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Context-sensitive cell behaviors during gastrulation

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Cell interactions are important during gastrulation in many systems. Sea urchin gastrulation provides a simple model system for understanding these cell interactions, and many of the cell behaviors that contribute to gastrulation have been analyzed. These include ingression, migration, and directed localization of primary mesenchyme cells, invagination of the archenteron, elongation of the archenteron via epithelial cell rearrangement, and attachment of the archenteron to the site of the future mouth by filopodia at the tip of the archenteron. These processes illustrate how the intrinsic behavior of a cell or group of cells can be modulated by the context in which the cells find themselves. Contextual influences include the local mechanical environment, the geometry of the embryo, the juxtaposition of one type of cell or tissue with another in space and/or time, the extracellular matrix, and localized regions containing guidance information for mesenchyme cells. All of these constraining influences play a role in sea urchin gastrulation, and serve to emphasize the importance of understanding both autonomous cell behaviors and the regulation of these behaviors by the embryonic environment during gastrulation.

Key words: gastrulation / invagination / mesenchyme / filopodia / sea urchin

IN HIS INTRODUCTION to the first volume of *Archiv für Entwicklungsmechanik der Organismen*, Wilhelm Roux outlined the nature and scope of the new science of 'developmental mechanics' (*Entwicklungsmechanik*) or 'causal morphology' (*causale Morphologie*), which he considered the proper approach to the study of problems in embryology. Roux coined the German term with the intent that it should encompass the experimental investigation of both the *mechanisms* and *mechanics* of embryonic development at various levels of organization.[1] Because it results in such a dramatic restructuring of the early embryo, gastrulation was a focal point for applying the new *Entwicklungsmechanik*. Numerous pioneering studies, particularly those of Holtfreter, firmly established that

> "the directed movements of embryonic regions can actually be traced back to basic faculties of the single cells and to their specific response to changes in environment. The unitarian character of their combined effort is mainly the result of the *predisposed arrangement of cells with a locally different kinetic behavior.*"[2]

For Holtfreter, gastrulation involved the interplay of the behavior of single cells with a particular 'supracellular' context in which the cells perform their behavior.[2] Gastrulation then can be thought of as a mosaic of region-specific processes which are coordinated in time and space. Individual cells in the gastrula comprise sheets of tissues, and the properties of these tissues in turn constrain and regulate the behavior of their constituent cells. As a result, cells are influenced in ways that can only be discovered by examining both intrinsic cell behaviors and how those behaviors are modulated by the context in which the cells find themselves. Contextual influences include the local mechanical environment, the geometry of the embryo, the juxtaposition of one type of cell or tissue with another in space and/or time, the extracellular milieu (including the extracellular matrix), and sites for adhesive contact between cells. The 'astonishingly integrative character of the gastrulation process'[2] can be easily forgotten amid the molecular focus of modern biology. The aim of this article is to illustrate the importance of context-sensitive cell behaviors in bringing about the successful completion of gastrulation in a simple system, the sea urchin embryo.

Overview of sea urchin gastrulation

Because of its simple organization, optical properties, and relative ease of experimental manipulation, the sea urchin embryo is a convenient model system for studying gastrulation. Historically, the study of sea urchin gastrulation has been influential in shaping

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ideas about how morphogenetic movements occur (in particular see reviews by Gustafson and Wolpert[3,4]). In the sea urchin embryo, just prior to gastrulation the epithelium at the vegetal pole of the embryo flattens and thickens to form the *vegetal plate* (Figure 1a). Following the flattening of the vegetal plate, *primary mesenchyme cells* ingress into the blastocoel (Figure 1a). Then the vegetal plate begins to bend inward to form a short, squat cylinder, the archenteron (Figure 1b). During this initial phase of invagination (*primary invagination*), the archenteron extends 1/4-1/2 of the way across the blastocoel. A short pause follows primary invagination, after which the archenteron resumes its elongation (*secondary invagination*; ref 5). At about the time secondary invagination begins, cells at the tip of the archenteron (*secondary mesenchyme cells*) become protrusive, extending long filopodia into the blastocoel (Figure 1c). Eventually the archenteron elongates across the blastocoel, and its tip attaches to the ventral ectoderm near the animal pole (Figure 1d). By this time the primary mesenchyme cells have localized into two major clusters in the ventrolateral ectoderm to form spicule rudiments, so that the embryo has a morphologically obvious dorsoventral axis (Figure 1d). Ultimately the tip of the archenteron fuses with the ectoderm to form the larval mouth (Figure 1e). As the pluteus larva develops, the archenteron becomes tripartite, and a host of differentiated tissues appear (Figure 1e). What cell behaviors generate these morphogenetic changes, and how are these behaviors controlled? Both 'classical' and more recent experiments and observations shed light on these questions. The following sections briefly review what is currently known about the major events in sea urchin gastrulation.

Control of primary mesenchyme motility and pattern

Primary mesenchyme cells (PMCs) are descended from the micromeres that form at the fourth division, and appear to be committed very early in development to form the skeletal elements, or spicules, of the pluteus larva (reviewed in refs 6-8). The ingression of primary mesenchyme cells is accompanied by a loss of affinity for neighboring epithelial cells and the hyaline layer,[9] alterations in cell polarity and loss of the epithelial phenotype (refs 10, 11; see also the chapter by Hay, this issue) and the appearance of new cell surface determinants and transcripts.[12-14] When they begin to ingress, PMCs become bottle-shaped in profile as the surface area of their apical ends is reduced.[10,15] Eventually they detach from the hyaline layer, sometimes leaving a portion of their apical end behind; PMCs may be aided in their detachment by lateral squeezing of adjacent epithelial cells in the vegetal plate, as these cells seek to maintain the integrity of the epithelium.[11,15] PMCs

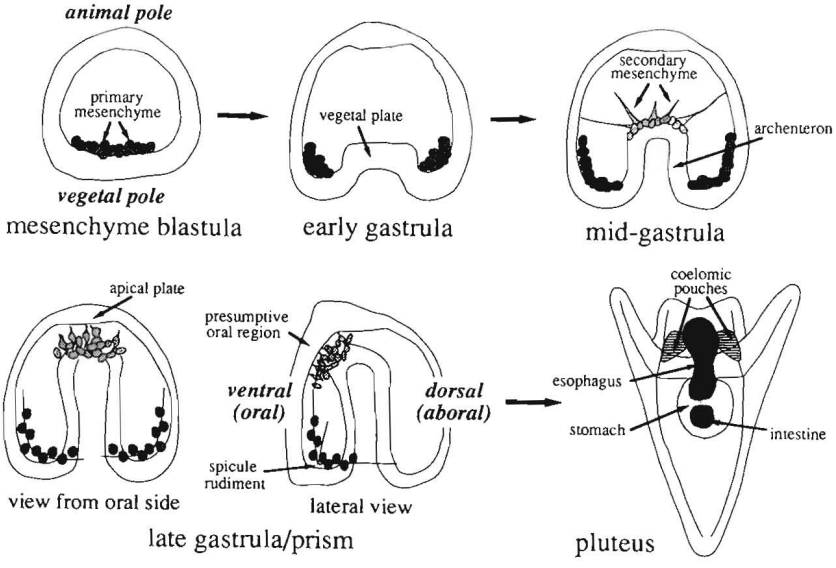

Figure 1. An overview of sea urchin gastrulation. For an explanation of the stages of gastrulation, see the text.

migrate via seemingly stiff, bristle-like filopodia.[4,6,16] Sulfated proteoglycans on the surfaces of PMCs and/or in the blastocoel are important for their migratory competence.[17-19] PMCs migrate away from the vegetal plate, but eventually form a ring in the vegetal pole region of the embryo. Ultimately, two clusters of PMCs form in the ventrolateral ectoderm, and these clusters give rise to the spicule rudiments of the larva. The cellular mechanisms of PMC migration and skeletogenesis have been extensively reviewed elsewhere.[4,6-8,20-22] The changes in motility and adhesion that take place in PMCs also occur in micromere descendants cultured *in vitro*,[9,23-25] indicating that this lineage can perform much of its developmental program autonomously.

Although micromeres are committed early in development to form PMCs, it is also clear that PMC behavior is modulated by the embryo in a number of ways. In particular, the remarkable pattern that these cells adopt leads naturally to a consideration of what might control this pattern. Okazaki *et al*[26] observed that PMCs localize at sites in the ectoderm where the epithelial cells are thickened, producing a fan-like optical effect. When this belt of cells is shifted along the animal-vegetal axis by classic 'vegetalizing' agents such as lithium chloride, PMCs localize to the shifted ectoderm.[26,27] These experiments suggest that regionally specific information is contained within the ectoderm that specifies the pattern of PMCs. Gustafson[28] suggested that the localization of PMCs to the fan-like arrays was due to mainly physical factors. In compressed *Psammechinus miliaris* embryos, filopodia appear to insert at cell boundaries, and so Gustafson suggested that filopodia might preferentially attach at these sites of thickened ectoderm, where more of these boundaries are presumably concentrated.[28] However, a recent ultrastructural study by Amemiya did not find filopodia that penetrated the basal lamina, and he instead suggests that regionally specific extracellular matrix material may be responsible for the localization of PMCs.[29]

While it is not clear at present what molecular signals are responsible for localization of PMCs, it is clear that the embryo can exercise remarkable regulatory control over the construction of the larval skeleton. Single micromeres added ectopically to host embryos produce PMCs that ingress on schedule and incorporate into the host skeleton normally,[30] demonstrating that there is nothing special about the site at which PMCs ingress that is important for skeleton formation. When PMCs are displaced from the vegetal pole by centrifugation, the displaced cells migrate back to the vegetal pole region.[26] Similarly, if PMCs are transplanted from older donor embryos into younger hosts, the donor PMCs localize to the vegetal pole.[31] Furthermore, even though they are competent to ingress and begin formation of the skeleton (as do their siblings in control embryos), older transplanted PMCs remain at the vegetal plate until the host PMCs ingress. The donor PMCs then join the host PMCs to produce a normal skeleton.[31] 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.[32] These results indicate that the embryo restricts the number of spicules, the sites at which they form, and the timing of their formation. Thus the embryonic environment can constrain mesenchyme motility and differentiation, even in the case of a highly stereotyped cell population such as the micromere/PMC lineage.

Invagination of the archenteron

What mechanisms are responsible for the seemingly simple process of primary invagination of the archenteron? The initiation of the invagination is not dependent on the prior ingression of the primary mesenchyme cells, since invagination occurs on schedule in embryos in which micromeres have been removed or prevented from forming[33,34] and in embryos of the cidaroid urchin, *Eucidaris tribuloides*.[30,35,36] In these cases spiculogenic cells are released from the tip of the archenteron well after gastrulation has begun. Furthermore, there are no forces outside the immediate vicinity of the vegetal plate required for its invagination. When the animal half of the embryo is removed, the first phase of invagination occurs normally, indicating that forces within the vegetal plate itself, and not constraints imposed by surrounding tissues, are responsible for its invagination.[37,38] The vegetal plate can be isolated several hours before primary invagination begins,[38] so the program of differentiation that leads to the initiation of gastrulation is established well before an invagination is evident.

But what promotes the inward bending of the archenteron? One model of historical importance involves 'mitotic pressure', i.e. pressure generated by local proliferation of cells, resulting in a buckling of the epithelium (see refs 39, 40 for general reviews of

proposed mechanisms of epithelial invagination). Mitotic pressure can be ruled out in the case of *Lytechinus pictus*, because embryos that have been mitotically arrested well before ingression of primary mesenchyme cells by treatment with the DNA synthesis inhibitor, aphidicolin, go on to gastrulate and to form early pluteus larvae with less than half the normal complement of cells.[41] In other species, however, mitosis may be more important.[42] Another model proposed to account for primary invagination invokes changes in cell adhesion in the vegetal plate.[4] In this model, cells in the vegetal plate lose affinity for the hyaline layer and/or gain affinity for one another, resulting in increased lateral contact and decreased apical area, ultimately producing an invagination. However, a careful analysis of cell shapes in the vegetal plate of *L. pictus* indicates that the cell shape changes required in this model do not seem to occur in *L. pictus*.[38]

Another model put forward to account for primary invagination is *apical constriction* of cells in the vegetal plate (refs 43, 44; see also the chapter by Schoenwolf and Smith, this issue). Is there evidence for apical constriction or some other active mechanism that might generate an invagination? Moore found that the central region of the invagination persists in osmotically swollen embryos, but that the peripheral region of the plate everts. Based on the appearance of such inflated embryos, Moore postulated that a central, active region is responsible for the invagination, and that the rest of the plate is passively distorted.[45] Consistent with the suggestion that stress is transmitted within the vegetal plate, a radial cut made in an isolated vegetal plate prevents invagination.[37] There are apically constricted, bottle-shaped cells in the center of the vegetal plate, and cells adjacent to these apically constricted cells actually have expanded apices, which suggests that they are under tension.[46] Similar morphology is seen in the case of the bottle cells that initiate formation of the blastopore lip in *Xenopus* where it has been shown experimentally that the cells are actively contracting.[47,48] In the case of bottle cells, local stretching and simultaneous bending of nearby tissues occurs as a result of their apical contraction.[48] It may be that the center of the vegetal plate actively generates bending via a similar mechanism, with the result that the rest of the vegetal plate is stretched and bent inward to produce an invagination. In summary, however, it must be said that the basic mechanisms of this seemingly simple invation are still obscure, and any proposed mechanisms to account for it remain speculative.

Elongation of the archenteron

Following primary invagination there is a pause prior to the elongation of the archenteron.[5] This observation hints at the possibility that primary and secondary invagination may employ different cellular mechanisms, and this in fact seems to be the case. There is good evidence to show that *epithelial cell rearrangement* occurs during archenteron elongation. Cell rearrangement was initially inferred from the observation that as the archenteron elongates, the number of cells around the circumference of the archenteron decreases (refs 49, 50; Figure 2). More direct evidence comes from the deformation of fluorescently labeled chimaeric clones of endoderm cells introduced into the vegetal plate prior to invagination. A sector of labeled cells is gradually resolved into a longer, narrower strip, as cells intercalate circumferentially to extend the archenteron (ref 46; Figure 2). As cells in the archenteron rearrange, the integrity of septate junctional domains appears to be maintained,[49,51] similar to junctional domains in other rearranging epithelia (reviewed in refs 40, 52, 53). Furthermore, cell rearrangement appears to be the dominant means by which the archenteron elongates, since secondary invagination does not require mitosis,[41] and no involution of additional cells occurs.[46] These observations establish that cell rearrangement occurs in the archenteron; however, they say nothing about the mechanisms of cell rearrangement or the forces that bring it about.

A striking feature of the latter part of sea urchin gastrulation is the intense protrusive activity of secondary mesenchyme cells (SMCs) at the tip of the archenteron.[5,54] The filopodia appear to exert tension where they attach, pulling out 'cones of attachment' in the ectoderm.[55] In some species, the tip of the archenteron occasionally rips away from the base, suggesting the presence of considerable tension.[54] In *Pseudocentrotus depressus*, the entire embryo flattens along the animal-vegetal axis during gastrulation, apparently due to filopodial traction.[56] The appearance and vigor of filopodial activity seem to be well correlated with the onset of secondary invagination.[5,55] Furthermore, under a variety of experimental conditions, including treatment with pancreatin, low calcium sea water, lithium chloride, osmotic swelling with sucrose, and injection of lectins or proteases into the blastocoel, a failure to elongate the archenteron is correlated with poor filopodial attachment.[54,57,58] These observations led to the proposal

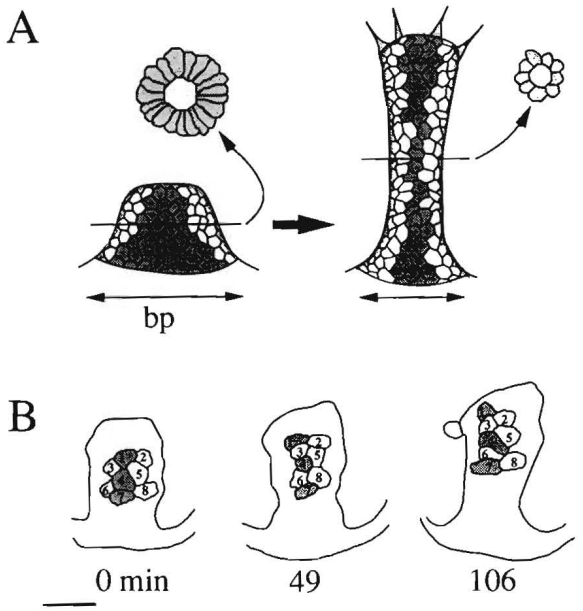

Figure 2. Cell rearrangement in the archenteron. A. Early in gastrulation, a transverse section through the archenteron reveals 20-30 around its circumference; late in gastrulation this number drops to as few as 6-8 in the narrowest region of the archenteron.[49,50] Fluorescently labelled chimaeric clones of cells (schematically depicted in gray) are wedge-shaped early in gastrulation; as gastrulation proceeds they elongate and narrow, becoming one cell wide in the narrowest region of the archenteron.[46] B. Direct observation of cell rearrangement in the cidaroid, *Eucidaris tribuloides*. A group of eight cells was followed over approximately 2 h. The array of cells gradually elongates and narrows, accompanied by intercalation of cells. Cells 1, 4, and 7 have been shaded for clarity. Adapted from ref 46 with permission. Bar = 25 μm.

that filopodial traction exerts sufficient tension on the archenteron to cause it to elongate.

However, several lines of evidence reveal that filopodial traction alone is insufficient to account for the elongation of the archenteron. Experiments indicate that cell rearrangement in the archenteron can occur via two mechanisms: *active, autonomous rearrangement* and *filopodia-dependent rearrangement.* Several experiments imply that the archenteron can elongate autonomously, without traction exerted by filopodia. First, if the filopodia are ablated with a laser microbeam, elongation continues to roughly two-thirds of the complete length.[59] The rate of elongation in such ablated embryos is initially comparable to control embryos, but decreases as the archenteron reaches two-thirds of its normal, final length. Second, the archenteron extends to about two-thirds of the completed length in exogastrulae, where secondary mesenchyme cells fail to reach any anchoring substrate upon which to pull.[50,59] Interestingly, the final length of archenterons in exogastrulae and in laser ablated embryos is virtually identical,[59] suggesting that in both cases autonomous extension of the archenteron can result in its partial elongation.

What cellular processes might generate this autonomous rearrangement? In *L. pictus,* lamellipodia are transiently extended towards the animal pole by cells in the wall of the archenteron just prior to the onset of secondary invagination.[46] These protrusions provide evidence that *all* cells in the archenteron wall undergo a dramatic change in their program of motility when cell rearrangement begins, and that the cells in the archenteron are polarized along its axis of extension prior to the onset of secondary invagination. The motility associated with cell rearrangement can be observed directly in the archenteron of the cidaroid, *Eucidaris tribuloides,* using DIC videomicroscopy. In *Eucidaris* filopodia extended by cells at the tip of the archenteron are few in number well into gastrulation and they never acquire an appreciably upward orientation, so filopodial traction is presumed to be of little mechanical importance to archenteron elongation in this species.[35,46] Cell rearrangement occurs in *Eucidaris* as it does in euechinoids (Figure 2b); as they rearrange, the cells of the archenteron undergo vigorous motile activity. Cortical bleb-like protrusions are continually sent out, they rotate part of the way around the basal periphery of the cell, and then disappear after approximately 30 s. As cells continually 'squeeze' up and down and back and forth, blebs can traverse as much as 180°, causing local displacements of neighboring cells. As a result the cells 'jostle' against one another continually as they rearrange.[46] Such basal, rotating cortical protrusions are similar to the 'cortical tractor' envisioned by Jacobson *et al* as a means of cell rearrangement in the amphibian neural plate.[60] The motility exhibited by endoderm cells in *Eucidaris* suggests that such motility may play a role in mediating individual rearrangement events in the archenteron. However, nothing is known about how such individual rearrangements are coordinated to produce global, directional extension of the archenteron.

Completion of archenteron elongation in euechinoids requires the participation of secondary mesenchyme cells, since if all SMCs are ablated the archenteron only reaches 2/3 of its full length.[59] If a few filopodia are left intact, the archenteron will con-

tinue to elongate after the two-thirds gastrula stage, but more slowly than in controls.[59] It is possible that the last phase of elongation is active in the same sense as the early phase, but that signals passed to the archenteron by secondary mesenchyme cells are somehow required to stimulate further rearrangement. However, the changes in shape of the cells in the archenteron and the overall contours of the archenteron late in gastrulation suggest that the final phase of elongation is driven by tension generated by filopodia. Measurements of cell shape during archenteron extension in *L. pictus* indicate that at about the time that SMCs reach the animal pole, there is a transient stretching of endoderm cells, apparently in response to filopodial traction.[46] In some cases, the central region of the archenteron becomes very narrow, as the cells in this region undergo additional rearrangement and become markedly stretched.[46] The transient elongation of the cells and the 'necking' behavior observed in this region of the archenteron can be accounted for by assuming that filopodial traction generates tension within the archenteron. This tension in turn results first in elongation of the cells, and then their passive rearrangement, as they attempt to accomodate the axial stress and at the same time remain as nearly isodiametric as possible. In support of this hypothesis, a mechanical model of rearrangement in response to applied stress accounts well for the observed changes that take place in the archenteron late in gastrulation (see the chapter by Oster and Weliky, this issue).

Attachment of the archenteron

How does the behavior of SMCs contribute to the attachment of the archenteron? The general motile behavior of individual SMCs consists of continual cycles of filopodial extension, attempted attachment, and withdrawal.[4,61] At the end of gastrulation the tip of the archenteron makes contact with the ectoderm near a thickened region of epithelium, the *apical plate*. At this time the exploratory behavior of the filopodia largely ceases, marking the end of gastrulation. Based on real-time analysis of the residence times of attached filopodia, protrusions that make contact with the ectoderm in the apical plate region remain attached 20-50 times longer than attachments observed at any other site along the blastocoel wall.[61] The SMCs bearing the long-lived filopodia eventually change their behavior as they flatten and spread onto this region. In some species, such as *Lytechinus variegatus*, this region lies near the animal pole; in other species, such as *Strongylocentrotus purpuratus*, it is located on the ventral side of the animal hemisphere.[61]

The normal behavior of SMCs suggests that the apical plate region may serve as a target for filopodial attachment, and that SMCs may be programmed to extend filopodia from the onset of archenteron elongation until the archenteron reaches this region. Several lines of evidence indicate that SMCs do in fact respond to such a 'target' region. First, the animal pole region can be pushed toward the tip of the archenteron with a micropipette so that contact is forced precociously. When the animal pole is presented to filopodia several hours ahead of schedule, the filopodial behavior of the SMCs ceases ahead of schedule, and a precocious, stable attachment of the tip of the archenteron results.[61] In contrast, when other areas of the blastocoel wall are indented close to the tip of the archenteron, temporary contacts are made, but the archenteron continues past the indentation to make stable attachments to the normal site (Figure 3). Thus the SMCs appear to respond specifically to contact with the animal pole region. Second, contact of SMCs with the animal pole can be prevented by squeezing embryos into narrow diameter capillary tubing, so that the embryos are abnormally elongated along the animal-vegetal axis and the archenteron cannot reach the animal pole. In this case SMCs continue their explorations for a longer period of time than they do in control embryos.[61] Third, if the archenteron is prevented from reaching the animal pole for several hours, SMCs eventually detach from the archenteron. Some migrate to the animal pole, and they undergo the change in behavior seen in normal embryos[61] (these experiments are summarized in Figure 3). Finally, ectopic combinations of animal pole ectoderm and archenterons in fused multiple embryos and chimaeras result in attachment of archenterons to the nearest available apical plate region.[61] Thus, filopodial extension appears to be programmed to continue until the appropriate target is reached on the wall of the blastocoel.

The experiments just described indicate that once filopodia can interact with the animal pole region, they rapidly attach to it, thereby anchoring the archenteron and helping to end gastrulation. But how is target recognition related to the morphogenetic movements that are proceeding concurrently in the archenteron? Measurements indicate that the maximum length that filopodia can achieve is ~35 μm.[61] However, in normal embryos the tip of the archenteron is ~50 μm away from the animal pole in *Lytechinus variegatus* at the onset of secondary invagi-

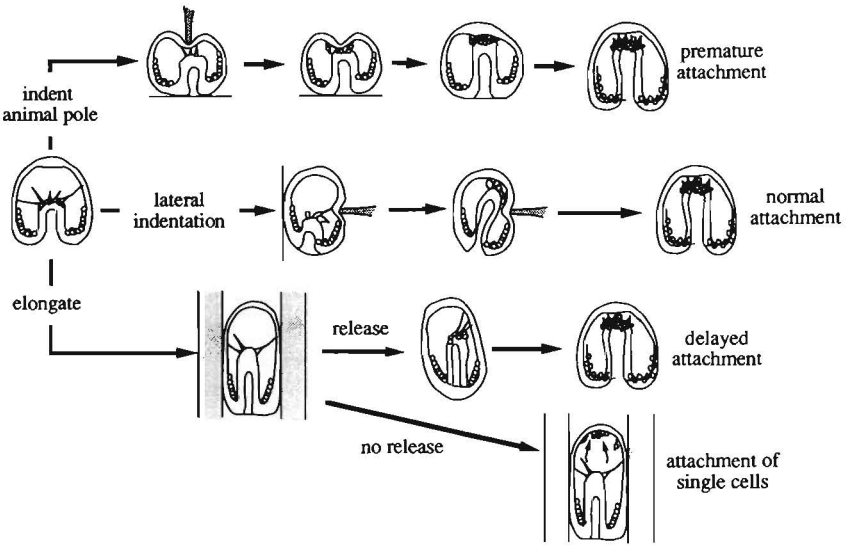

Figure 3. Experimental demonstration of target recognition by secondary mesenchyme cells late in gastrulation.[61] Indentation of the animal pole places it within reach of filopodia early in gastrulation, resulting in premature attachment of the archenteron. A lateral indentation does not produce this effect; instead, the archenteron attaches at the normal location. When embryos are elongated by aspirating them into capillary tubing, secondary mesenchyme cells continue their random exploratory behavior longer than control embryos. If the embryos are released from the tube, they regain a normal shape, and attachment of the archenteron soon follows. If the embryos are kept in the tube for several hours, individual secondary mesenchyme cells leave the tip of the archenteron; those that migrate near the animal pole localize there. Adapted from ref 78 with permission.

nation. As a result, although filopodia are extended in all directions, they can only successfully attach to lateral ectodermal surfaces, which are ≤35 μm from the tip of the archenteron. In contrast, filopodia rapidly attach to the animal poles of midgastrulae whose shape is altered such that the animal pole is <35 μm from the tip of the archenteron.[61] Thus filopodial attachment is sensitive to the geometry of the embryo. In normal *Lytechinus variegatus* embryos filopodia can only reach the animal pole at the 2/3-3/4 gastrula stage, when the distance to the animal pole is ≤35 μm. Since autonomous extension of the archenteron is occurring at the same time, it appears that this extension is required to place filopodia close enough to the animal pole to allow them to interact with it. Once filopodial attachments become concentrated in the animal pole region, filopodial traction in turn appears to exert tension on the archenteron, helping to complete its elongation.

The extracellular matrix and the initiation of gastrulation

The epithelial tissues of the sea urchin embryo are in contact with two extracellular matrix layers: the *apical lamina* or *hyaline layer*, on their apical surfaces, and the *basal lamina* on their basal surfaces. The hyaline layer has been proposed to be important as a structural support and mechanical integrator of epithelial sheets in the sea urchin embryo,[3,4,62] in a manner analogous to that at one time attributed to the 'surface coat' in amphibians (ref 63; see ref 64 for a critical evaluation of this notion in amphibians). If the hyaline layer did indeed serve this function, then perturbing the association of cells with it would be expected to block epithelial morphogenesis at the gastrula stage, particularly invagination of the archenteron. When the hyaline layer is experimentally disrupted by incubating fertilized eggs in Fab fragments of a monoclonal antibody that binds to the protein hyalin (a major component of the hyaline layer), cell binding to the hyaline layer is disrupted. The hyaline layer visibly delaminates from the epithelium, the epithelium becomes abnormally thickened, and invagination of the archenteron is blocked.[65] If blocked embryos are removed from the antibody, development resumes, and a normal pluteus larva results.[65] If the antibody is applied at the mes-

enchyme blastula stage, invagination is still blocked, but primary mesenchyme cells ingress into the blastocoel to produce spicules, and pigment cells eventually appear (J. Hardin, unpublished observations). These results suggest that the antibody interferes with the mechanical and structural integrity of the epithelium, but also with more general epigenetic cues necessary for the initiation of gastrulation.

Treatments affecting the basal lamina also block gastrulation. Incubation of fertilized eggs in β-aminoproprionitrile (BAPN), an inhibitor of lysyl oxidase (an enzyme involved in collagen crosslinking), allows embryos to develop normally to the mesenchyme blastula stage, but the archenteron fails to invaginate.[66,67] If the BAPN is removed, even after the embryos have been arrested at the mesenchyme blastula stage for more than 24 h, the embryos begin to gastrulate and complete development normally. BAPN prevents proper construction of the basal lamina, but it does not affect the synthesis of collagenous molecules,[66-68] nor does it interfere with the *de novo* expression of a number of genes in the ectoderm.[69] In contrast, antigens that are normally expressed in the archenteron fail to appear as long as the embryos are incubated in BAPN.[66] If the drug is applied after gastrulation has commenced, then invagination proceeds, but secondary mesenchyme cells show impaired motility, and the archenteron is often flaccid.[67,68] These data suggest that, in addition to the direct mechanical or structural effects that disruption of either extracellular matrix layer may have, a critical period precedes gastrulation during which the vegetal plate must be in normal contact both with the basal lamina and with the hyaline layer in order for gastrulation to commence.

Phylogenetic variation and the cellular processes of gastrulation

The results presented above suggest that the shape of the embryo at the onset of gastrulation can impose significant constraints on how gastrulation progresses. In some cases a given cell behavior is incapable of completing its normal function, without the concurrent execution of other morphogenetic events. An example of this sort of limitation is the interplay between filopodial exploration and autonomous extension of the archenteron. In some cases, reliance on a single cell behavior could result in disastrous distortions of tissues. Indeed, mechanical simulations suggest that in the sea urchin embryo filopodial traction alone would result in widespread indentation of the blastocoel roof and a wide blastopore, which is not seen in most species *in vivo*.[50] These limitations can be overcome by combining a small set of relatively simple cell behaviors (e.g. autonomous cell rearrangement, random exploration by filopodia, and target recognition). As a result, gastrulation can be successfully completed despite a wide range of variations in embryonic shape and the positions of interacting tissues.

As an illustration of such flexibility, a survey of a number of sea urchin species reveals several ways in which the archenteron can elongate, all apparently accounted for by differences in embryonic shape and target placement, in conjunction with the simple cell behaviors outlined above. These include 'central elongators', in which the archenteron is equidistant from all lateral ectodermal surfaces (e.g. *Lytechinus variegatus, L. pictus*), 'dorsal crawlers', in which the dorsal ectoderm is near the tip of the archenteron (*Psammechinus miliaris, Echinus microtuberculatus*), and 'ventral crawlers', in which the ventral side is closer (e.g. *S. purpuratus*; ref 61). In other species, the stomadeum does not form at the animal pole, but lateral to it; in these species, such as *E. tribuloides* and the Japanese sand dollar, *Clypeaster japonicus*, filopodia never reach the animal pole.[20,46] Instead, they appear to extend laterally towards the stomadeal invagination directly. In all of these cases, the simple cell behaviors that are responsible for archenteron elongation and attachment are inherently flexible enough to permit such phylogenetic variations in embryonic shape.

Phylogenetic variations point out another salient feature of a cellular analysis of gastrulation: cells with the same fate may not play the same morphogenetic role in different species. For example, secondary mesenchyme cells seem to be important mechanically in many euechinoid species, but in the cidaroid, *Eucidaris*, they seem to have little direct mechanical role during gastrulation. Nevertheless, molecular markers indicate that these cells give rise to similar differentiated mesenchymal populations in euechinoids and *Eucidaris*.[30] In other cases, seemingly similar cells may have divergent fates as well as differing roles in morphogenesis. An example of this sort of divergence is the role and fate of bottle cells during amphibian gastrulation. Bottle cells in *Xenopus* remain epithelial as they constrict, initially aiding invagination of the archenteron and rolling of the blastopore lip. Later, they respread to become part of the roof of the archenteron.[48] In contrast, many bot-

tle-shaped cells in urodeles are mesenchymal, undergoing ingression to become part of the axial mesoderm.[2,70]

In addition to variations in embryonic shape, there are also variations in the timing of developmental events with respect to one another (*heterochronies*). Events that are temporally correlated in one species but functionally independent might be expected to display heterochrony when the same processes are compared in another species. Ingression of spiculogenic cells, which has been shown experimentally to be dissociable from invagination of the archenteron (see above), is a good example of this sort of variation. Spicule-producing cells ingress at the mesenchyme blastula stage in many sea urchin embryos, but in *Eucidaris* ingression of similar cells occurs many hours after invagination of the archenteron has begun.[30,35,71] It might be expected that more radical alterations in early development, such as the omission of a larval stage, might result in wholesale alterations in or deletion of an entire ensemble of these basic cell behaviors. Such radical alterations seem to be the case in direct developing sea urchins, in which the production of a functioning larval gut is obviated.[72] Among amphibians, similar radical changes seem to have occurred in the embryos of marsupial frogs, in which gastrulation occurs in a blastodisc, rather than in the spherical embryo characteristic of amphibians that possess a typical larval stage.[73,74]

Conclusions: the contextual nature of gastrulation

An understanding of the molecular events associated with morphogenesis is important, and molecular analyses are beginning to provide insights into the basic mechanisms cells use to change the shape of the embryo. However, by briefly reviewing what is and is not known about gastrulation in the specific case of the sea urchin embryo, this article has attempted to underscore the importance of understanding the 'supracellular' influences that affect cell behavior. Specifically, the analyses described here illustrate several generalization that seem to be helpful in understanding gastrulation.

First, *mechanical interactions are significant* during gastrulation. Cells exert forces which are used to shape the embryo. Mechanical interactions involve force production by single cells (e.g. individual secondary mesenchyme cells) or groups of cells (e.g. bottle cells that form along the blastoporal pigment line in *Xenopus*; ref 48), and accommodation of the stresses these cells produce by integrated sheets of tissues (e.g. the archenteron). Our current knowledge of the mechanical properties of embryonic tissues, particularly their quantitative properties, is far from complete (see chapters by Koehl; Oster and Weliky, this issue, for further discussions of these issues). However, transmission of stresses within tissue sheets, rearrangement of cells in tissues under stress, and the possible role of the extraembryonic matrix in integrating the mechanical behavior of epithelial sheets are all illustrated in simple systems such as the sea urchin embryo; such systems should continue to be helpful in elucidating the characteristics of both force-producing cells and responding tissues. Second, *one event may serve multiple morphogenetic functions.* An example is seen in the role played by secondary mesenchyme cells. Their motility seems to be important for archenteron extension, but also simultaneously for correct attachment and positioning of the tip of the archenteron in preparation for mouth formation. A third generalization is that *analysis of cell populations is indispensible* for understanding gastrulation. An important property of tissue sheets undergoing the massive changes in shape that are ubiquitous during gastrulation is that their constituent cells rearrange. Since cell rearrangement by definition requires examination of more than one cell at a time, it seems clear that coordinated morphogenetic movements cannot be fully understood by reducing them to the study of single cells in isolation. Furthermore, specific juxtapositions of interacting cells in time and space are important. This point has been recognized as crucial in the case of inductive interactions during early development (e.g. reviewed in ref 75), but is also an important contribution to the movements of gastrulation. During sea urchin gastrulation, the proximity of various regions of the ectoderm to the tip of the archenteron affects the function of secondary mesenchyme cells, and filopodial motility appears to interact with archenteron morphogenesis in a reciprocal fashion. Many examples from other systems could be cited as well. For example, Keller and colleagues have shown that the various region-specific behaviors at work during *Xenopus* gastrulation must operate in a temporally and spatially coordinated manner to complete gastrulation.[48,64,76,77] These sorts of sequential and hierarchical interactions are one of the hallmarks of the process of gastrulation. As our grasp of these interactions improves, it will be possible to ask even more probing questions

about mechanism at the molecular level. Such molecular analyses will in turn lead to a deeper understanding of the cellular basis of gastrulation.

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