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Local cell interactions and the control of gastrulation in the sea urchin embryo

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The sea urchin embryo is a good model system for studying the role of mechanical and cell-cell interactions during epithelial invagination, cell rearrangement and mesenchymal patterning in the gastrula. The mechanisms underlying the initial invagination of the archenteron have been surprisingly elusive; several possible mechanisms are discussed. In contrast to its initial invagination, the cellular basis for the elongation of the archenteron is better understood: both autonomous epithelial cell rearrangement and further rearrangement driven by secondary mesenchyme cells appear to be involved. Experiments indicate that patterning of freely migrating primary mesenchyme cells and secondary mesenchyme cells residing in the tip of the archenteron relies to a large extent on information resident in the ectoderm. Interactions between cells in the early embryo and later cell-cell interactions are both required for the establishment of ectodermal pattern information. Surprisingly, in the case of the oral ectoderm the fixation of pattern information does not occur until immediately prior to gastrulation.

Key words: sea urchin / epithelial invagination / cell rearrangement / mesenchyme

TO UNDERSTAND gastrulation in any satisfying sense, its cellular basis must be understood. As the specific cellular behaviors that contribute to the shaping of the gastrula are elucidated, further questions can be posed about how these behaviors are regulated and integrated in time and space, and how specific molecular interactions influence these behaviors. Historically, the sea urchin embryo has been an important model system for studying cell behavior during gastrulation, and recent experiments have added substantially to our understanding of the cellular events of gastrulation in this simply organized embryo. The following sections examine several important aspects of sea urchin gastrulation, and what progress has been made in understanding them.

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The sea urchin archenteron: a 'simple' invagination?

The hatched blastula consists of a ciliated epithelial monolayer (Figure 1); its interior surface comprises the basal ends of the epithelial cells and their associated basal lamina, and its outer surface comprises the apical ends of the cells. Its apical surface is covered by at least two, but possibly more, distinct layers.[1] The outermost layer, the *hyaline layer*, is constructed after release of cortical granules at fertilization.[2] The innermost layer, the *apical lamina*, consists of three major glycoproteins, the fibropellins.[3] In addition, specialized structures are associated with the tips of microvilli, which project into the apical extracellular matrix.[1,4]

The epithelium at the vegetal pole of the hatched blastula flattens and thickens to form the *vegetal plate*. The vegetal plate consists of clonally distinct cells that will ultimately give rise to all of the major mesenchymal cells of the embryo, as well as the archenteron. The first mesenchymal cells to leave the vegetal plate are the *primary mesenchyme cells* (PMCs) which lose the epithelial phenotype and ingress into the interior. As they ingress, PMCs undergo both structural[5,6] and adhesive[7,8] changes. PMCs remain at the vegetal region and ultimately adopt a highly stereotyped pattern within the lateral ectoderm (see below).

Following the ingression of PMCs, the vegetal plate undergoes *primary invagination* to form the archenteron. Initially, the archenteron is a shallow depression, but the invagination deepens to form a cylinder with a flat top. How is the initial invagination of the archenteron produced, and what force(s) cause it to continue to invaginate? Unlike the ventral furrow of *Drosophila*[9,10] or the blastoporal pigment line of *Xenopus*,[11] the sea urchin archenteron is a cylindrically symmetric invagination, suggesting that the forces that produce it must be so as well. Classic microsurgical experiments by Moore and Burt[12] and repeated more rigorously by Ettensohn,[13] indicate that the forces responsible for invagination are local in character: when the vegetal plate is

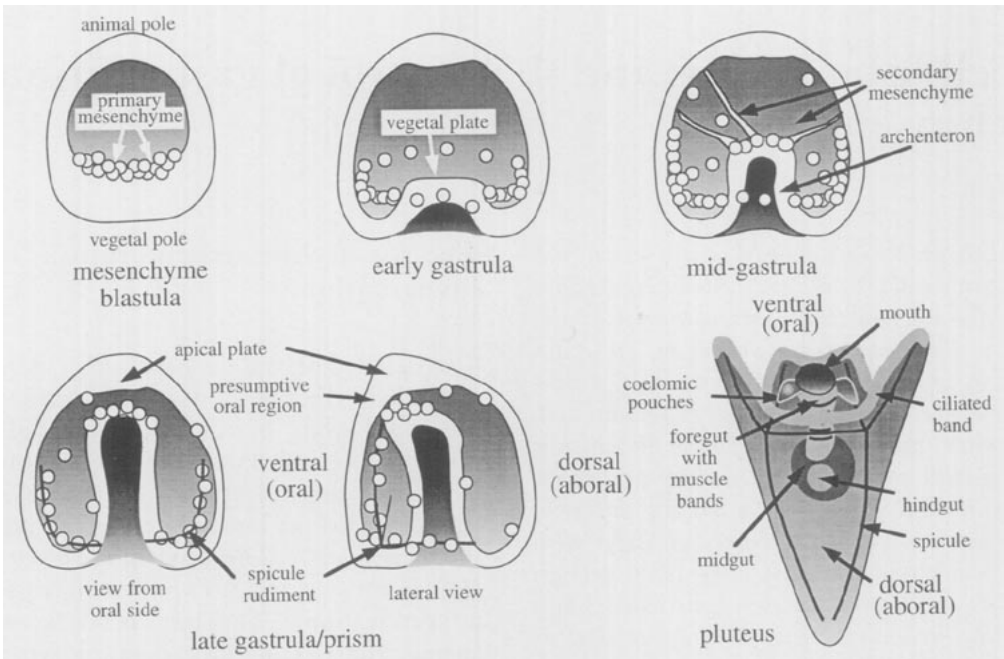

Figure 1. An overview of gastrulation in the sea urchin embryo.

isolated prior to or during primary invagination, it will still invaginate or remain invaginated. Thus long-range mechanisms such as global epiboly of the ectoderm cannot account for primary invagination. Disruption of microtubules[14,15] or inhibition of DNA synthesis and subsequent mitoses[16] do not prevent invagination, indicating that in at least some species, oriented cell division and resulting 'mitotic pressure' are not important for invagination.

A number of models have been proposed that attempt to account for the local, presumably actin-mediated invagination of the archenteron (Figure 2). The best known of these is active *apical constriction*. Ultrastructural data[13] and confocal microscopy (J. Hardin, unpublished observations) indicate that an apical actin network is present in the vegetal plate, as it is in the ventral furrow of *Drosophila* at the time of its invagination.[17] Furthermore, scanning electron microscopic data indicate that some cells in the vegetal plate acquire a flask or bottle shape as their apices constrict.[18,19] Such shape changes are consistent with active constriction, but they could equally well be an effect of other forces acting on the vegetal plate. Persistent cytochalasin treatment results in dissociation of the cells of the early sea urchin gastrula,[13] so it cannot be used to disrupt microfilaments in an interpretable fashion. In addition, there is no evidence that isolated cells of the vegetal plate will autonomously constrict their apices, which might be expected if active apical constriction were operating. Such autonomous constriction can be demonstrated in the case of bottle cells at the blastopore lip of *Xenopus*.[11] Thus, currently there is no compelling evidence

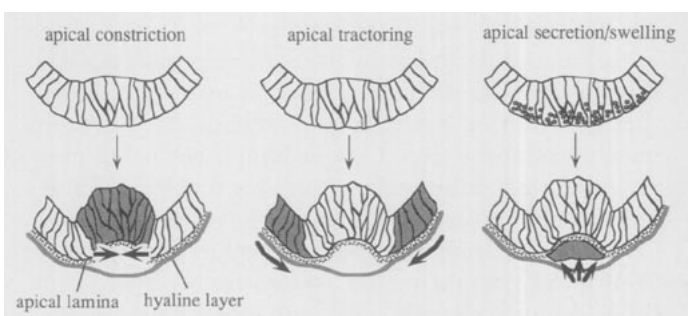

Figure 2. Proposed mechanisms of primary invagination. Proposed force-producing cells or materials are shown in gray. In the apical constriction model, cells in the center of the vegetal plate constrict their apices, resulting in local bending of the vegetal plate. In the apical tractoring model, cells adjacent to the vegetal plate converge towards the plate; the resulting compression buckles the plate. In the apical secretion/swelling model, vesicles containing proteoglycans are stored in the vegetal plate; when secreted, they assemble and swell to produce a plug of material that pushes the vegetal plate inward. See text for further explanation.

that active apical constriction operates within the vegetal plate.

A second proposed mechanism for producing an invagination is '*apical tractoring*'. This model was prompted by the observation that epithelial cells flanking the vegetal plate proper appear sheared, with their apical surfaces 'pulled' toward the vegetal plate in mesenchyme blastulae of *Strongylocentrotus purpuratus*.[20] Burke and colleagues have suggested that cells actively extend apical protrusions, using the apical extracellular matrix as a substratum; as they converge toward the vegetal plate, the plate itself would experience compression and buckle inward to produce an invagination.[20] In support of this hypothesis, treatment of hatched blastulae with antibodies recognizing fibropellins results in failure of primary invagination and lack of convergence of material towards the vegetal plate.[20] An alternative explanation is that an intact apical lamina is simply required for the mechanical integration of the epithelium near the vegetal plate; if the integrity of the tissue were perturbed, stresses produced within the plate would not be properly transmitted. A general effect of this sort appears to result when embryos are treated with antibodies recognizing hyalin; in this case cells detach from the hyalin layer and the epithelium loses its stability.[21]

Finally, *localized secretion* and *swelling* of proteoglycans has been suggested recently as another mechanism that could produce an invagination. In support of this model, a monoclonal antibody that recognizes a vertebrate chondroitin sulfate proteoglycan stains material at the vegetal plate.[22] Invagination can be precociously produced by treating embryos with calcium ionophores, and such treatments appear to result in premature release of this material from intracellular vesicles. Conversely, treatments that block secretion in general (including the proteoglycan that appears at the vegetal plate) tend to inhibit precocious invagination. This model would seem to require the mechanical resistance of the hyalin layer, which would presumably force the proteoglycan to push inward. However, thus far nothing is known about how hyalin or other extracellular matrix proteins interact structurally or biochemically with the released material.

None of the models discussed above excludes the others, so it is possible that a combination of them could account for primary invagination. Unfortunately, virtually nothing is known about the motility of the populations of cells that may be involved in producing an invagination. A detailed, dynamic analysis of primary invagination is still needed. Beyond this much needed descriptive analysis, additional, interpretable experiments are required to provide persuasive evidence for or against these models.

The elongating archenteron: directional cell rearrangement

In most species, the archenteron ceases its invagination after achieving a length of 1/3-1/2 of the diameter of the embryo. Following a noticeable pause,[23] the archenteron resumes its elongation, at about the time *secondary mesenchyme cells* (SMCs) become protrusively active at its tip. Whereas the cellular mechanisms underlying primary invagination of the archenteron remain surprisingly elusive, a reasonably satisfying picture of the second phase of invagination has recently emerged (Figure 3). The major means by which the archenteron elongates is through the rearrangement of its epithelial cells. Epithelial cell rearrangement was initially inferred from tissue sections and scanning electron micrographs by Ettensohn in *L. pictus*.[24] More recently, cell rearrangement has been directly observed in the pencil urchin, *Eucidaris tribuloides*,[19] and cell rearrangement appears to be a ubiquitous phenomenon during sea urchin gastrulation.[25] Rearrangement occurs despite the presence of septate and adherens-type junctions at the apices of the

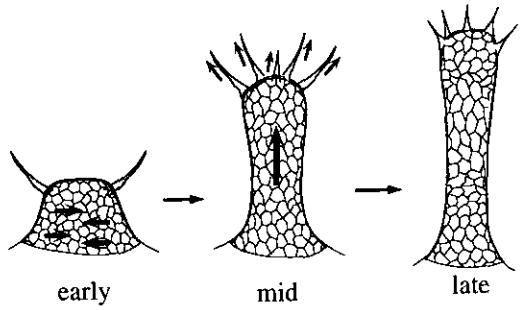

Figure 3. A summary of proposed mechanisms responsible for elongation of the archenteron. During the early phase of elongation, autonomous rearrangement (intercalation) of epithelial cells in the wall of the archenteron results in significant lengthening. Midway through its elongation, the archenteron begins to experience tension generated by secondary mesenchyme cells, which results in further rearrangement and noticeable stretching of the cells in the narrowest region of the archenteron late in gastrulation.

rearranging cells,[1,13] as it does in other situations where epithelial cell rearrangement has been demonstrated.[26,27] As the archenteron elongates, cell rearrangement occurs simultaneously at the blastopore, resulting in a decrease in its diameter.[19]

What is the motile basis for the cell rearrangement observed during archenteron elongation? Gustafson and coworkers,[28,29] and Dan and Okazaki[30] proposed that tension exerted by SMCs could provide a sufficient explanation for the elongation of the archenteron. Numerous observations indicate that filopodia extended by SMCs do exert tension: (1) they pull out 'cones of attachment' where they attach to the ectoderm; (2) when individual SMCs detach the archenteron often visibly retracts (ref 30; J. Hardin, unpublished observations); (3) in some species, SMCs appear to rip out of the tip of the archenteron (ref 30; J. Hardin, observations). In addition, general treatments that result in poor attachment of SMCs often result in impaired elongation of the archenteron.[30,32] Although Trinkaus pointed out that most of this evidence is open to other interpretations,[33] these results nonetheless provide evidence for an important role for SMCs in elongating the archenteron.

More recent experiments have clarified the cellular mechanisms of archenteron elongation. First, it now seems clear that filopodial traction is not required during the early phase of elongation, since the archenteron can nearly double in length after laser ablation of all SMCs.[34] In *Eucidaris tribuloides*, filopodia attach laterally throughout gastrulation, making it unlikely that they produce significant tension along the axis of extension of the archenteron.[19] However, the laser ablation experiments also indicate that SMCs are required late in gastrulation. When most, but not all, SMCs are ablated late in gastrulation, the archenteron continues to elongate at a reduced rate directly related to the number of actively protrusive cells at its tip.[34] Thus archenteron elongation seems to involve a combination of both autonomous extension and continued elongation in response to filopodial traction.

Little is known about the motility that produces active epithelial cell rearrangement in this or any system.[26,27] In *Eucidaris tribuloides*, the basal surfaces of cells in the archenteron display basal bleb-like cortical protrusions as they rearrange.[19] Scanning electron microscopy[19] and, more recently, computer-assisted and laser scanning confocal microscopy of cells labeled with $DiIC_{16}$ have revealed that as active rearrangement begins, the basal surfaces of cells in the archenteron extend numerous lamellipodial protrusions; when visualized with DiI, these protrusions can extend several cell diameters.[35] Likewise, cells at the blastopore appear to extend numerous lamellipodial protrusions; in many cases these appear to be oriented towards the blastopore.[35] Although the signals that entrain cells to rearrange directionally remain elusive, these observations indicate that rearranging epithelial cells are a 'hybrid' sort of cell, exhibiting both epithelial and mesenchymal characteristics. As suggested by Mittental and Mazò,[36] one possible orienting cue might be the differential distribution of adhesion molecules along the length of the archenteron. A similar conjecture has been made recently to account for germ band extension in *Drosophila*.[37]

A detailed understanding of how filopodial traction produces continued cell rearrangement is also lacking. However, several elements of this picture appear to be emerging. First, cells are visibly stretched due to filopodial traction late in gastrulation, especially in *L. pictus*.[19] The stretching is transient; additional cell rearrangement appears to relieve the stresses within the archenteron.[19] In this sense, the tissue sheet undergoes deformation analogous to the plastic deformation of a polymer in response to unusually high stress. Adding plausibility to this notion, computer simulations have been performed by Oster and Weliky[38] in which junctions between cells in a model epithelium can be remodeled when the stress on a given cell-cell connection is too high. When applied to the sea urchin archenteron, the model closely approximates what is seen in actual embryos. While such models suggest ways in which epithelia might respond to mechanical stress, the cellular machinery that might mediate such behavior is not known.

Target recognition by mesenchyme cells: the interaction of motility and pattern

The two major populations of non-pigmented mesenchyme, PMCs and SMCs, engage in distinctly different pattern forming processes during and immediately after gastrulation. PMCs form two large clusters on the ventral side of the embryo, and intervening PMCs adopt a characteristic ringed pattern; ultimately they secrete the larval skeleton (reviewed in refs 39, 40). SMCs, on the other hand, give rise to a number of different cell types, including muscle cells, pigment, and large, spindle-shaped

blastocoelar cells.[41,42] Some SMCs remain in the tip of the archenteron throughout gastrulation and attach to a predictable site near the animal pole in the ventral ectoderm.[43] What controls the pattern adopted by these two populations of mesenchyme? Experiments suggest that pattern information resides in the ectoderm in both cases.

Several experiments indicate that PMCs respond to local pattern information in the ectoderm. First, when recently ingressed PMCs are displaced by centrifugation or transplantation, they migrate to the vegetal plate to rejoin unperturbed PMCs.[44,45] Second, PMCs that form the ventrolateral clusters rest atop two ventrolateral ectodermal thickenings.[44] When these thickenings are shifted towards the animal pole in embryos vegetalized with LiCl, the PMCs are shifted as well.[46] Third, when embryos are radialized by treatment with $NiCl_2$, their PMCs adopt a radialized pattern.[47] However, when PMCs from $NiCl_2$-treated embryos are transplanted into normal embryos devoid of their own PMCs, the transplanted cells adopt a normal, bilateral pattern. Conversely, normal PMCs transplanted into nickel-treated embryos adopt a radialized pattern.[48] These experiments indicate that the differentiation of the ectoderm along both the dorsoventral and animal-vegetal axes is crucial to the pattern adopted by PMCs. More, global regulation of PMC pattern has also been demonstrated. When 2-3 times the normal number of PMCs are added to embryos, they produce a normal skeleton, although the spacing of PMC cell bodies along the spicules of such embryos is 2- to 3-fold more dense than usual.[49] In half- and quarter-sized dwarf larvae, the pattern produced by PMCs is proportionately correct,[50] again indicating that regulation of the size of the skeleton occurs. The molecular basis for PMC patterning is unknown, although a number of possibilities have been put forward. (Consult refs 39 and 51 for a more detailed discussion of the merits of these hypotheses.) Clearly, more careful analysis is needed to clarify the sorts of cues that could be operating at the molecular level to control PMC pattern.

In contrast to PMCs, the cellular cues directing SMCs appear to be better understood. SMCs extend filopodia repeatedly; when filopodia attach to lateral regions of the embryo, they remain attached for several minutes on average, but are eventually withdrawn and collapse, only to be re-extended.[43] When SMCs contact the ventral ectoderm near the animal pole, however, their behavior abruptly changes: they cease cyclical extension of protrusions and remain attached for long periods of time to this 'target' region.[43] This change in motility can be delayed by preventing contact with this region, and precociously induced by forcing contact with this region earlier than usual.[43] As filopodial attachments become focused near the animal pole, the vectorial contribution of each filopodium to the axial tension exerted on the archenteron increases, precisely at the time that such tension appears to be most significant within the archenteron.[19] The net result is that the archenteron completes its elongation and is positioned in the correct location for mouth formation.[43] Here again, however, the molecular nature of this guidance information is not known.

How does the pattern information that is clearly present within the embryo during gastrulation arise developmentally? Based on studies by Hörstadius[52] and Cameron and colleagues (reviewed in ref 41), it is clear that lineage founder cells that give rise to the major tissue territories of the embryo can be distinguished by the 5th-6th cleavage. It is also clear that local cell-cell interactions between cells from different tissue territories can influence the expression of particular cell fates in dramatic ways. For example, studies using 16- and 32-cell embryos[52-54] have shown that micromeres in contact with mesomeres can induce them to form an archenteron, even though they do not normally do so.

Do additional territorial interactions occur later to establish mesenchymal 'targets'? Classic experiments by Hörstadius[52] as well as more recent studies suggest that at least two distinct cell-cell interactions may be important (Figure 4). First, transplantation of veg_2 cells to an ectopic location results in the autonomous production of a second archenteron by the implanted cells.[52] In addition, a single ectopic veg_2 cell can induce two new bilateral sites of spicule formation by host PMCs; this appears to result from lateral induction of host ectoderm to produce two new ectodermal patterning centers (Figure 4A; J. Hardin, H. Benink, G. Wray, manuscript in preparation). Second, Hörstadius found that the cells destined to give rise to most of the ectoderm (the an_1 and an_2 tiers in his terminology) formed *Dauerblastulae* when isolated at the 32- or 64-cell stage. However, when the next more vegetal (veg_1) tier is included, the an_1/an_2 progeny form a stomodeal invagination (the ectoderm's contribution to the mouth; Figure 4B).[52]

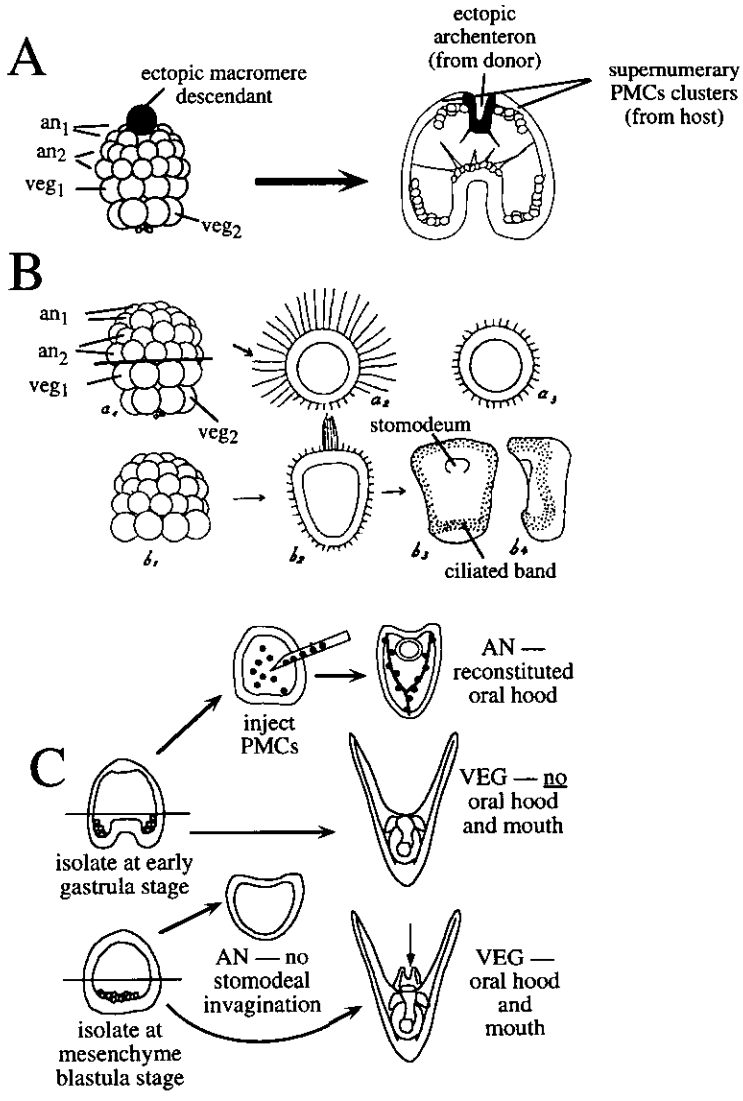

Figure 4. Experiments demonstrating interactions between different tissue territories are important for establishment of 'targets' for mesenchyme cells in the sea urchin embryo. (A) When labeled veg_2 cells are ectopically incorporated into an unlabeled host, the labeled tissue gives rise to an ectopic archenteron. In addition, two new bilateral sites appear in the ectoderm, resulting in an extra pair of spicule rudiments (J. Hardin, H. Benink, G. Wray, manuscript in preparation). (B) When the veg_1 tier and the animal half of the embryo are isolated from more vegetal tiers of blastomeres, they form a stomodeum (adapted from ref 52). (C) Experiments showing that fixation of the oral field does not occur until the gastrula stage. When animal halves are isolated at the mesenchyme blastula stage, they do not produce a stomodeum; when isolated slightly later, they will. In addition, when primary mesenchyme cells are transplanted into such a fragment, they form structures resembling normal oral rods. Conversely, vegetal fragments show a decreasing ability to regulate to produce a new site of mouth formation.[55]

This suggests that interactions occur between the veg_1 progeny and the adjacent tiers, resulting in induction of the oral field. Surprisingly, isolations performed by Hardin and Armstrong[55] at progressively later stages indicate that this interaction does not fix the oral field until relatively late in development. Prior to the early gastrula stage, the remaining vegetal tissue can regulate to produce

a new site of mouth formation, whereas the animal hemisphere does not produce a stomodeum (Figure 4C). Transplantation of PMCs into animal hemispheres isolated at various times indicates that the pattern information required for the production of the parallel skeletal rods flanking the mouth is coordinately regulated with the oral field[55] (Figure 4C).

Conclusions

The major cellular events underlying sea urchin gastrulation have been and are being identified. While this is gratifying, the challenge for the future will be to identify specific molecules that mediate these cellular behaviors and interactions. The dearth of molecular information in the sea urchin system is in part due to the lack of a practical means of isolating and characterizing mutations affecting gastrulation. However, given the ease with which extracellular matrix molecules can be isolated in this system (e.g. ref 56), and given the power of the polymerase chain reaction and the remarkable homology between adhesion and signaling molecules in other diverse organisms, tools should soon be available that will aid our molecular understanding of this appealingly 'simple' embryo.

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