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Cell Interactions as Epigenetic Signals in Morphogenesis of the Sea Urchin Embryo

DAVID R. McCLAY, MARK C. ALLIEGRO, AND
JEFFREY D. HARDIN

The classic blastomere transplantation experiments of Hörstadius showed that many sea urchin blastomeres are influenced by their position relative to other cells in the early embryo (Hörstadius, 1935). Since individual sea urchin blastomeres respond to external information and have the capacity to progress along a variety of developmental pathways, their differentiation is said to be *regulative* (for recent reviews see Wilt, 1987; Davidson, 1989). By contrast, in animals that develop in a determinate manner (e.g., Caenorabditis, leeches and tunicates), there is very little plasticity in the developmental fate of a cell and external cues do not appear to be as prominent (even in these embryos, however, external cues are necessary; e.g., Sternberg and Horvitz, 1986). A predictive developmental fate map can be constructed for most embryos, including the sea urchin (see Cameron et al., 1987; Davidson, 1989). However, the realization of a particular fate, in regulative eggs, rests heavily on the reception of information from extracellular sources such as neighboring cells.

What are the cues for differentiation in regulative embryos? When are they delivered to responding cells and from where? How do the cues signal changes in morphogenesis? This chapter examines these developmental signals in a continuing effort to determine how morphogenesis works in regulative embryos.

What constitutes a "cue" in the sense used in this chapter? Broadly speaking, we define a *cue* as any signal or organizing influence that modulates a behavior or set of behaviors performed by a cell or group of cells during morphogenesis. We have avoided using the term "induction" because that term often implies that some unique or specific instruction is being passed on to the responding tissue. Examples below will illustrate cases in which a cue may be a normal substrate that simply coordinates or permits the continuation of a developmental sequence that has already been initiated. However, the concept of a developmental cue also encompasses the classical ideas of inductive signals in which certain instructive signals are transmitted to a cell.

BLASTOMERE AGGREGATION AND REGULATION OF THE EMBRYONIC AXES

It is known from experiments with egg fragments (Maruyama et al., 1985), observations on the pattern of cleavage, and experiments on blastomere rearrangements (Hörstadius, 1939) that one of the embryonic axes, the animal–vegetal axis, is specified prior to fertilization. Little is known about the time of establishment of the other major axis, the dorsal–ventral (D–V) axis, although some workers have suggested that it too may be specified as early as the eight-cell stage (Czihak, 1971; Cameron et al., 1987; Davidson, 1989). Regulation of the D–V axis can clearly occur after this time since experimental changes in the normal arrangement of blastomeres, by a variety of methods, still result in normal embryos (Driesch, 1893; Hörstadius, 1973; Davidson, 1989). Nevertheless, once the animal–vegetal and the dorsal–ventral axis are established, they give the positional information necessary for organization of the embryo.

Perhaps the most dramatic disruption of the normal axes is seen if one prepares cell aggregates from early embryos. If one dissociates an embryo at the four-cell stage and allows the cells to reaggregate at random immediately, normal embryos are recovered with a high frequency (Fig. 5.1). Performing the same experiment one cleavage stage later results in embryos that often have gross abnormalities in organization (Nelson and McClay, 1988). Typically, embryos that are reassociated from eight-cell stage blastomeres have a normal epithelium, often a normal-appearing gut, but they usually have an abnormal skeleton with little recognizable symmetry (the appearance of the skeleton primordia is one of the first recognizable signs of bilateral symmetry during development in this organism). In aggregates begun from progressively older embryos, a careful study has noted a steady reduction in the capacity for restoration of normal structure and symmetry (Freeman, 1988). Although these experiments do not address the question of precisely how the overall symmetry of normal embryos arises, they do indicate that the ability of dissociated blastomeres to reconstruct an embryo with organized embryonic axes is gradually restricted during early stages of development. Nothing is currently known about the molecular nature of this restriction in the sea urchin embryo.

APICAL–BASAL POLARITY DURING MORPHOGENESIS

Another axis that is established early in development is the apical–basal polarity of blastomeres. Schroeder describes the morphological appearance of this polarity (Schroeder, 1988), and it can be shown experimentally that blastomeres maintain their polarity even when isolated as single cells (Nelson and McClay, 1988; Schroeder, 1988). If the cells are reassociated they appear to rotate to reestablish an apical–basal orientation.

One manifestation of apical–basal polarity is the release of distinct groups of

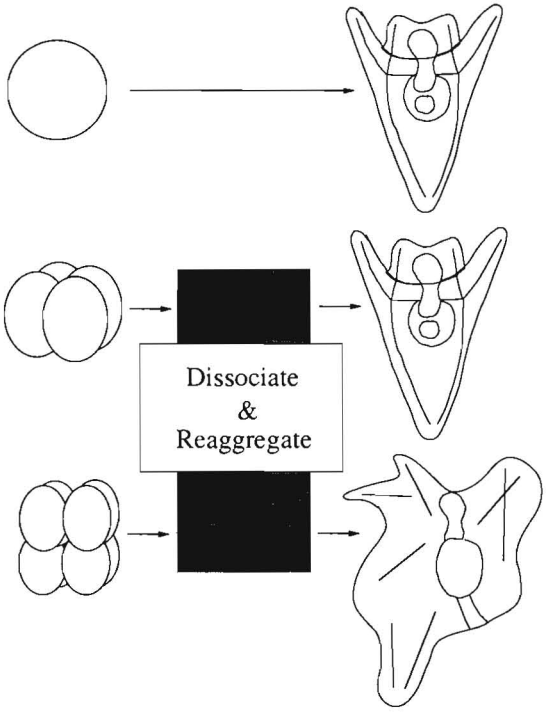

Figure 5.1. Summary of reaggregation experiments. Normally, a fertilized egg results in the formation of a pluteus larva in about 24 hours (top). If an embryo is dissociated at the four-cell stage (middle), and immediately reassociated, most of the embryos that result appear to develop with normal axes. If the embryo is dissociated at the eight-cell stage or beyond (bottom), the aggregates reassemble a normal epithelial layer, often a normal gut, but the axial information determining the skeletal pattern is missing or confused.

proteins at the two cell surfaces. Proteins characteristic of the basal lamina are released at the basal surface and other proteins are released into the hyaline layer located at the apical surface of the cells (Wessel et al., 1984; McClay et al., 1989).

Functionally, the polarized location of extracellular matrix proteins may be important in the maintenance of the blastocoel. Dan (1960) incubated cleavage-stage embryos in hypotonic sea water and noticed that cells continued to adhere to the hyaline layer after all other adhesions were lost. He suggested that the interaction of cells with the hyaline layer might be important for establishment of the blastocoel. Analysis of the hyaline layer has revealed two proteins that are adhesive substrates for sea urchin blastomeres. Hyalin, first isolated by Kane and Stephens (1969), was later shown to serve as an adhesive substrate for cells (McClay and Fink, 1982). A monoclonal antibody to the cell-binding region of hyalin has been described (Adelson and Humphreys, 1988). Incubation of embryos in the antibody results in a withdrawl of cells from the hyaline layer and a partial collapse of the blastocoel (Fig. 5.2) (Adelson and Hum-

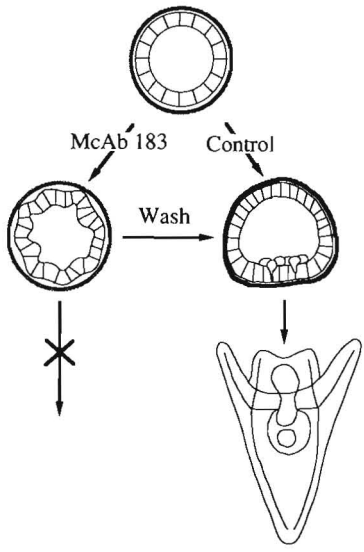

Figure 5.2. Treatment of embryos with a monoclonal antibody to hyalin. If the antibody is added to early blastula-stage embryos, the cells withdraw from hyalin and the blastocoel partially collapses. Embryos in the antibody do not progress beyond the blastula stage. If embryos are inhibited by the antibody, then washed free of the antibody, they recover and develop to the pluteus larva stage.

phreys, 1988), again suggesting that adhesion to hyalin is important for the maintenance of the blastocoel.

The second hyaline layer protein involved in adhesion is echinonectin (Alliegro, et al., 1988). This protein is secreted after fertilization into the innermost lamina of the hyaline layer. The protein has been shown to serve as an adhesive substrate for sea urchin cells in much the same way as hyalin (Alliegro et al., 1988). Thus, at least two proteins in the hyaline layer appear to provide an adhesive substrate during cleavage, and may aid in blastocoel formation.

CELL FATES ARE FIXED AT DIFFERENT TIMES IN DEVELOPMENT

Though the basic organization of the whole embryo may be specified early, individually transplanted cells can adapt to their new surroundings. At the 32-cell to 128-cell stages individual blastomeres were vitally stained and incorporated into ectopic positions of unstained whole embryos (Wray and McClay, 1988; Bernacki and McClay, 1989). The embryos then were analyzed at the pluteus stage. When single mesomere descendants were inserted into the host embryo at random, the mesomeres appeared to have their fate respecified by adapting to their new surroundings (Fig. 5.3). These experiments indicate that although the overall axial organization of the embryo might be established very early, at least in the case of mesomere derivatives, individual blastomeres continue to retain the capacity for (re)specification of fate until later in development. The experiments of Freeman (1988) appear to support the same conclusion. In experiments where a clump of undissociated tissue was purposely included in cell aggregates, the probability of aggregate reorganization was greatly improved. Freeman observed that an intact group of cells somehow

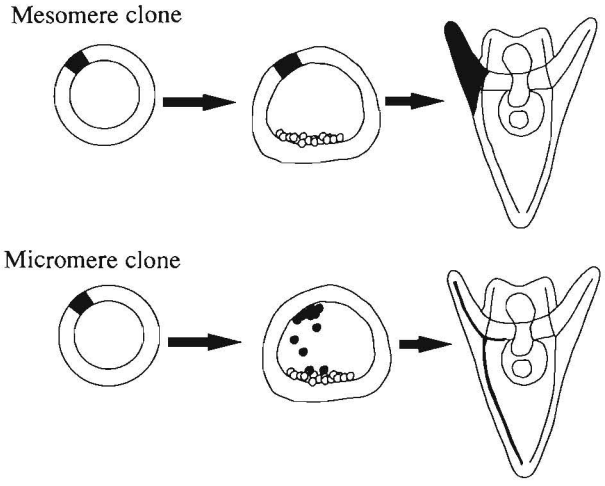

Figure 5.3. Results of ectopic insertion of blastomeres. If mesomeres are labeled with a fluorescent dye, then inserted into ectopic positions in unlabeled embryos (top), the cells become a part of the ectoderm and differentiate spatially, according to their adopted position. By contrast, ectopically inserted micromeres (bottom) retain their specification, ingress at the mesenchyme blastula stage, and contribute to a normal skeleton.

retained axial information, whereas this information was lost or respecified in isolated cells that joined the aggregates.

Although presumptive ectodermal cells retain the capacity for fate change for a long time, the micromere lineage becomes restricted very early. Four micromeres appear at the vegetal pole as a result of an unequal division at the fourth cleavage. Many experiments indicate that the micromeres are determined when they appear at the 16-cell stage. A clear indication of that determination can be seen in experiments by Okazaki (1975). The micromere lineage eventually gives rise to the group of cells, the primary mesenchyme cells (PMCs), that produce the skeleton of the larva. Okazaki (1975) showed that micromeres will produce spicules if cultured *in vitro* from the 16-cell stage onward, demonstrating that from the time of their appearance, micromeres do not require external signals from the embryo in order to differentiate into spicule-producing cells.

In addition to their role in spicule production, the PMCs can influence the pattern of differentiation of other cells in the embryo. Hörstadius (1939) transferred a quartet of micromeres from the vegetal pole (their usual location) to the animal pole, resulting in the formation of a supernumerary gut rudiment at the animal pole. Hörstadius's experiment was interpreted to suggest the existence of a gradient of some component that specifies the animal–vegetal axis. As a result of the addition of the micromeres at the animal pole, presumptive ectoderm was considered to be respecified. By contrast, transplantation of single micromeres to the animal pole fails to introduce a secondary archenteron

(Wray and McClay, 1988). This suggests that there is a threshold requirement for some factor for the presumptive ectoderm to be respecified.

The single micromere transplant experiments reveal another property of the micromeres. Ectopically incorporated micromeres do not change their fate, again indicating that the micromere lineage is determined. Furthermore, ectopically incorporated single micromeres undergo differentiation into primary mesenchyme cells (PMCs) at the same time as the normally located micromeres, and the ectopic PMCs ingress at the same time no matter where they are located in the embryo (Fig. 5.3). They then migrate to the vegetal pole where they join the host PMCs. These observations indicate that single micromeres differentiate independently of any surrounding cells, and that they are programmed to ingress no matter where they are located in the embryo. They also indicate that there is not a special apparatus provided at the vegetal plate that allows ingression movements to occur (Wray and McClay, 1988).

DIFFERENTIATION OF THE MICROMERE LINEAGE

At the mesenchyme blastula stage the PMCs ingress into the blastocoel and begin to produce a number of proteins that are unique to this lineage (McClay et al., 1983; Harkey et al., 1988; Davidson, 1989). The morphogenetic movement that places the PMCs inside the blastocoel involves changes in both adhesion and motility. Here again the methods of Okazaki (1975) are useful for revealing changes in cell behavior as PMCs defferentiate. When the cells are cultured they change behavior in the same sequence that occurs *in vivo*. They become motile (Okazaki, 1975), undergo three simultaneous adhesion changes (McClay and Fink, 1982; Fink and McClay, 1985), migrate, and later the cells associate into syncitia and produce spicules (Okazaki, 1975). The three adhesion changes are (1) a loss of an affinity for hyalin and for echinonectin, (2) a loss of an affinity for neighboring cells, and (3) an increased affinity for the basal lamina, specifically for a fibronectin-like protein in the basal lamina (Fig. 5.4). That these changes can occur in cultured micromeres suggests that this transformation is entirely internally programmed.

Although experiments in which PMCs differentiate in culture and the micromere transplantation experiments show that PMCs can differentiate autonomously, PMCs nevertheless are influenced by the embryo in a number of ways. If PMCs are transplanted from older donor embryos into younger embryos, the donor PMCs migrate to the vegetal pole (Fig. 5.5) (Ettensohn and McClay, 1986). Despite their competence to ingress and begin formation of the skeleton (as seen in the donor control embryos), transplanted PMCs remain stationary until the host PMCs ingress. The donor PMCs then join in with the host PMCs to produce a normal skeleton (Ettensohn and McClay, 1986). Thus, the embryo can impose changes in timing of a specific behavior of PMCs.

The imposition of this change in the temporal sequence of PMC differentiation is not a unique case. A number of other cues also influence the development of the PMC lineage. First, experiments suggest the presence of a target

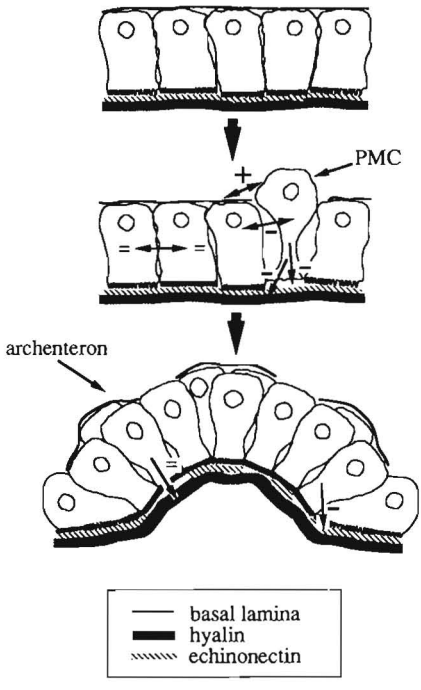

Figure 5.4. Sequence of adhesive changes occurring to the mesenchyme blastula stage and during gastrulation. Prior to primary mesenchyme cell ingression (top), all cells of the embryo are in contact with the basal lamina, with other cells, and with echinonectin and hyalin in the hyalin layer. At ingression, the primary mesenchyme cells lose an affinity for echinonectin, hyalin, and for other cells. At the same time these cells have an increased affinity for the basal lamina (middle). Meanwhile the presumptive ectoderm retain the same affinities for these four substrates all through the mesenchyme blastula stage. At gastrulation (bottom), the endoderm cells change shape and begin to rearrange as invagination begins. During this process the cells lose an affinity for echinonectin but retain an affinity for hyalin.

on the floor of the blastocoel to which the donor PMCs respond. PMCs can be injected anywhere into the blastocoel and yet they migrate specifically to the vegetal plate (Ettensohn and McClay, 1986). No other cell of the embryo will perform this behavior if injected, showing this behavior to be specific to the PMC lineage. Ectopic micromeres also move to the vegetal pole after their ingression (Wray and McClay, 1988).

The third influence of the host embryo on the transplanted PMCs involves pattern formation. When supernumerary PMCs are transplanted into host embryos, the pattern of the skeleton that results is indistinguishable from the normal pattern, even though as many as two to three times the normal number of PMCs participate in skeleton production (Fig. 5.5) (Ettensohn and McClay, 1986; C. Ettensohn, unpublished observations). Thus, the embryo can some-

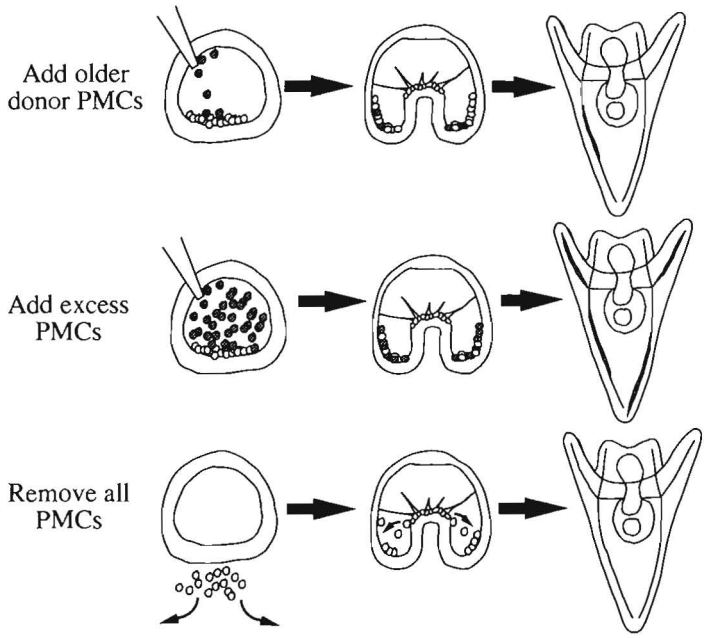

Figure 5.5. Summary of experiments involving primary mesenchyme cell transfer. If older PMCs are added to younger embryos (top), the older cells adapt to the developmental schedule of the younger host embryo. The donor PMCs move to the vegetal pole, join with the host PMCs, and collaborate to make a normal skeleton on the schedule of the host embryo. If excess numbers of PMCs are added to an embryo (middle), the cells again collaborate with the host PMCs but make a normal skeleton. In spite of many additional PMCs, the pattern of the skeleton that results is normal. If all PMCs are removed from embryos (bottom), secondary mesenchyme cells move from their position at the tip of the archenteron, convert to become PMCs, and produce a normal skeleton.

how regulate the expression of skeletal pattern. *In vitro*, these same PMCs routinely initiate more than two spicule primordia per 60 cells (there are about 60 PMCs per embryo and these produce only two spicule primordia *in vivo*). Thus, the embryo somehow restricts the number of spicule primordia to two.

If one experimentally radializes the embryo using a variety of techniques (reviewed by Czihak, 1971), more than two spicule primordia are produced. Similarly, if one disrupts the embryo at any time after the eight-cell stage and allows the cells to reaggregate, the differentiated aggregates often produce more than two spicule primordia (Nelson and McClay, 1988). These experiments demonstrate that the embryo normally constrains spicule formation to two sites in a pattern that reflects the bilateral symmetry of the embryo. The influence seems to be a restrictive one in that when freed from the constraints, PMCs have the capacity to originate more than two primordia per 60 PMCs. The molecular nature of the restrictive influence is unknown, but it is likely to reside in the vicinity of the vegetal plate.

The primary mesenchyme cells produce restrictive cues of their own. This can be demonstrated experimentally by removing all PMCs (Fig. 5.5). After removal, a skeleton arises from a subpopulation of secondary mesenchyme cells (SMCs), which become spiculogenic and produce a normal skeleton (Ettensohn and McClay, 1988). Normally, the SMCs never participate in spicule production. Therefore the PMCs must provide some restrictive signal preventing the SMCs from differentiating into spicule-producing cells. The nature of the restrictive signal is, as yet, unknown but it can be analyzed quantitatively. In *Lytechinus variegatus* if one removes 10, 20, 30, or 40 PMCs, about 50, 40, 30, or 20 SMCs, respectively, will become spiculogenic (Ettensohn and McClay, 1988). The restrictive influence must operate over a distance of tens of microns since the PMCs and SMCs are some distance from one another in the embryo. One way that SMCs could become restricted is through contact with PMC-released matrix material (known to be in the extracellular matrix); by failing to encounter such matrix material, the SMCs could convert from their normal fate and replace the PMCs. It is known that the PMCs normally produce extracellular matrix molecules that are deposited over the entire wall of the blastocoel (Wessel et al., 1984; Wessel and McClay, 1985, 1987), and thus SMCs send out filopodia that presumably come in contact with the molecules produced by PMCs. These data do not, however, rule out the possibility that a diffusible molecule might be released by PMCs, which promotes restriction of the SMC lineage.

FORMATION OF THE ARCHENTERON

Since invagination of the archenteron has been so easy to observe in the sea urchin embryo, there have been many speculations as to how this morphogenetic movement occurs. To begin the invagination, cells in the vegetal plate change shape and bend inward. The shallow invagination soon begins to elongate into a hollow cylinder, and elongation continues until the tip of the archenteron reaches a region somewhere near the site where the stomodaem will form. The initial invagination is apparently autonomous, i.e., there are no forces outside the immediate vicinity of the vegetal plate required for its invagination. This is best shown by experiments in which the animal half of the embryo is removed. Under such conditions the first phase of invagination occurs normally (Moore and Burt, 1939; Ettensohn, 1984). But what promotes the inward bending of the archenteron? Although various models have been proposed, including models involving an apical constriction of microfilaments (Odell et al., 1981) or cell adhesion changes in the vegetal plate (Gustafson and Wolpert, 1967), what drives this morphogenetic movement remains unknown.

Although little is known about the motile mechanisms of invagination, several kinds of evidence support the hypothesis that specific cell recognition changes occur during the invagination process. First, after the beginning of invagination endoderm cells begin to express cell surface antigens that are

unique to this lineage (McClay and Hausman, 1975; McClay et al., 1977, 1983; McClay and Marchase, 1979). Second, in cell aggregation studies, the beginning of gastrulation signals the beginning of the ability of endoderm cells to sort from ectoderm cells when mixed in aggregates (McClay and Marchase, 1979; Nelson and McClay, 1988). Third, during archenteron invagination endoderm cells lose an affinity for the extracellular matrix protein echinoectin (Alliegro et al., 1988). Fourth, time-lapse observations of the basal surfaces of cells in the vegetal plate indicate that they undergo intense cortical blebbing at the time of invagination (Kinnander and Gustafson, 1960). At the same time, isolated presumptive endoderm cells become motile in culture, extruding numerous blebs. It is not known what role this behavior plays during the early stages of invagination, but this behavior could be an important component of the cell movements that occur.

There is good evidence to show that epithelial cell rearrangement occurs during archenteron elongation. Indirect support comes from ultrastructural studies in which it is observed that as invagination continues, the number of cells around the circumference of the archenteron decreases. The best explanation for this observation is that the cells have rearranged (Ettensohn, 1985; Hardin and Cheng, 1986). More direct evidence comes from studies in which a group of endodermal cells is labeled prior to the beginning of invagination (Fig. 5.6). A patch of cells is gradually resolved into a narrow row of cells during archenteron elongation, by intercalation of the labeled cells with one another. The only possible explanation for this observation is that the cells rearrange during invagination. It is possible the rearrangements involve a combination of motil-

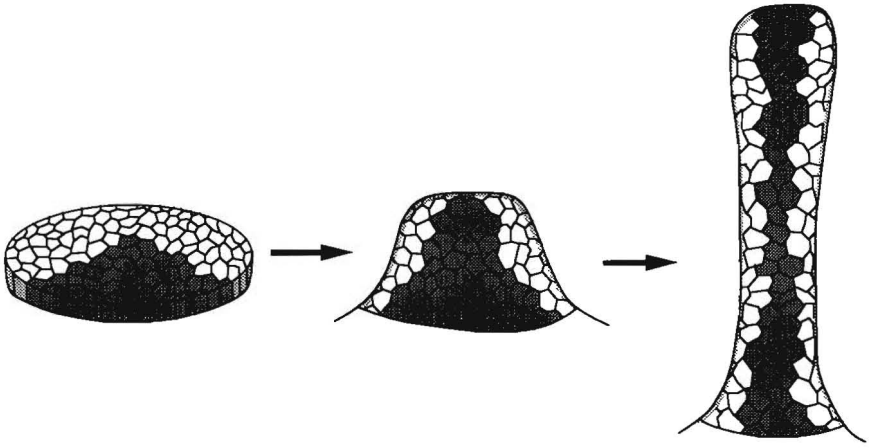

Figure 5.6. Diagram of endodermal invagination to show pattern of cell rearrangements. Labeled presumptive endoderm cells were added to the vegetal plates of early blastulae. These formed a patch of presumptive endoderm cells in the vegetal plate just prior to invagination (stipled cells at left). During invagination this patch of labeled cells became rearranged to form a linear array of cells along the length of the archenteron (right).

ity and cell adhesion changes during archenteron formation, but the experiments fall short of any demonstration of the extent to which the cell rearrangements actually use these properties.

A striking feature of the latter part of sea urchin gastrulation is the intense protrusive activity of secondary mesenchyme cells at the tip of the archenteron. Time-lapse films reveal that the secondary mesenchyme cells extend filopodia that often come into contact with the wall of the blastocoel (reviewed by Gustafson and Wolpert, 1967). This observation led Gustafson and Wolpert to propose that filopodia provide a pulling force that allows the archenteron to elongate. However, two kinds of evidence argue that more than half of the elongation of the archenteron is autonomous. First, if the filopodia are ablated with a laser microbeam, elongation continues to roughly two-thirds of the complete length (Hardin, 1988). Second, the archenteron extends to about two-thirds of the completed length in exogastrulae, where secondary mesenchyme cells fail to reach any anchoring substrate on which to pull (Hardin and Cheng, 1986; Hardin, 1988).

However, completion of archenteron elongation requires the participation of secondary mesenchyme cells. If all SMCs are ablated, or if exogastrulation occurs, the archenteron does not reach its full length. Measurements of filopodia and of the shape of endoderm cells during archenteron extension in *Lytechinus pictus* indicate that at about the time that SMCs reach the point of contact on the blastocoel wall that signals an end to invagination, there is a transient stretching of endoderm cells as the filopodia pull the archenteron to its final length.

How do the SMCs participate in archenteron elongation and what controls their behavior? The general motile behavior of SMCs during invagination appears to be specified at the beginning of gastrulation. This behavior consists of continual cycles of filopodial extension, attachment, and withdrawal on the part of many SMCs at once (Gustafson and Kinnander, 1956; Hardin and McClay, 1989). The filopodial activity continues until, at the end of gastrulation, the SMCs reach a specific region, or "target," at an experimentally demonstrable site somewhere along the wall of the blastocoel, usually near the animal pole (in *Lytechinus* the target is at or very near the animal pole). On striking the target the SMCs become quiescent, and shortly thereafter can be seen to begin to differentiate into a variety of mesodermal structures. The normal behavior of SMCs suggests that they are programmed to extend filopodia from the onset of archenteron elongation until reaching the target. If this were true then perhaps reaching the target would provide the signal for cessation of filopodial extension. To test this possibility, the putative target region was pushed toward the tip of the archenteron so that contact was forced precociously (Fig. 5.7). When the target was presented to filopodia several hours ahead of schedule, the filopodial behavior of the SMCs ceased precociously. Is the target site unique in its ability to provide the correct cue to stop the SMC behavior? When the tip of the archenteron was forced to fuse with other (nontarget) regions of the blastocoelic wall, temporary contact was made, but then was followed by persistent, further extension toward the target (Fig. 5.7). Once contact with the actual target region was made, the filopodia exhibited stable

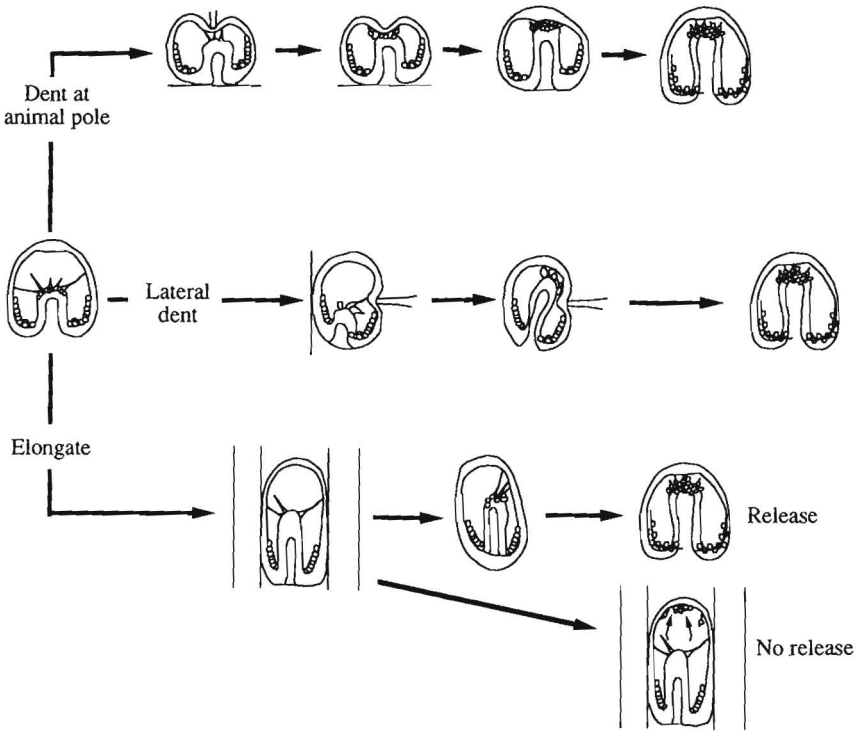

Figure 5.7. Summary of experiments to identify the target for archenteron invagination. It was observed that when SMCs reached the animal pole they became quiescent after having exhibited repeated rounds of filopodial extension all through the invagination movements. To test whether contact with the animal pole was important for the change in cell behavior, embryos were dented from the animal pole to bring the tip of the archenteron precociously into contact with the basal lamina at the animal pole (top). SMCs were observed to become quiescent precociously. Dents were made laterally to determine whether there was anything special about the animal pole in bringing about the change in SMC behavior (middle). The SMCs made contact with the lateral dents but did not change their behavior and proceeded to the animal pole where they became quiescent. If embryos were elongated to prevent SMCs from reaching the animal pole (bottom), the filopodial extension behavior continued long beyond the time of normal SMC–animal pole contact. If released from the elongated shape, the SMCs made contact with the animal pole and became quiescent. If maintained in the elongated state (no release), the SMCs ultimately broke free from the archenteron and migrated to the animal pole on their own where they became quiescent.

attachments, and they then appeared to direct the tip of the archenteron to the target region.

By squeezing embryos into narrow diameter glass capillary tubing, embryos can be abnormally elongated along the animal–vegetal axis such that the archenteron is forced to travel a greater distance before reaching the target at the

animal pole (Fig. 5.7). In this experiment SMCs extended filopodia for a longer period of time than they did in control embryos. If the archenteron was prevented from reaching the animal pole for an extended time, SMCs finally broke away from the archenteron, migrated to the target region at the animal pole on their own, and became quiescent when they reached the target. Thus, filopodial extension appears to be programmed to continue until the appropriate target is reached on the wall of the blastocoel.

DIFFERENTIATION OF ENDODERM AND SECONDARY MESENCHYME CELLS

Presumptive endoderm cells and secondary mesenchyme cells both are involved in invagination. Does this mean that they rely on the same developmental cues for their differentiation? To address this question we examined the patterns of differentiation of the two cell types. A number of experiments suggested the presence of at least two critical regulatory periods, the first just prior to the beginning of invagination, and the second at or near the time of the interaction of the tip of the archenteron with the target region at the end of invagination.

A number of reagents prevent the initiation of invagination. For example, treatment of fertilized eggs with β-aminoproprionitrile (BAPN), an inhibitor of lysyl oxidase (an enzyme involved in collagen cross-linking), allows embryos to develop normally to the mesenchyme blastula stage, but the endoderm fails to invaginate (Fig. 5.8) (Wessel and McClay, 1987; Butler et al., 1987). If the BAPN is removed, even after the embryos have been arrested at the mesenchyme blastula stage for more than 24 hours, the embryos begin to gastrulate and complete development normally. The BAPN has been shown to prevent proper construction of the basal lamina but the inhibitor does not appear to interfere with the expression of genes that are active up until invagination begins, nor does it interfere with the new expression of genes in the ectoderm (Wessel and McClay, 1987; Wessel et al., 1989). In contrast, marker proteins that normally are expressed in the archenteron fail to be expressed as long as the embryos are incubated in BAPN. These markers are expressed shortly after removal of the embryos from BAPN, suggesting that a critical interaction is necessary at the beginning of invagination for a subset of endodermal genes to be transcribed (Wessel and McClay, 1987; Wessel et al., 1989). The BAPN data suggest a critical interaction with the basal lamina is necessary for invagination and for the expression of archenteron-specific genes. A similar block to invagination and endoderm-specific gene expression can be obtained when the hyaline layer is experimentally disrupted by incubating fertilized eggs in a monoclonal antibody that binds to hyalin (Adelson, 1985; Adelson and Humphreys, 1988). As with BAPN, the antibody blocks archenteron formation reversibly, and marker endodermal proteins fail to be expressed when embryos are incubated in the antibody from fertilization onward (Adelson, 1985). These data suggest that a critical event occurs at the beginning of gastrulation, and that

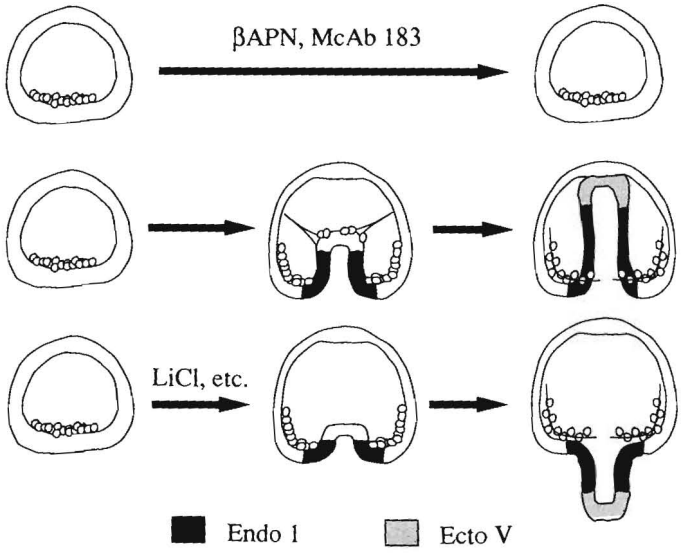

Figure 5.8. Summary of experiments on sequence of expression of endodermal monoclonal markers. If embryos were treated with compounds that prevent initiation of archenteron invagination (top), endodermal marker proteins fail to be expressed. Control embryos, or embryos released from the inhibitors (middle) express endo 1 and then later express ecto V at about the time of archenteron completion. If archenteron completion is prevented (bottom), the two endodermal markers are still expressed in the control sequence.

when that event occurs the vegetal plate must be in normal contact both with the basal lamina and with the hyaline layer.

Once the embryo passes through this critical period, a number of endodermal genes are expressed in a temporal sequence. For example, Endo 1 (McClay et al., 1983; Wessel and McClay, 1985) is expressed at, or shortly after, invagination begins. A second marker, Ecto V (McClay et al., 1989), is expressed, normally, at the completion of invagination in the foregut (Fig. 5.8). In experiments in which invagination begins, but the tip of the archenteron is prevented from reaching the animal pole target, both antigens are still expressed in the correct sequence (Fig. 5.8). Correct sequential expression of these markers also occurs in exogastrulae, in which both the complete extension of the archenteron and the attachment to the target are prevented. These data suggest that the endoderm is somehow programmed at the beginning of gastrulation to express a temporal sequence of genes irrespective of the ultimate successful completion of archenteron elongation and oral attachment.

Secondary mesenchyme cells (a loose, collective term for mesenchyme cells that do not normally produce spicules, and that are located at the tip of the archenteron) may follow different regulatory cues. A subset of SMCs, the pigment cells, differentiate during gastrulation (Gibson and Burke, 1985), but other SMCs do not appear to be irreversibly determined until a time that may

approximate reaching the target region at the animal pole. The lineage conversion experiments described above (Ettensohn and McClay, 1988) reveal that until some time during archenteron invagination, the SMC population (that does not differentiate into pigment cells) retains the capacity to become spiculogenic and therefore is not restricted in fate. Shortly after the SMCs change behavior on contact with the target area they can be observed to begin their differentiation. Thus, in contrast to the endoderm, which undergoes a critical determinative event at the beginning of gastrulation, SMC determination is delayed and may be associated with, or occur shortly after, the SMC–target interaction.

CONCLUDING REMARKS

From early cleavage stages through gastrulation in the sea urchin embryo our experiments suggest that there are a series of critical interactions that influence cells to develop along particular pathways. The several developmental cues described in this chapter probably represent just a few of an abundant group of epigenetic regulatory elements that act during morphogenesis. Added to this is a growing list of nuclear regulatory elements that have been described in the sea urchin embryo (Calzone et al., 1988), and in other embryos, to provide a hierarchy of control during development. Characterization of homeotic genes in *Drosophila* and *C. elegans* has indicated that these genes may serve as master switches to progressively restrict developmental pathways in these organisms. The sea urchin embryo may have master switches as well [e.g., homeobox sequences have been described in the sea urchin, though nothing yet is known about what they might regulate (Angerer et al., 1989)]. Even if there are such master switching mechanisms, the data indicate that sea urchin development also relies on a whole series of epigenetic interactions that are critical for morphogenesis. This chapter has reviewed the evidence for the existence of a few of those cues. It is important now to identify these cues, to learn how these epigenetic signals relay information, how these signals impinge on determinative regulatory pathways, and how they help to orchestrate the complex morphogenetic changes that transform the egg into an embryo.

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