolated halves is close to the normal pattern, suggesting that *HE* expression is unaffected by equatorial dissection of the embryo at the 8-cell stage.

HE spatial pattern in Li^+-treated animal and vegetal halves from 8-cell stage embryos

When vegetal halves were treated with Li^+, the *HE* gene was almost completely repressed (not shown). This could be expected from our results on whole embryos, whatever the mechanism of patterning along the AV axis. This was not investigated further and we focussed mainly on embryoids derived from animal halves.

Animal halves (n=57) were continuously exposed to 30 mM Li^+ beginning 30 minutes after fertilization, a classic vegetalizing treatment. The pattern observed in the animal halves was dramatically altered. The *HE* expression was reduced to a small domain as shown in Fig. 5B. A similar result was obtained when the Li^+ treatment was begun only after the animal halves were isolated (n=51; Fig. 5C). In order to position the residual expression domain, a few blastomeres from the vegetal area of 16-32 cell-stage embryos were implanted at the animal pole of recipient embryos (n=36). As shown above, these exogenous cells and their descendants do not express the *HE* gene and thus were expected to appear as a cleared area labeling the animal pole. An embryo treated in this manner is shown in Fig. 5D. The cleared area always occupies the center of the labeled domain, indicating that the residual domain is radially symmetrical around the AV axis. This is strongly reminiscent of what we observed with intact embryos. We have shown that increasing Li^+ concentration progressively shifts the border of the *HE* domain towards the animal pole, decreasing its size to a small polar cap (Ghiglione et al., 1993).

Therefore, the spatial restriction induced by Li^+ in intact embryos also takes place within animal halves, suggesting independence from the vegetal half. In the first series of experiments, Li^+ is present from the beginning and could act through the vegetal half before separation from the animal half. This is not the case, however, as shown in the second series of experiments since Li^+ can act in the animal half even if added after separation of the 2 halves. Thus the vegetal half *per se* is not required for the Li^+ effect on *HE* expression. However, the responding animal half has been in contact with the vegetal half prior to the addition of Li^+ and this could be required for Li^+ to act later.

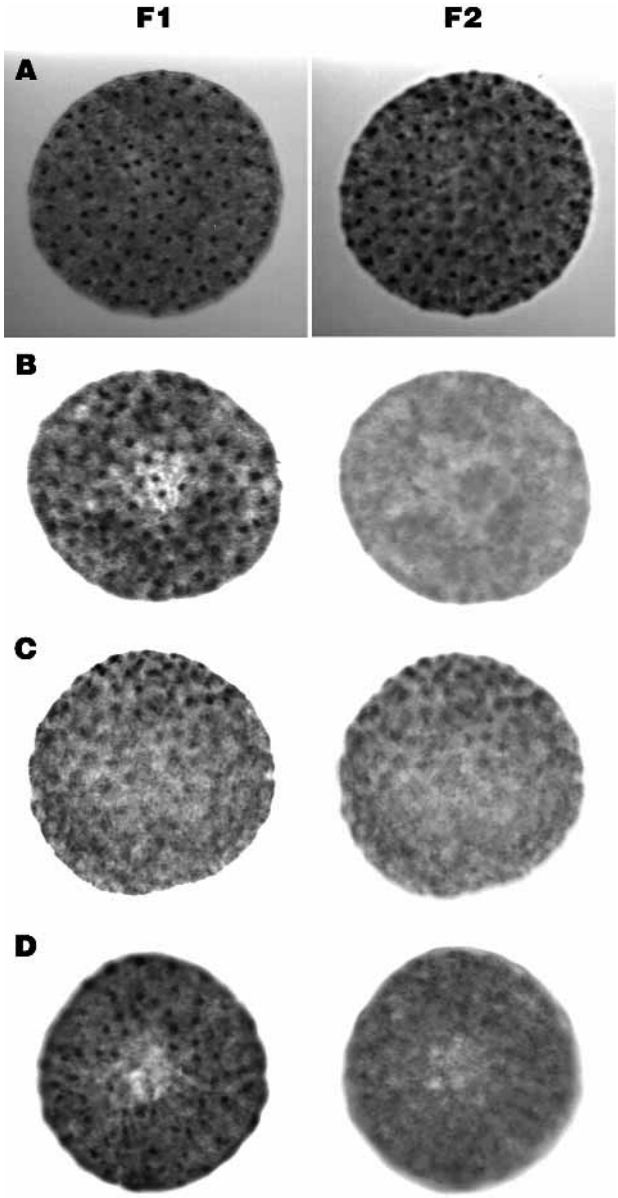

Fig. 5. Effect of Li^+ on *HE* gene expression in blastula-like embryoids derived from 8-cell stage animal halves. Immunolocalization of the hatching enzyme was carried out on embryoids about 10 hours after fertilization. Photograph pairs Fl and F2 show the two faces of the same embryoid. (A) Untreated embryoid, (B) embryoid treated continuously with 30 mM LiCl beginning 30 minutes after fertilization. (C) Embryoid treated continuously with 30 mM LiCl beginning after the separation of the animal and vegetal halves. (D) embryoid treated with LiCl as in B. To orient the expression domain, non-expressing cells were grafted at the animal pole of early cleavage embryos (see text) which appears as a clear area. Thus, B and C are animal pole views and C is a lateral view.

HE spatial pattern in untreated and Li^+-treated animal halves isolated from unfertilized eggs

Even when separated as early as feasible – at the 8-cell stage – blastomeres from the animal and vegetal halves are briefly in direct contact, between the time they form and the surgical separation. Such a limited contact might be long enough to allow interactions between the newly formed daughter-cells each belonging to one of the 2 halves. To completely eliminate such potential interactions we separated the animal and vegetal hemispheres of unfertilized eggs. We took advantage of a natural marker of the egg polarity, the subequatorial pigmented band visible in some egg batches of *P. lividus*. The unfertilized egg, once oriented, could be cut approximately through

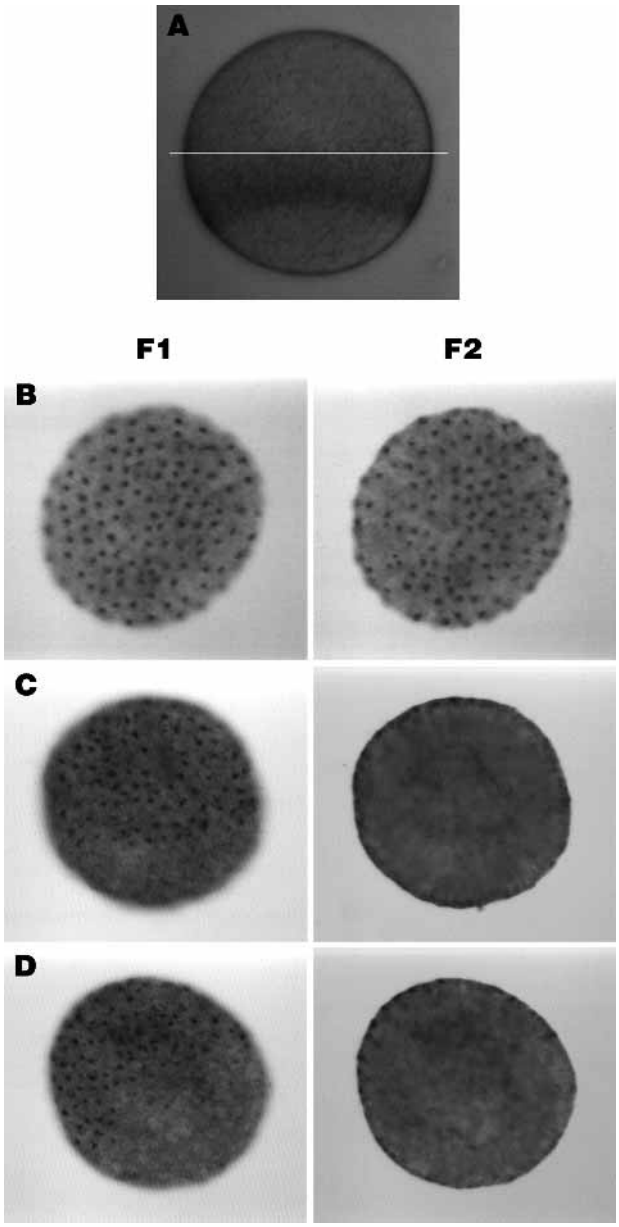

Fig. 6. Effect of Li^+ on *HE* gene expression in blastula-like embryoids derived from egg animal halves. (A) Video-enhanced recording of a unfertilized egg showing the sub-equatorial pigmented band, allowing positioning of the cutting plane (white line) to separate animal and vegetal halves. Immunolocalization of the hatching enzyme was carried out on embryoids about 10 hours after fertilization. Photograph pairs Fl and F2 show the two sides of the same embryoid. (B) Untreated embryoid. (C,D) Embryoids treated continuously with 30 mM LiCl beginning 30 minutes after fertilization.

the equatorial plane and the two halves fertilized (Fig. 6A). Animal halves developed into small blastula-like embryoids very similar to those obtained from 8-cell stage animal halves.

In all untreated animal halves (*n*=31), the domain of *HE* expression extends to every cell, exactly as observed for animal halves isolated at the 8-cell stage (Fig. 6B). This is also a good indication that animal hemispheres were correctly isolated, and contained only cytoplasm from the animal half or only slightly more. When the embryoids (*n*=64) were continuously treated with 30 mM Li^+ beginning 30 minutes after fertilization, the *HE* gene expression became restricted in all cases (Fig. 6C,D), again in the same manner as in embryoids prepared from the 8-cell stage. As markers are lacking, it was not possible to position the residual domain, but it is very likely oriented as in 8-cell stage halves. Thus, animal halves derived from unfertilized eggs or from 8-cell stage embryos express the *HE* gene to a similar extent and respond to Li^+ in a similar way.

DISCUSSION

We have previously shown that in unperturbed embryos, the *HE* gene is activated during cleavage and spatially restricted to a spherical segment of the blastula consisting of two-thirds of the blastomeres and centered at the animal pole. The border of this domain is perpendicular to the AV axis and coincides approximately with the clonal separation line between the presumptive ectoderm and endoderm (Lepage and Gache, 1990; Lepage et al., 1992b). Analysis of *HE* expression during altered development revealed two specific features (Ghiglione et al., 1993).

Firstly, in permanently isolated blastomeres the timing and level of *HE* transcription are almost unaltered. Whether the spatial regulation was also conserved could not be assayed in the absence of markers allowing identification of blastomere types. We were limited to comparing the ratio of expressing to non-expressing cells which was found to be similar in embryos and in the population of isolated blastomeres. *HE* is thus likely to have an autonomous expression.

Secondly, Li^+ progressively shifts the border of the *HE* domain towards the animal pole, indicating a gradient of sensitivity to Li^+ along the AV axis. This shift parallels the change in cell fate which occurs in Li^+-vegetalized embryos.

Further investigations with completely dissociated blastomeres were limited. *HE* expression appeared to be more sensitive to Li^+ in isolated blastomeres than in embryos, as judged by the low signals measured. Unfortunately, combining dissociation and Li^+ treatment altered cell division rates and prevented comparisons from being made. Although complete dissociation experiments give useful information, it is worth comparing with less extreme situations as reported, for example, by Henry et al. (1988), who established that the developmental potential of mesomeres is dependent on the size of the fragment isolated (mesomeres, mesomere pairs, aggregates, animal caps) and on the time of separation. Furthermore, interpretation of dissociation experiments may be less straightforward than expected. It has been shown in *C. elegans* that some early blastomeres behave cell-autonomously in isolation but are determined through cell interactions within the embryo (Schnabel, 1994, 1995). Even though this appears to be a rare phenomenon, attempts should be made to look directly for cell-cell interactions within the embryo whenever possible. In order to shed some light on the relationship between gene spatial control, cell interactions and Li^+ effect, *HE* gene expression was probed under two experimental situations: after grafting micromeres as pioneered by Hörstadius and recently revisited and improved by Ransick and Davidson (1993) and after separation of embryos into 2 equatorial halves combined with Li^+ treatment.

By transplantation, Ransick and Davidson (1993) demonstrated the inductive capacity of the micromeres to alter specific gene expression and to change ectodermal cell fate. Transplantation allows direct assay of the potential impact of the micromeres on the *HE* gene. If the *HE* gene conformed to the Davidson's model, it would be shut-off in most vegetal cells as the result of inductive events starting from the micromeres. In our experiments, the micromeres grafted at the animal pole are present at the right time (even earlier than the endogenous ones) and are able to induce a large change in cell fate. We might expect the ectopic micromeres to shut-off the *HE* gene in the animal pole area to the same extent as they do in the vegetal hemisphere, thus reducing the *HE* territory to a narrow equatorial belt. This was not observed. Cells from the animal pole could be less sensitive than cells from the vegetal area. However, this is not the case since micromeres implanted among veg 1 blastomeres in vegetal halves are also unable to decrease *HE* gene expression in the neighboring cells. In addition, the Li^+ effect remains unchanged in embryos with micromeres present at both the animal and vegetal poles: the shift of the *HE* domain border occurs in the vegetal to animal direction and the blastomeres adjacent to the graft still express the *HE* gene. Apparently, the *HE* gene is not affected by the inductive cascade, and may not be an intrinsic target for those inductive processes. It may be potentially sensitive, but expressed too early to be influenced on time. It is still possible that micromeres can repress *HE* only in cells from the veg 2 layer, but this would imply some kind of gradation along the AV axis. Therefore, either the micromere cascade is not the unique patterning element along the AV axis, or it has no role in the spatial control of the *HE* gene, or both.

The development of isolated parts of the sea urchin embryo and the drug-induced abnormal development of whole embryos has been investigated. Experiments combining embryo fragmentation and Li^+ treatment have been also reported (see Hörstadius, 1973). In particular, von Ubisch (1929) observed formation of invaginations and spicules in Li^+-treated animal caps and sometimes obtained complete plutei but reduced in size. Recently, Wikramanayake et al. (1995) obtained gastrula-like larvae by treating *L. pictus* animal halves with LiCl. All the older studies relied exclusively on morphological observations. Several groups have since reinvestigated the behavior of isolated embryo fragments and the influence of Li^+ using molecular markers. Livingston and Wilt (1989, 1990) worked with mesomere pairs isolated from 16-cell stage embryos. They used several markers specific for vegetal differentiation (alkaline phosphatase and endo-1 for the gut, SM50 for the primary mesenchyme) and the ectoV antigen as a marker not restricted to the descendants from vegetal blastomeres. They showed that Li^+ strongly increases formation of spicules and gut-like invaginations in animal embryoids and evokes expression of alkaline phosphatase and SM50. EctoV expression is abnormal since it is uniformly distributed instead of being localized. Li^+ partially restored a normal pattern. Wikramanayake et al. (1995) have investigated the behavior of Spec 1 (*S. purpuratus*) and its homolog LpS1 (*L. pictus*) in mesomere pairs and animal caps from the 2 species. Spec 1 is produced to a nearly normal level and is localized to one side of the embryoids. This apparently reflects a correct differentiation along the oral-aboral axis. In contrast, LpS1 is not expressed in embryoids. Li^+ evokes LpS1 expression but the spatial pattern was not reported. In both species, ectoV expression was found to be abnormal as indicated above. Thus, clearly some genes can be correctly expressed but different genes have different behavior and surprisingly, important differences exist between species, making generalizations hazardous.

Some of these results were obtained on mesomere pairs, a situation which obviously does not preserve all cell associations within the animal half, and in some cases, the potential influence from the micromeres was not completely eliminated, since mesomere pairs were obtained from 16-cell stage embryos. Furthermore, the available molecular markers used to monitor gene expression in animal cells were not perfectly adequate as far as the AV axis is concerned. EctoV is first uniformly expressed and later restricted to the oral ectoderm, which is of mesomere descent, and to the foregut, which derives from the macromeres (Coffman and McClay, 1990). The early expression of ectoV is not localized, and its late localization is not restricted to a single lineage and not simply related to the AV axis. Spec 1 is a lineage restricted marker (Tomlinson and Klein, 1990) expressed only in the aboral ectoderm, which is a subset of the ectoderm. The oral and aboral ectodernn are separated by a curved surface sectioning the AV axis. This boundary is generated through the invariant cleavage pattern which positions the founder cells of these polyclonal territories and their descendants and allows adequate interactions to pattern the oral-aboral axis (Davidson, 1989). Thus in both cases, the expression of these markers is probably not directly or uniquely linked to the AV axis. Furthermore, as all the markers used were late markers, observations were often made on embryoids after several days in culture during which numerous regulative processes may occur.

The *HE* gene instead has a very early expression, being completely shut-off by less than 10 hours after fertilization, well before hatching, when the blastula has about 350 cells. The shape of its domain of expression is simple and has a clear border perpendicular to the AV axis.

In whole embryos, Li^+ shifts the *HE* domain border as well as the ectoderm/endoderm limit along the AV axis without altering orientation and direction of the axis. In isolated animal halves, Li^+ restricts *HE* expression in a way which resembles that in whole embryos and can trigger regulative events leading to construction of a complete larva. Li^+ thus can alter the *HE* spatial pattern within the animal half in the total absence of the vegetal half and in the absence of any type of interaction between cells from both halves.

Li^+ and micromeres have been considered to function in a parallel manner. Firstly, the effects of Li^+ (von Ubisch, 1929) and of micromere grafts (Hörstadius, 1973) on isolated animal halves are similar. Both treatments provoke animal halves to form gut and spicules and sometimes to develop into normal larvae. Secondly, it has been shown that Li^+ interferes with signal transduction pathways (Berridge et al., 1989). Thus Li^+ might mimic or potentiate the inductive effect of micromeres. However, nothing is known of their mechanism of action and it has not been demonstrated that they work along the same pathway. The signal emanating from the micromeres has not been identified and the targets of Li^+ in the sea urchin embryo are not known. Our results clearly show that Li^+ is not simply amplifying micromere influence but is active by itself in animal

halves, as in the classic experiment of von Ubisch (1929). It is also likely that micromeres are not capable of affecting *HE* expression in whole embryos, while Li^+ can. Li^+ and micromeres may have different effects on the *HE* gene because of the strong influence from maternal components, while their actions on later inductive events might be more similar. There is another obvious and fundamental difference. Micromeres have a directional action from their normal position or from any ectopic site. Their inductive power is so strong that they can induce a second axis, practically at any angle to the normal one (Hörstadius, 1973; Ransick and Davidson, 1993). In whole embryos or animal halves, effects of Li^+ on *HE* expression and cell fate are not uniform or randomly dispersed, but spatially oriented. Li^+ in all cases evidently acts isotropically and is only expected to reveal or modulate preexisting anisotropy. This suggests that the early animal half is patterned along the original AV axis independently of interactions with cells from the vegetal half. In other words, the AV axis could not be reduced to a small vegetal center.

Most of the known genes which are differentially expressed in the cleavage embryo are restricted to one of the five polyclonal territories defined by lineage (Cameron and Davidson, 1991). These presumptive territories (except the prospective skeletogenic mesenchyme) derive from founder cells which are conditionally specified by a series of cell interactions triggered by the micromeres (Davidson, 1989). Our results establish that such interactions are not required for Li^+ interference with the *HE* gene spatial control in the animal half, and suggest they are not crucial for *HE* control in normal whole embryos. They provide evidence that the spatial regulation of the earliest strictly zygotic gene expressed in the sea urchin embryo is autonomous and strongly linked to the AV-axis maternally established throughout all of the mature egg.

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