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CELL BEHAVIOUR DURING ACTIVE CELL REARRANGEMENT: EVIDENCE AND SPECULATIONS

RAY KELLER*
Department of Zoology, University of California at Berkeley, Berkeley, CA 94720, USA

AND JEFF HARDIN
Group in Biophysics and Medical Physics, University of California at Berkeley, Berkeley, CA 94720, USA

SUMMARY

The cell behaviour and motility underlying cell rearrangement during gastrulation in amphibian and sea-urchin embryos are discussed. In particular, the cell behaviour of deep (non-epithelial) and epithelial cell populations that undergo cell rearrangement is compared and contrasted. Deep cell rearrangement in *Xenopus laevis* involves both convergence of cells towards the future dorsal midline and simultaneous axial extension of the mesodermal cell mass. Time-lapse cinemicrography and scanning electron microscopy suggest that asynchronous, repetitive motions of individual deep cells, involving local extensions and retractions of their margins, may provide the motive force for rearrangement. Such protrusive activity may be guided by local differences in cell–cell contacts in the marginal zone. Epithelial cell rearrangement in the sea-urchin embryo both elongates the archenteron and simultaneously closes the blastopore. Cell rearrangement is accompanied by stage-specific changes in protrusive activity and cell shape of the basal surfaces of cells in the wall of the gut rudiment, in contrast to the apical surfaces, which show little activity. These basal protrusions may be involved in the rearrangement process.

INTRODUCTION

The geometric necessity of cell rearrangement

The fate maps of early amphibian embryos (Vogt, 1929; Keller, 1975, 1976) show that embryonic tissues undergo massive changes in shape over relatively short periods of time. Waddington (1940, p. 109) pointed out that the dorsal sector of the early amphibian embryo elongates greatly through gastrulation and neurulation; he offered two explanations: either that the component cells change shape to reflect the elongation and narrowing of the tissue mass, or that the cells rearrange and, in the end, retain their original shape. If Waddington's thinking on this subject seems trivial, one has only to remember that the notion of cell rearrangement languished as a morphogenetic mechanism for 30 years, until it was revived by Fristrom's observation that *Drosophila* imaginal (limb) discs elongate during their evagination by cell rearrangement (see Fristrom, 1976). Geometric considerations suggest that any situation in which a disc is converted into a cylinder (Fristrom, 1976), in which

* Author for correspondence.

narrowing and elongation (convergence and extension) occur (Keller, 1978; Keller *et al.* 1985*a*,*b*), or in which a circumference such as the mouth of a blastopore (Hardin, 1986) or the margin of a cell sheet (Keller & Trinkaus, 1987) decreases markedly, is a prime candidate for cell rearrangement (see Keller, 1986).

Cell rearrangement occurs during many morphogenetic processes

There are many examples of epithelial cell rearrangement during morphogenesis. During evagination of *Drosophila* imaginal limb discs, the folded disc transforms into an elongated, segmented cylinder; this transformation is accompanied by a decrease in the number of cells in the circumference and an increase in the number of cells in the length of specific segments of the limb (Fristrom, 1976). During regeneration of transverse fragments of *Hydra*, the fragment regains the proper proportions by reorganizing itself to form a longer, narrower tissue, apparently by cell rearrangement (Bode & Bode, 1984). During secondary invagination of the archenteron of the sea-urchin gastrula, the archenteron becomes longer and narrower by rearrangement of epithelial cells, so that the archenteron consists of fewer cells in cross-section and a greater number along the length of the archenteron (Ettensohn, 1985; Hardin & Cheng, 1986). Nardi & MaGee-Adams (1986) have found that the scale patterns in the moth wing arise by cell rearrangement: the cells of the scale primordia are initially irregularly distributed and then become aligned in regularly spaced rows, probably through the action of long filiform basal processes that extend across several cell diameters. Similarly, Locke (1985) has found that contraction of basal 'feet' probably causes the shape transformations and cell rearrangement associated with insect pupal segment morphogenesis.

The notoplate region in the central part of the neural anlage of the urodele lengthens and narrows greatly during neurulation, a process that is accompanied by and perhaps driven by rearrangement of the epithelial cells of the notoplate (Jacobson & Gordon, 1976; Jacobson, 1982). During gastrulation and neurulation in *Xenopus*, the narrowing and lengthening (convergence and extension) of the dorsal sector of the embryo is accompanied by rearrangement of the epithelial cells, as shown by direct time-lapse cinemicrographic analysis (Keller, 1978). During teleost gastrulation, the dramatic epibolic movements of the enveloping layer involve marginal and submarginal cell rearrangement as the enveloping layer decreases in circumference as it approaches the vegetal pole of the egg (Kageyama, 1982; Keller & Trinkaus, 1982, 1987). Healing of wounds in the corneal epithelium of the cat is also accompanied by cell rearrangement (Honda *et al.* 1982).

Non-epithelial cells also rearrange during morphogenesis. During epiboly, the deep cells of *Xenopus* undergo radial intercalation to form fewer layers of greater area (Keller, 1980). Likewise, the deep cells of the circumblastoporal region of *Xenopus* gastrulae undergo circumferential intercalation to form a longer, narrower array (Keller, 1984; Keller *et al.* 1985*a*,*b*). Cell rearrangement continues at the neurula stage with the narrowing and lengthening of the notochord by cell intercalation (Keller *et al.* 1985*a*,*b*), a process that also occurs in the ascidian (Cloney, 1964; Miyamoto & Crowther, 1985). The elongation of the pronephric duct of the axolotl,

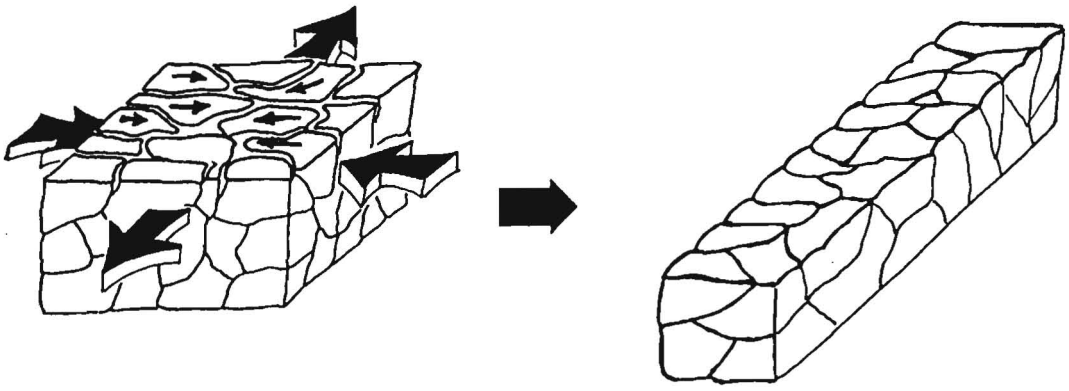


Fig. 1. Narrowing (convergence) and lengthening (extension) of the marginal zone of the early amphibian embryo occurs by active cell rearrangement. The deep cells of the marginal zone actively move between one another (small arrows) to produce a longer, narrower array (large arrows). (From Gerhart & Keller (1986).)

Ambystoma, involves rearrangement of cells to form a longer, narrower array (Poole & Steinberg, 1981).

CELL BEHAVIOUR DURING DEEP CELL REARRANGEMENT IN *XENOPUS* GASTRULATION

The function of cell rearrangement in Xenopus *gastrulation*

A major part of *Xenopus* gastrulation involves convergence and extension of both the involuting marginal zone (IMZ) and non-involuting marginal zone (NIMZ) (Keller *et al.* 1985*a*,*b*; Keller & Danilchik, 1987). Blastopore closure and involution are brought about as the IMZ and NIMZ, particularly their dorsal sectors, narrow in the circumblastoporal direction (convergence) and lengthen in the animal–vegetal direction (extension). The involuted part of the IMZ converges circumferentially and extends toward the inside of the blastoporal lip by a process of active intercalation of deep cells to form a longer, narrower array (Fig. 1). Cells from the part of the IMZ not yet involuted are added to the vegetal end of this intercalating array and presumably they join in the process of intercalation as they are added. The overlying NIMZ is stimulated to converge and extend, but only when its basal surface establishes contact with the involuted IMZ; then it extends and converges at a rate faster than the IMZ and thus tends to push the remaining uninvoluted IMZ over the blastopore lip (Keller *et al.* 1985*a*,*b*; Keller & Danilchik, unpublished). The result of the operation of this double convergence and extension machine is the involution of the IMZ and the simultaneous closure of the blastopore. Results to date show that the deep, non-epithelial cells are probably the cells that generate the force for extension and convergence of these regions (Keller & Danilchik, unpublished) and that the superficial cells passively rearrange to accommodate the tissue distortion

driven by the deep region (see Keller, 1978, 1986). Here we will only deal with deep cell behaviour in the IMZ; the deep cell behaviour in the NIMZ appears to be different (Keller & Danikchik, unpublished).

Method of analysis of deep cell behaviour during intercalation

The cell behaviour involved in bringing about intercalation of deep cells was analysed by direct time-lapse micrography of sectors of the IMZ-NIMZ explanted into culture in a solution that mimics the blastocoel fluid (Keller *et al.* 1985*a*,*b*). Cell behaviour is recorded with high resolution and contrast by producing a video image with a Dage high-resolution camera (model 81, set for 1300×1050 lines) and monitor (model 2000). This image is then recorded on 16 mm film off the monitor in time-lapse with an Arriflex time-lapse camera, using a 40 mm macro lens. The resulting film has superb resolution and contrast. Under these culture conditions, deep cells of the dorsal sector will not only rearrange to form a longer, narrower array. Later they will segregate into a notochord and somitic mesoderm; the latter forms individual somites, which undergo the usual cellular rotation. Video-films can be made at high (×40) magnification to resolve cellular details and at low (×10) magnification to determine macroscopic patterns of movement.

Kneading motion due to repetitive, asynchronous shape change among deep cells

Deep cells of the animal cap, the IMZ and the NIMZ show repetitive changes in shape. Their deep aspects are constantly changing in shape by the advancing and retraction of their margins, first along one axis and then another (Fig. 2). The area, the length/width ratio and the perimeter of contact with adjacent cells change as each cell undergoes these repetitive extensions and retractions of its margins. Deep cells are intimately connected to one another by multiple filiform protrusions, both in explants and *in situ* (Fig. 3A), such that changes in the shape of one cell produce accommodating changes in the position and shape of adjacent cells (Fig. 4). Thus the behaviour of an individual cell, at any instant, is partly a product of endogenous contractile or protrusive events and partly a result of these same processes being exercised in adjacent cells. But exercise of these processes in adjacent cells is usually out of phase, both in timing and in the axial orientation of the extension–retraction cycles, so that a 'kneading action' results, and individual cells appear to be constantly massaged one way and then another.

The exercise of this kneading action in the dorsal IMZ, a region that shows convergence and extension, results in the cells jostling together towards the dorsal midline, and rearranging to form a longer, narrower array in the process (see Keller *et al.* 1985*a*,*b*). In contrast, in the animal cap region, which shows no convergence and extension, there is no net movement. We do not yet know why jostling of dorsal cells results in mediolateral intercalation instead of jostling in place. The cycles of marginal extension and retraction, and the resulting jostling process may be random and function only to permit cells to sample new cell–cell contacts. Adhesion in the tissue array could be anisotropic in such a way that some cell–cell contacts are more stable and thus are favoured in the course of the jostling. It is not clear what kind of

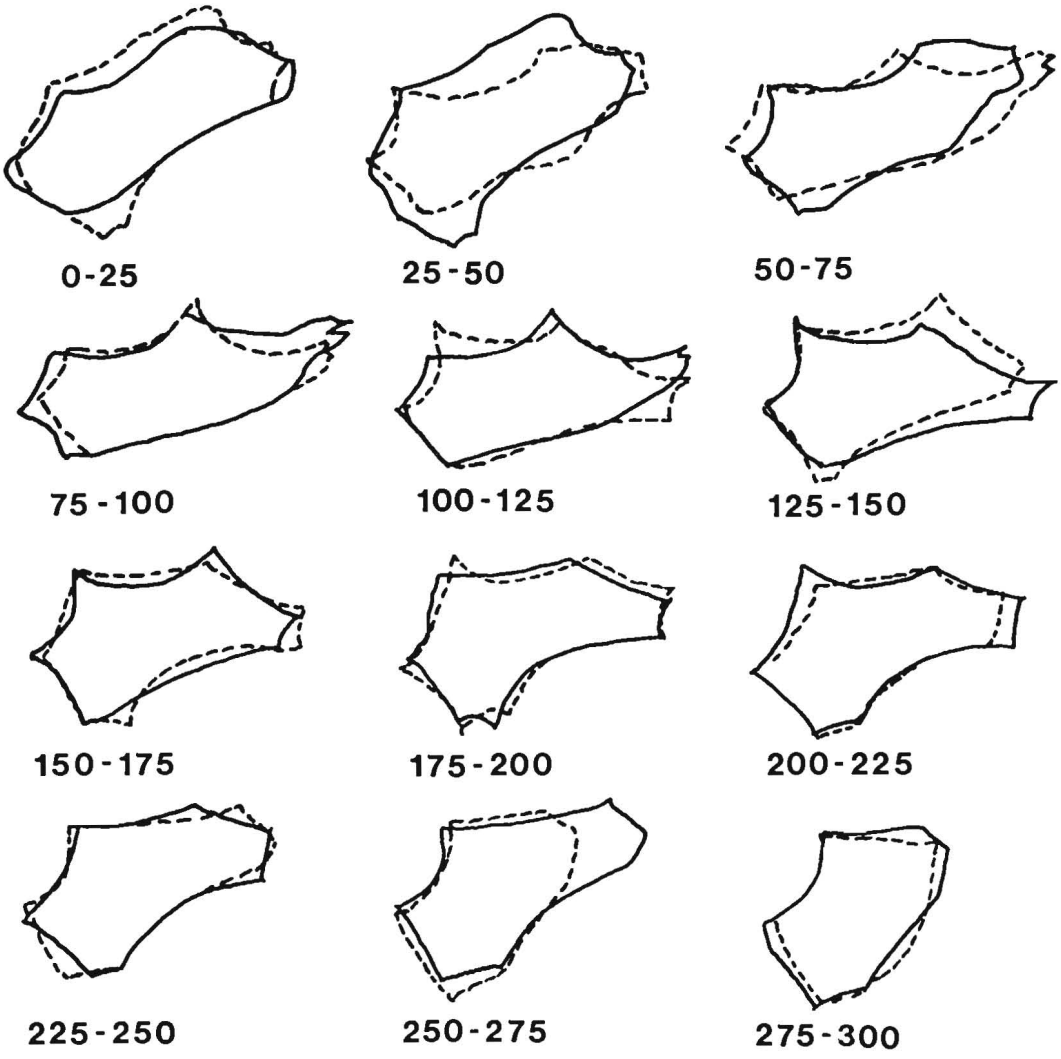
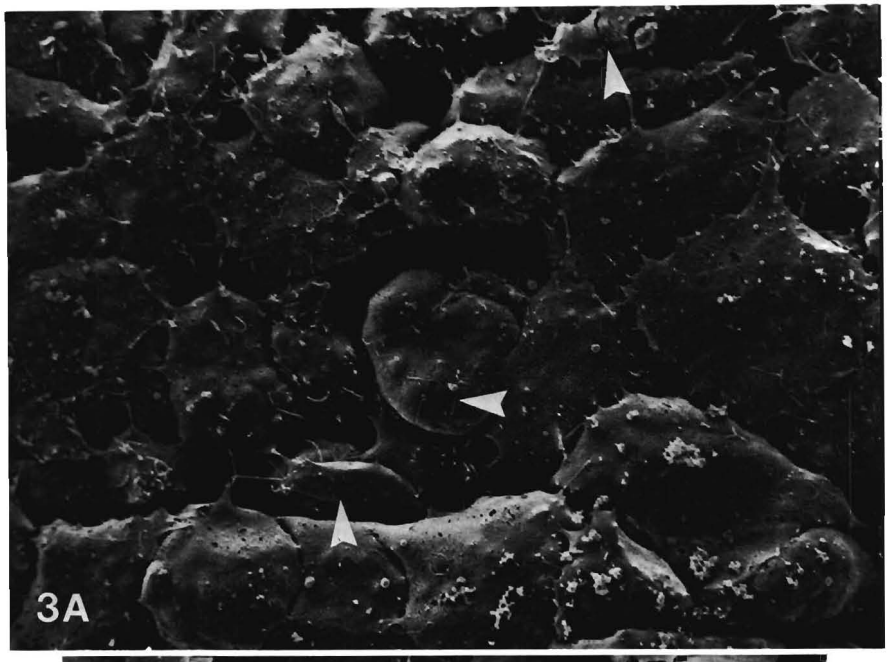


Fig. 2. Change in shape of the inner aspect of a single deep cell is shown by tracings from time-lapse recordings of a cultured explant of the involuting marginal zone of a midgastrula. The continuous line indicates the shape at the first frame number given below each outline and the broken line indicates the shape at the second frame number. The frame interval is 20 s.

pattern of adhesion would result in the mediolateral or circumferential intercalation of cells observed, but some ideas have been presented by Mittenthal & Mazo (1983).

Alternatively, the axial orientation of the cycles of extension–retraction and the resulting boundary relationships between cells may be inherently biased in regions showing directed intercalation of cells. Such is definitely the case after the notochord–somite boundary forms. The extension–retraction cycles of the notochord cells are then oriented mediolaterally or circumferentially (with respect to the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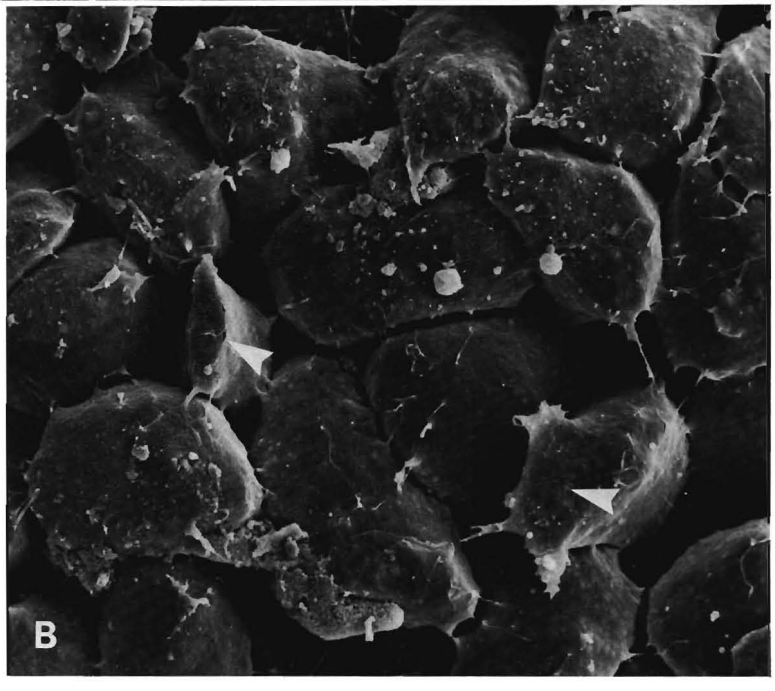
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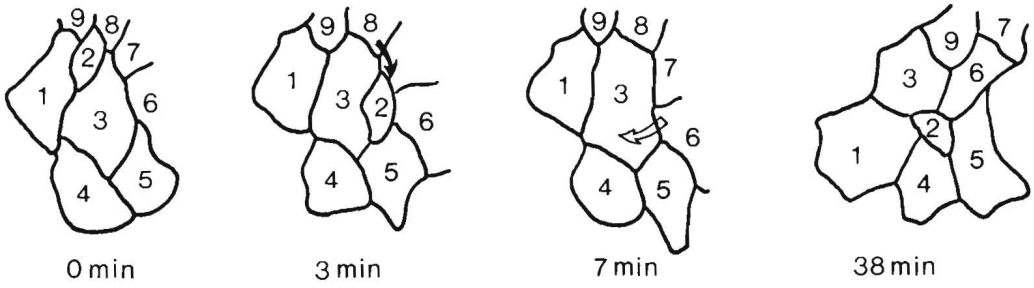


Fig. 4. Outlines of the inner aspects of deep cells of the involuting marginal zone of a late midgastrula traced from time-lapse recordings show the rapid changes in cell position and contact relations during the 'kneading motion' imparted by the exercise of the extension–retraction cycles illustrated in Fig. 2. The less-common rapid movement of individual cells is illustrated by movement of the inner aspect of cell no. 2 around no. 3 (filled arrow), then under 3 (open arrow) to emerge and take up a new position between 3 and the juncture of no. 4 and no. 5. The axis of extension is vertical. Bar, 50 μm.

blastopore) and the cells intercalate transversely with respect to the long axis of the notochord (Keller *et al.* 1985*a*,*b*; also see Miyamoto & Crowther, 1985). We suspect that the same is true at earlier stages of extension and convergence (between stage 10·5 and 11·5), but because of the complexity of the movements in this period we have no proof at present.

Relation of periodic shape change to limnicola movement and intercalation by cytoplasmic flow

Dissociation of gastrula cells by calcium-free media or mechanical means will result in individual, isolated cells showing the 'limnicola' or 'circus' movement described by Holtfreter and others (see Holtfreter, 1947), in which a large bleb forms and rotates around the cell, finally disappearing into the cell body after a good part of a full rotation or more. The rotating bleb is usually clear and inclusion-free, but it may be invaded by yolk platelets and other inclusions. Holtfreter (1947) suggested that the limnicola movement was abnormal behaviour due to loss of cell–cell contact.

However, in the course of the transient extension–retraction events at the margins of deep cells, an extension occasionally results in the formation of a large, blunt protrusion that rotates around part or all of the perimeter of the cell. The protrusion

Fig. 3. The inner aspects of cells of the involuting marginal zone of the late midgastrula are interconnected by filiform protrusions (A). Occasional cells show large, rounded lobopodial structures (central arrowhead) and elongate protrusions extending between adjacent cells (top and bottom arrowheads). The inner aspects of these involuting marginal zone cells may also bear large protrusions that connected these cells to the overlying non-involuting marginal zone or roof of the blastocoel. These are apparent by their flattened morphology and extension out of the plane of the involuting marginal zone (arrowheads in B) in stereo micrographs. ×1000. (Fig. B is from Lundmark *et al.* (1984).)

may extend by the influx of cytoplasm and yolk, probe in several directions, and be abruptly withdrawn by reversal of cytoplasmic flow. In other cases, these protrusions are the initial stage of a rapid translocation of the cell (Fig. 4) in which the protrusion is extended continuously, the cell moves forward rapidly (up to 25 μm min^{-1}) by rapid flow of the cell body into the leading protrusion, and the cell insinuates itself between its neighbours with apparent ease. In some cases this involves simple arcing of the protrusion around an adjacent cell and flow of the cell body into the protrusion to produce intercalation of the cell between its immediate neighbors (see Keller *et al.* 1985*a*,*b*). In other cases, the cell may advance between other cells for a distance of five or six cell diameters and then abruptly resume the more common extension–retraction behaviour pattern. Such behaviour is infrequent and has been seen only in the IMZ to date. These 'sport' cells, so named because of their speed and manoeuvrability, often show putative 'exploratory' behaviour in that they rapidly probe several interstices before abruptly settling on a new position. In the course of this fast mode of movement, the cells appear to be unaffected by contact with their neighbours and move readily between them, often pushing their neighbours apart or even raising an intervening cell up and out of the plane of focus as they pass beneath (Fig. 4).

Because the pulsatile marginal extensions in the extension–retraction cycles lead directly to the fast movements using cytoplasmic flow, we interpret the extension–retraction cycles as reflecting a constant probing of the adjacent cell interstices in which stronger advances, or more favourable external conditions, result in net advance of the cell margin and concomitant change in neighbour relationships. But occasionally, and for reasons not yet understood, the constraints normally limiting the advance to a few micrometres fail, and rapid cytoplasmic flow and rapid, continuous advance of the cell occur until the constraints are reimposed and the cell returns to the extension–retraction cycle.

The rapid movement involving cytoplasmic flow is not likely to be an artifact of culture, but a characteristic though infrequent event in the IMZ of the intact gastrula. The inner surface of the involuted dorsal IMZ consists of cells connected by multiple, filiform and occasionally lamelliform protrusions, but on occasion deep cells will bear large, rounded protrusions (large arrowhead, Fig. 3). Such cells never have the symmetry of form or attachment of dividing deep cells, which are also rotund (see Keller & Schoenwolf, 1977). A continuum of morphologies exists from these short, large diameter protrusions with blunt ends to long, tapered protrusions often ending in filiform attachments to other cells (small arrowheads, Fig. 3). Although we cannot see inside the gastrula, we believe these morphologies to correspond to the transition of a cell *in vivo* from the extension–retraction cycle to the rapid translocation mode seen in time-lapse films of explants.

Coordinated motile phenomena

In general, local cycles of extension–retraction appear, at least in regions other than the dorsal IMZ, to be out of phase with one another, so that the characteristic kneading behaviour arises. However, occasionally contraction of cells and the

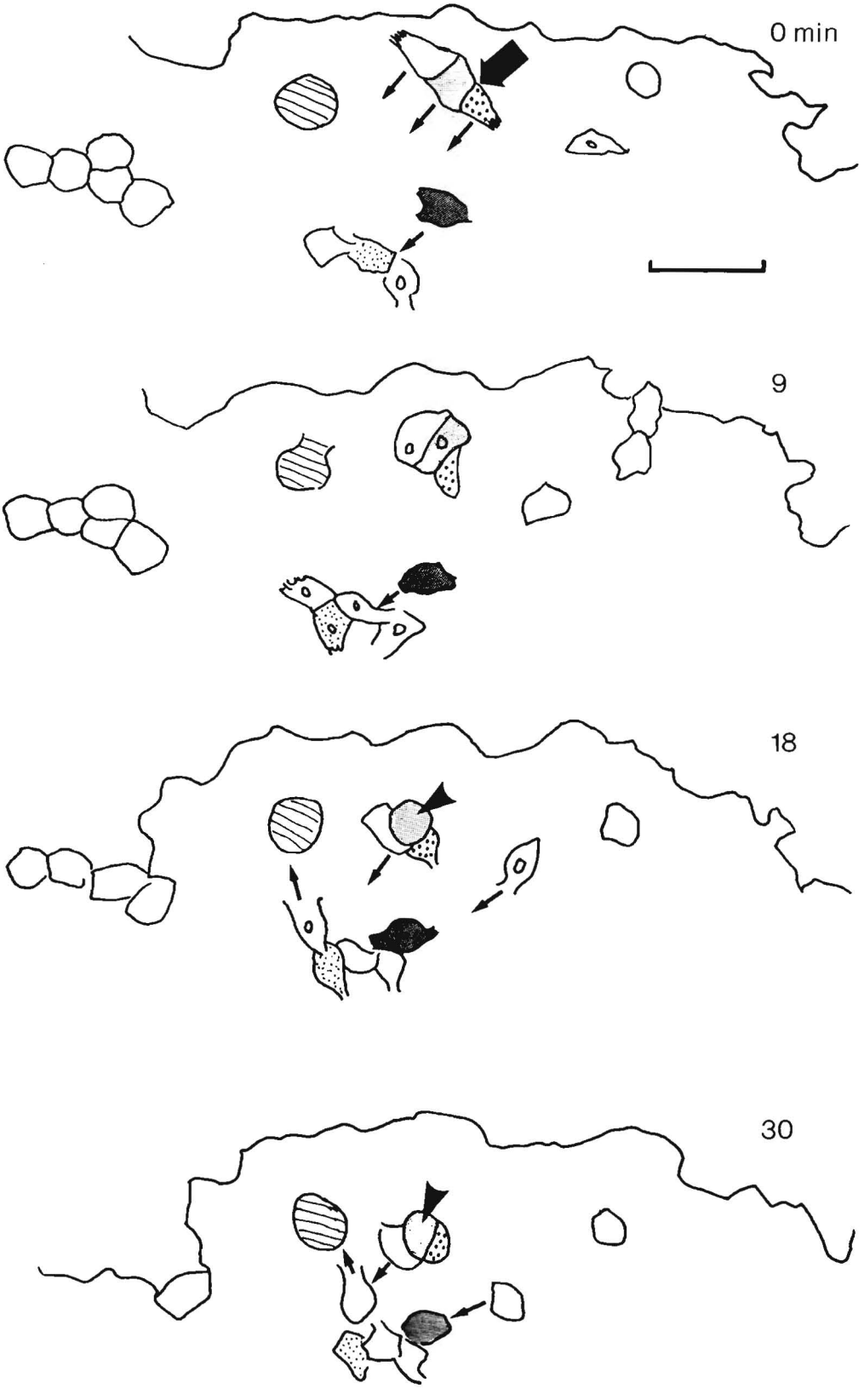
limnicola movement are coordinated in a transcellular pattern. Local contraction centres occur in which many cells contract together or sequentially, resulting in a displacement of adjacent cells larger than that seen when individual cells retract in extension–retraction cycles. Major contractions of this type will occur from once to a half dozen times, and smaller twitches occur more frequently in the course of extension and convergence of the entire dorsal, axial mesoderm (dorsal IMZ). The cell arrangement before and after these events does not change markedly and these contractions seem to have no obvious function. Similar twitches also occur in films of whole embryos, usually in the marginal zone, and with the same frequency as seen in explants; so it does not appear to be a phenomenon unique to cultured explants.

The second coordinated process is sequential blebbing or limnicola movement in a series of cells. In this situation, limnicola movement will occur in one cell after another in a linear series across the explant. In other cases, a wave of limnicola movement will pass across the tissue as a broad front hundreds of cells across. These types of behaviour appear to be propagated from cell to cell. In the case of a linear series of cells, the event has been observed in all directions for distances of 3–15 cells. In the case of the broad fronts, they pass roughly from the midline laterally and have been observed only in explants that are near or beyond the point of delineating the notochord–somite boundary. The rate of propagation is 40–90 μm min^{-1}. Overall, this phenomenon appears to have no lasting effect on the explant. In response to nudging, *Fundulus* deep cells in culture also propagate blebs from one to another (Tickle & Trinkaus, 1976).

Mesodermal cells of the IMZ are involved in two types of motility: intercalation and migration

In the explant, the circumferential (mediolateral) intercalation of deep cells of the IMZ occurs without benefit of an adhesive external substratum, presumably as a result of forces generated by the protrusions connecting these cells with one another. However, these same cells also have protrusions extending towards and adhering to the overlying NIMZ, which has been removed (see Keller & Schoenwolf, 1977) (arrowheads, Fig. 3). They intercalate between one another but supposedly migrate animal-polewards on the inner NIMZ (Nakatsuji, 1976; Keller & Schoenwolf, 1977). Thus these cells must have specialized behavioural properties, with one set of rules governing their interaction with one another to produce mediolateral intercalation (convergence and extension) and another set of rules governing their interaction with the overlying NIMZ or the animal cap to produce translocation on this surface.

We will now examine the interaction of dorsal IMZ cells with the overlying substrate for migration, the NIMZ or animal cap. If the involuted IMZ of a midgastrula is peeled away from the NIMZ so that individual cells of the IMZ are left attached to the NIMZ (Fig. 5), and cultured in the explant system (Keller *et al.* 1985*a*,*b*), IMZ–IMZ interactions and IMZ–NIMZ interactions can be studied directly by video- or cine microscopy. Individual IMZ cells may or may not move on the inner surface of the NIMZ, depending on circumstances that are not at present understood. If they do move, they can move in any direction (small arrows, Fig. 5).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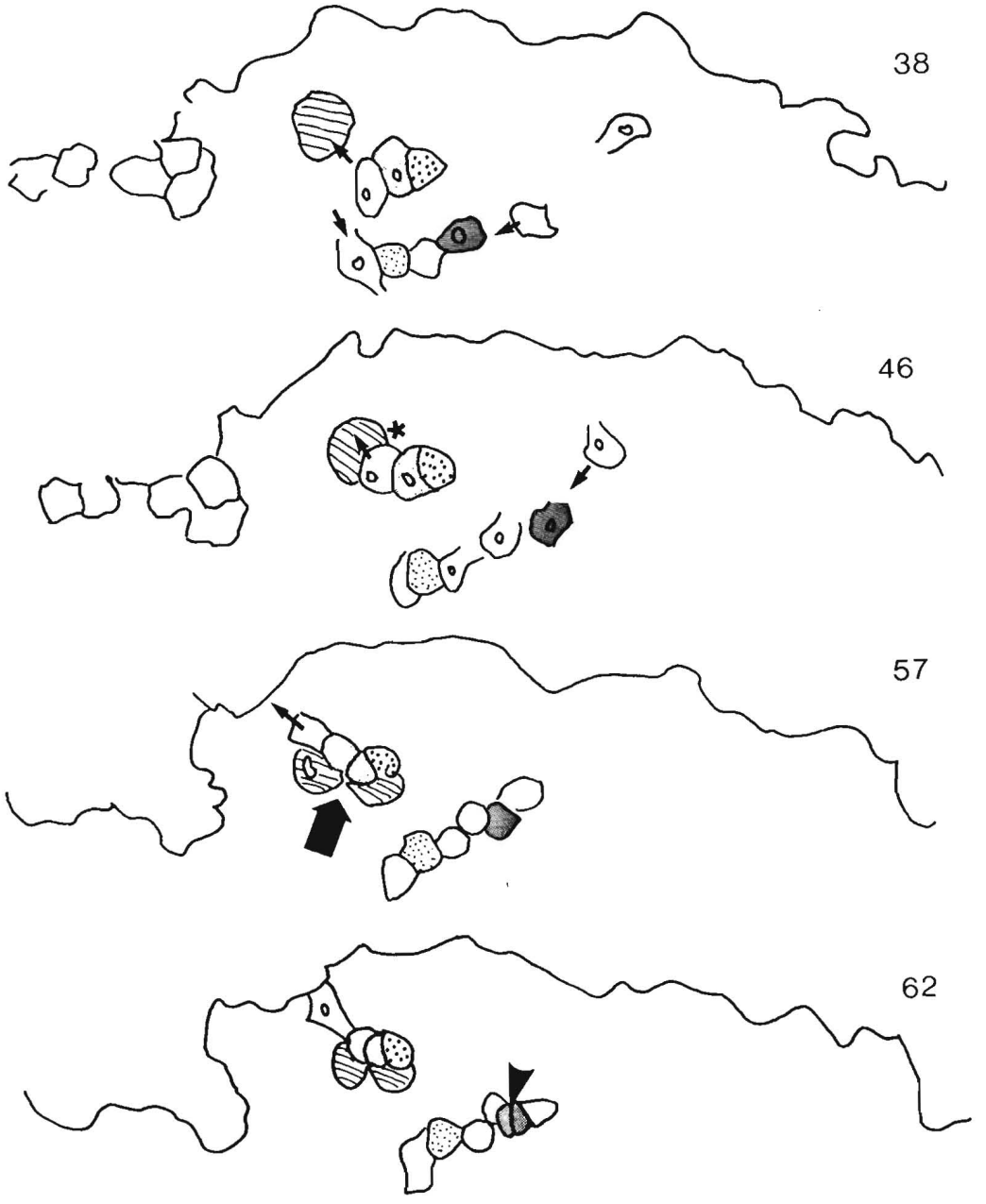

Fig. 5. The migration of individuals and small groups of involuted mesodermal cells on the inner surface of the non-involuting marginal zone of the late midgastrula are illustrated with tracings from time-lapse recordings. The non-involuting marginal zone was stripped off the involuting marginal zone of a stage 11 gastrula in such a way that individual mesodermal cells were left attached at the animal end of the explant (bottom of each figure) and a contiguous mass of involuted mesodermal cells was left attached at the vegetal end of the explant (top of each figure). The line in each figure is the animal-most boundary of the contiguous mass. Identity of individual cells is indicated by symbols and their major movements are indicated by arrows. The arrowheads indicate cells riding on top of cells underneath. Bar, 100 μm.

They may move as groups of several cells, side-by-side (group of three, large arrow, 0 h; Fig. 5), or one may move with another on its back (arrowheads throughout, Fig. 5). When meeting one another or the main mass of IMZ cells, they do not necessarily cease movement or retract as if they were under the influence of contact inhibition of movement (Abercrombie, 1970). They are capable of crawling upon their own kind (asterisk, 46 min, Fig. 5) and crossing over them (large arrow, 57 min, Fig. 5), not only alone but as groups. The migratory behaviour of large populations of mesodermal cells contrasts strongly with the behaviour of individual cells. If an explant retains a large contiguous population of postinvolution mesodermal cells on its inner surface instead of the scattered cells shown in Fig. 5, they move toward the cell-free space at the animal end of the mass as a coordinated unit (data not shown) or cell stream (see pp. 449–450, Trinkaus, 1984*a*). We could find no evidence for the substrate guidance of mesodermal cells seen in culture (see Nakatsuji & Johnson, 1984). In this respect our results are consistent with those of Kubota & Durston (1978) for the axolotl.

Mechanical properties of the involuted IMZ

The dynamic behaviour of the deep IMZ cells with respect to one another during intercalation, and with respect to the overlying NIMZ during migration, should not be taken to suggest that this region is insubstantial in the mechanical sense. The protrusions interconnecting the IMZ cells (Fig. 3) are so substantial that our attempts to break them with microneedles usually lead to dislodgment of the cell or tearing of the cell. The IMZ appears to become stiff and more difficult to bend after its involution, as shown by the fact that if it is transplanted outside the blastopore lip, and expected to involute again, it will not bend over the lip and involute, but instead remains at the lip like a canoe on the edge of a waterfall. Despite this apparent rigidity, the individual cells can move between one another, either slowly by the kneading action of individual pulsatile events or occasionally by the rapid cytoplasmic flow described above.

A WORKING HYPOTHESIS OF HOW CELL MOTILITY GENERATES REARRANGEMENT OF *XENOPUS* DEEP CELLS

We do not know how the motile activities uncovered to date bring about directional cell intercalation to produce convergence and extension. The following is our current, working hypothesis, which we feel incorporates most of what we know.

The kneading action seen among deep cells is a reflection of periodic extension and retraction of the cell margin. At any instant, the bulk of the cell population exercising this kneading action is stabilized by multiple filiform protrusions. As the extension–retraction cycles are repeated, contacts with adjacent cells are modulated at the microscopic level. In regions not showing convergence and extension, these modulations average out, but in regions undergoing convergence and extension, they do not, but are biased in such a way that cells tend to intercalate between those cells

that are medial or lateral to themselves. At present we favour a bias in the direction of extension–retraction cycles leading to directional intercalation, because directional bias is displayed by the same cells later, during notochord cell intercalation. The rapidly moving 'sport cells' may reflect abnormal loss of the constraints necessary to prevent all cells from attempting to move rapidly and at the same time.

The extending and converging dorsal, axial mesoderm is a stiff tissue array that may function as a 'skeleton' in the gastrula to close the blastopore and elongate the dorsal side, and it can elongate independently of an external substratum and actually push debris (see Keller *et al.* 1985*b*; Keller, 1986). This mechanical integrity is a necessary part of the function of the extending and converging cell population. Our notion is that to maintain this integrity, many of the cells at any one time must form a rigid tissue array, stabilized by the filiform protrusions connecting them, while a subpopulation of cells, cycling though a motile phase, advance by small increments between their neighbours. Too many cells moving too far at the same time might disrupt the mechanical integrity of the tissue array. In this context, the apparent asynchrony of extension–retraction cycles in a given region may be important. There may be a control system to regulate these cycles, specifically ensuring that adjacent cell populations or adjacent cells are out of synchrony. Alternatively, there may be a pattern in the control of these cells that we do not yet recognize. The coordinated blebbing and contraction that we have observed suggests that there may be a system capable of controlling motility but it is puzzling that these coordinated events are infrequent and have no obvious effects. We are currently analysing temporal and spatial patterns of extension–retraction cycles, contact relations, and fast movements to determine whether such order exists. Our observations on the interaction of involuted mesodermal cells with one another and with the overlying NIMZ or blastocoel roof, raise the possibility that these cells can participate in active intercalation among themselves and simultaneously participate in directional migration on an external substratum. Resolving the rules of interaction of IMZ cells with each other and with the overlying substratum will greatly enhance our knowledge of what constitutes an organized 'stream' of cells in morphogenesis.

CELL BEHAVIOUR AND PROTRUSIVE ACTIVITY DURING REARRANGEMENT OF EPITHELIAL CELLS DURING SEA-URCHIN ARCHENTERON ELONGATION

The role of cell rearrangement in archenteron elongation

The most dramatic change that occurs at the onset of archenteron elongation (secondary invagination) in the sea-urchin embryo is the appearance of long, filopodial protrusions extended by secondary mesenchyme cells at the tip of the gut rudiment. The coincident appearance of these protrusions as the second phase of gastrulation begins provide strong circumstantial evidence that filopodial traction is the dominant mechanism of archenteron elongation (see Gustafson & Wolpert, 1963, for a review). However, mechanical simulations of the effects of filopodial traction

suggest that filopodial pulling alone would produce deformations of the gastrula that are not seen in actual embryos (Hardin & Cheng, 1986). More importantly, using Fristrom's (1976) assay for cell rearrangement in a cylindrical epithelium, Ettensohn (1985) was the first to show that cell rearrangement is important during secondary invagination. As in other rearranging epithelia, cell rearrangement occurs despite the presence of typical septate junctions (Spiegel & Howard, 1983; Ettensohn, 1985). Cell rearrangement has been demonstrated in other species as well, and is accompanied by flattening of the cells in the wall of the gut rudiment (Hardin & Cheng, 1986).

Cell rearrangement alone can account for virtually the entire increase in length of the gut rudiment during secondary invagination in *L. pictus* (Hardin & Cheng, 1986). Archenteron elongation does not require cell division or DNA synthesis (Stephens *et al.* 1986), and little or no involution or addition of cellular material occurs during secondary invagination (Hardin, 1986). The extent of cell rearrangement, as judged by the decrease in the number of cells around the circumference of the archenteron, is very closely correlated with archenteron length. At the beginning of secondary invagination, there are approximately 24 cells around the circumference of the archenteron, and this number drops to as low as six to eight in the narrowest portion of the gut rudiment by the end of secondary invagination. The extent of rearrangement is most pronounced in the central region of the archenteron, although all regions participate in the rearrangement to some extent (Ettensohn, 1985; Hardin & Cheng, 1986).

Cell rearrangement also accounts for the closure of the blastopore during secondary invagination (Hardin, 1986). A strong inverse correlation exists between blastopore diameter and archenteron length, and the number of cells around the circumference of the blastopore decreases concomitant with the decrease in the number of cells around the base of the archenteron (Hardin, 1986), in a manner strongly reminiscent of blastopore closure during gastrulation in *Xenopus*. Cell rearrangement thus accounts for most of the tissue distortions that occur during secondary invagination in the sea urchin (Fig. 6).

Cell rearrangement and exogastrulation

The demonstration of cell rearrangement in the archenteron does not provide any information concerning the mechanism(s) by which such rearrangement might be occurring. However, the phenomenon of LiCl-induced exogastrulation can be used as a tool to examine whether cell rearrangement relies solely on filopodial traction, or whether the rearrangement is in some sense intrinsic to the cells of the gut rudiment. Significantly, cell rearrangement can occur without filopodial pulling in the case of LiCl-induced exogastrulation (Hardin & Cheng, 1986), and suggests that autonomous rearrangement of epithelial cells in the wall of the archenteron can cause it to elongate. Furthermore, it is not uncommon for these everted gut rudiments to exhibit a pattern of cell rearrangement similar to that of normal embryos, i.e. cell rearrangement is most pronounced in the central portion of the archenteron (Fig. 7A). Later, the everted gut rudiment differentiates into a typical tripartite gut

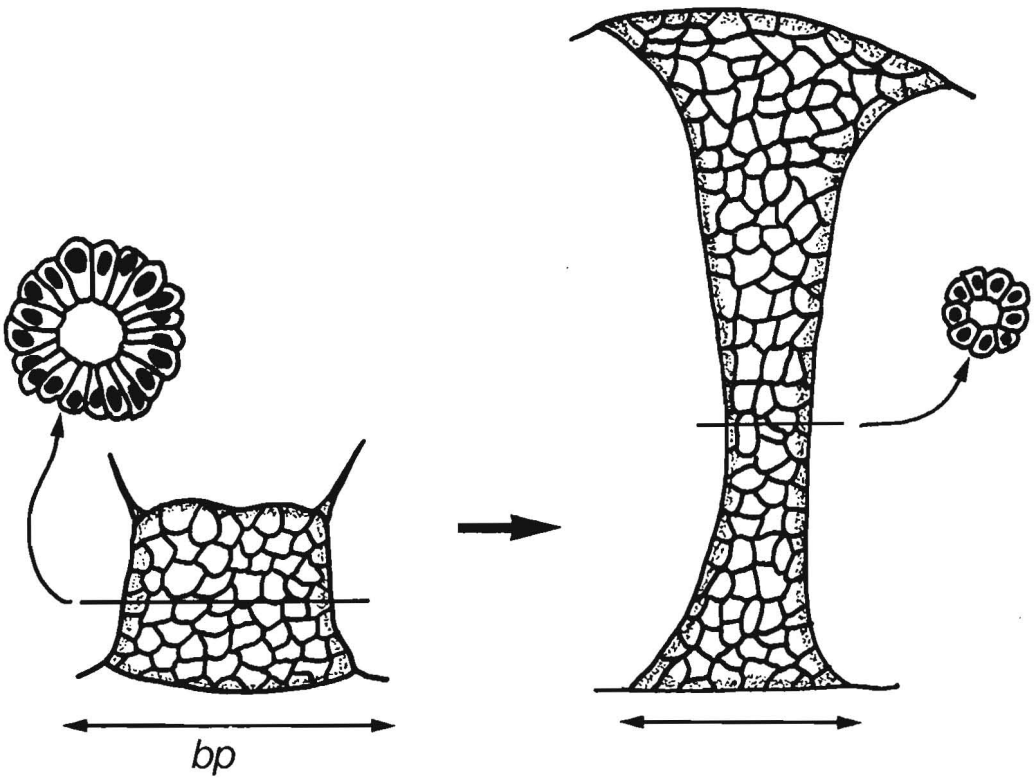

Fig. 6. The role of cell rearrangement in archenteron elongation in the sea-urchin embryo. At the onset of the second phase of gastrulation there are approximately 24 cells around the circumference of the gut rudiment. As the archenteron lengthens, cell rearrangement reduces the number of cells around the circumference, while simultaneously lengthening the cylinder and closing the blastopore (bp).

(Fig. 7B). These facts led to a model of archenteron elongation in which the cells of the archenteron actively rearrange, while the secondary mesenchyme cells at the tip of the gut rudiment serve primarily to guide the archenteron to the correct site to form the mouth primordium (Hardin & Cheng, 1986).

Microtubules and cell rearrangement

The cell rearrangement that occurs in the archenteron as it elongates does not seem to depend on intact cytoplasmic microtubules. Although earlier studies using colchicine had suggested that microtubules were necessary for secondary invagination to occur (Tilney & Gibbins, 1969), subsequent experiments using improved inhibitors combined with immunofluorescent detection of microtubules have demonstrated that microtubules are not crucial for elongation of the archenteron (Hardin, 1987; Fig. 8). Disruption of microtubules with nocodazole does not affect cell rearrangement in the gut rudiment, or spontaneous exogastrulation. On the other hand, treatment of embryos with the colchicine analogue, lumicolchicine, which

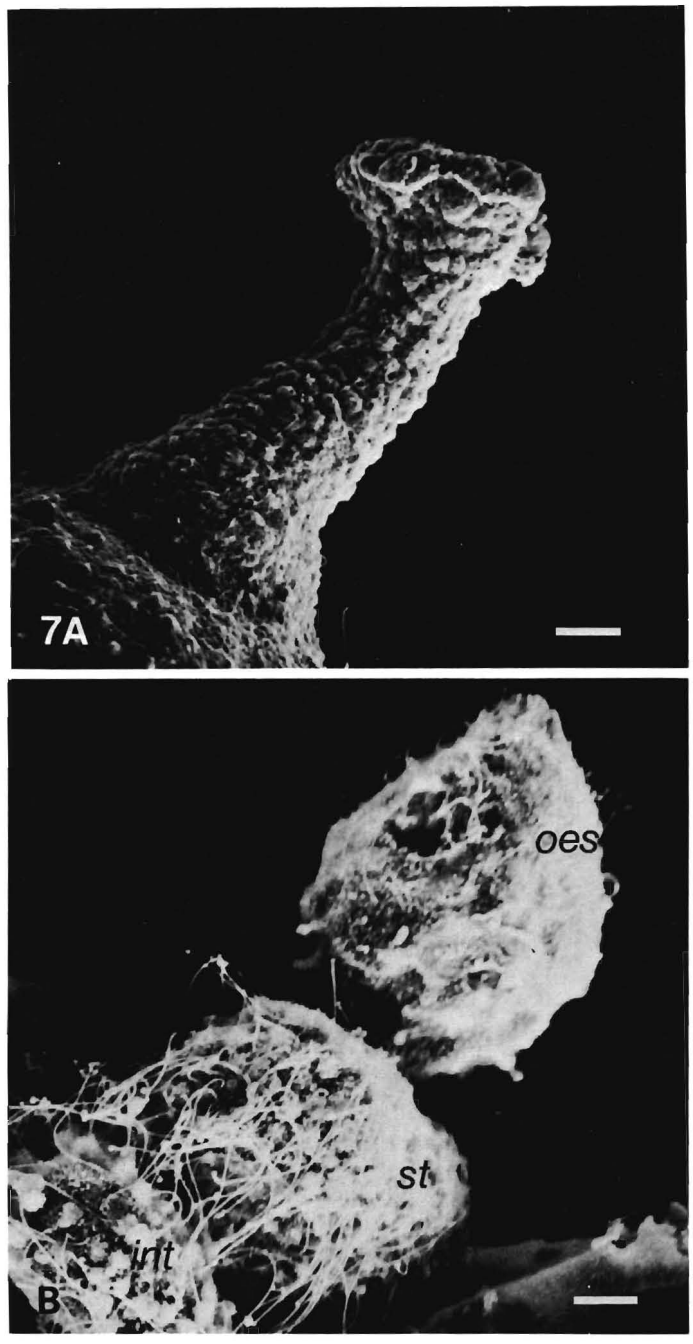

Fig. 7. LiCl-induced exogastrulation in *Lytechinus pictus*. A. SEM of an elongated exogastrula. Note that the central region of the archenteron undergoes more cell rearrangement than the rest of the gut rudiment. Bar, 10 μm. B. Gut rudiment of an exogastrulated pluteus larva. Note the tripartite differentiation of the gut into oesophagus (*oes*), stomach (*st*), and intestine (*int*). Bar, 5 μm.

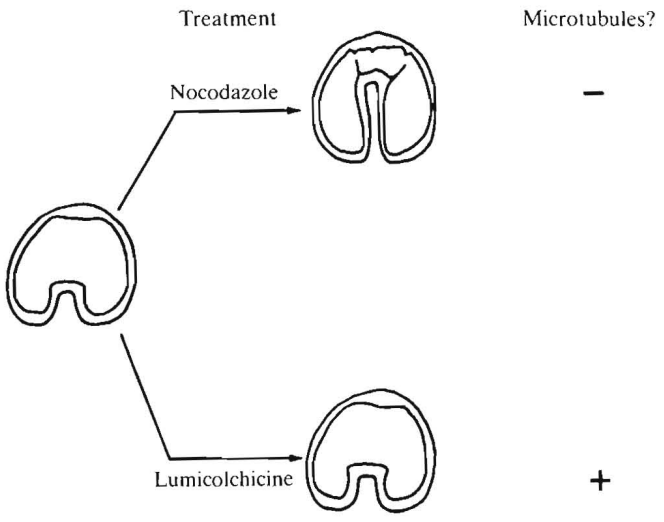

Fig. 8. The role of microtubules in archenteron elongation: 0·1 mM-β-lumicolchicine, an analogue of colchicine that does not bind tubulin, prevents elongation without disrupting microtubules (+); nocodazole (10 μg ml^{-1}) does not prevent elongation, but does disrupt microtubules (−), indicating that microtubules are not crucial for archenteron elongation.

does not bind tubulin, inhibits secondary invagination without disrupting microtubules, indicating that earlier results using colchicine are probably due to side effects of the drug, rather than its effects on microtubules (Hardin, 1987*a*).

Protrusive activity during archenteron elongation

The detailed motile behaviour, which presumably depends on actin microfilaments, of the rearranging epithelial cells in the wall of the archenteron is only beginning to be understood. The elegant use of time-lapse cinemicrography by Gustafson and coworkers (e.g. see Gustafson & Kinnander, 1956; Kinnander & Gustafson, 1960) revealed a general pulsatile behaviour of the basal surfaces of cells in the gut rudiment. As the archenteron elongates, this pulsatile behaviour becomes progressively localized to the tip of the archenteron (Kinnander & Gustafson, 1960). It is not known what relation this pulsatile behaviour has to cell rearrangement, and unfortunately the resolution afforded by the light microscope has to date prevented any further information from being gained using time-lapse techniques.

However, scanning electron microscopy has provided new information on the coordinated motility and shape changes that occur in the archenteron during cell rearrangement (Hardin, 1987*b*). Careful staging of gastrulae at various stages of secondary invagination reveals striking, stage-specific changes in the morphology of the basal surfaces of endoderm cells in the wall of the gut rudiment. At the end of the first phase of invagination, basal surfaces of endoderm cells are bulbous, and occasional lateral processes are seen between neighbouring cells (Morrill & Santos, 1985; Hardin, 1987*b*). When the archenteron begins to elongate, long, highly

oriented lamellipodial processes are extended by each cell towards the tip of the archenteron. These lamellipodia overlap one another, giving the archenteron a 'shingled' appearance (Fig. 9A). The broad lamellipodia end in shorter, filopodial processes that extend onto the basal surfaces of cells in overlying tiers, generally at a slight angle to the long axis of the archenteron (Fig. 9B). Similar changes in morphology have been observed by Ettensohn (1984*a*). It is not clear how these protrusions might function in cell rearrangement, but at the least they indicate that all of the cells of the archenteron have inherent directionality; they 'know' which way is 'up' (i.e. towards the animal pole). Furthermore, up no longer corresponds to the inherent apical–basal polarity of the cells of the gut rudiment, but is instead roughly perpendicular to the apical–basal axis.

When the archenteron has lengthened slightly, these oriented protrusions disappear; the basal surfaces of the cells become somewhat rounded, and a dense, overlapping network of cell extensions is visible between cells (Fig. 10A). These extensions make contact with the basal surfaces of neighbouring cells in a criss-crossing fashion. Many of these protrusions are not taut, but instead appear loosely curled (Fig. 10B). The angular distribution of these protrusions is markedly different from the oriented lamellipodia that precede them, and suggests that these smaller protrusions may represent alterations in cell–cell contacts as the cells begin to rearrange (Hardin, 1987*b*).

At the 1/2 to 2/3 gastrula stage, basal surfaces appear rounded, with little evidence of protrusions. By the 2/3 to 3/4 gastrula stage, basal surfaces elongate slightly (Hardin, 1986). The extent of elongation is not uniform; some cells in the thinnest portion of the gut rudiment show marked elongation, while cells in other regions are not nearly as elongated. By the 3/4 gastrula stage, the outer surface of the archenteron becomes covered with a thick layer of extracellular matrix material (Hardin, 1986).

Evidence for protrusive activity on lateral and apical (luminal) surfaces of endoderm cells is considerably more difficult to obtain compared to the basal surfaces, due to the difficulty of fracturing through the archenteron without causing its complete disintegration. However, some generalizations can be made. First, there is little evidence for protrusive activity on lateral cell surfaces during secondary invagination. Small cell processes can occasionally be seen over- or underlapping cells in different tiers at the mid-gastrula stage, but these are relatively infrequent (Hardin, 1987*b*). Second, tenuous apical extensions can sometimes be seen connecting one cell in a given tier with the upper (animal-poleward) region of the apex of a neighbouring cell in the same tier. These extensions may be reasonably (though tentatively) interpreted as domains of decreased contact between cells that are moving past one another as they rearrange.

In addition to any protrusive activity exhibited by the apical surfaces of cells in the archenteron, cell apices probably also modulate their shape and attachment to the hyaline layer during cell rearrangement. The hyaline layer maintains its attachment to the inner surface of the archenteron throughout secondary invagination, requiring not only that it change its shape as the dimensions of the archenteron change, but also

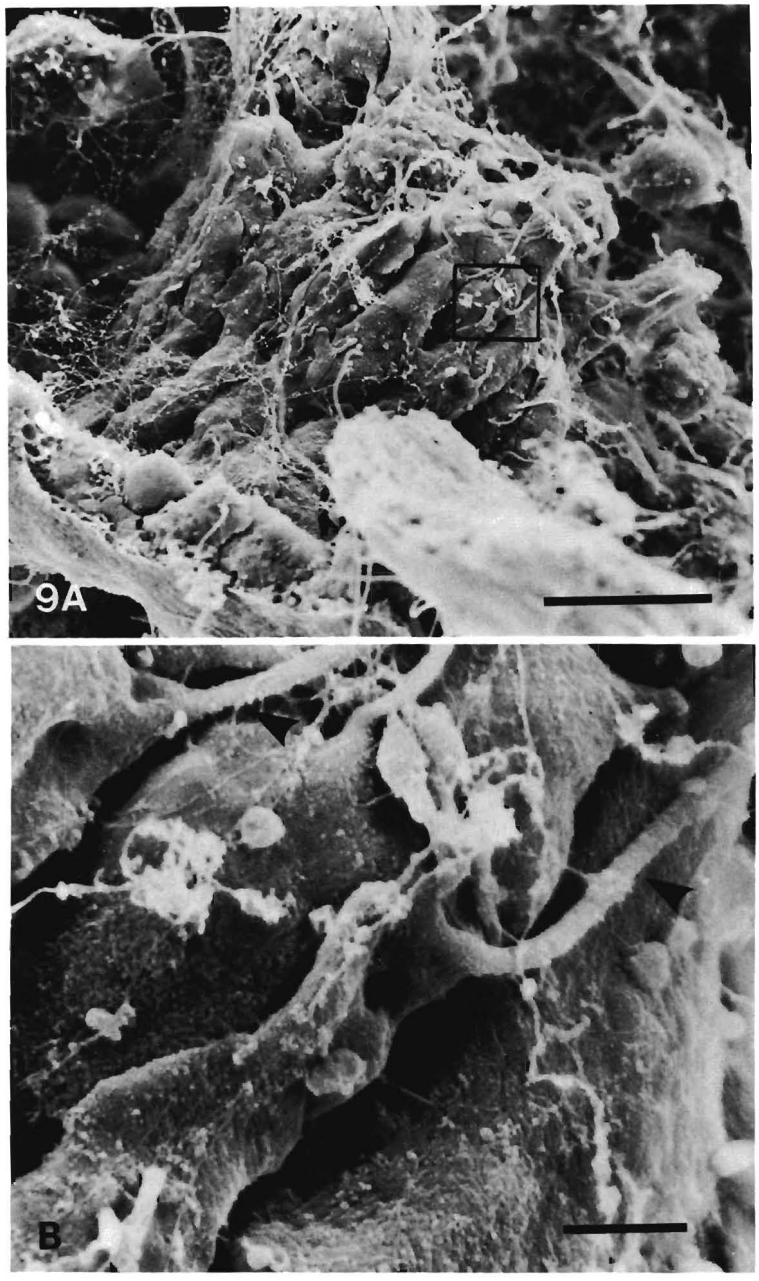

Fig. 9. Lamellar protrusions at the onset of secondary invagination. A. Long, oriented protrusions extend towards the tip of the archenteron. Bar, 10 μm. B. Higher magnification of the enclosed region in A. The lamellipodia end in slender filopodial processes (arrowhead). Bar, 1 μm.

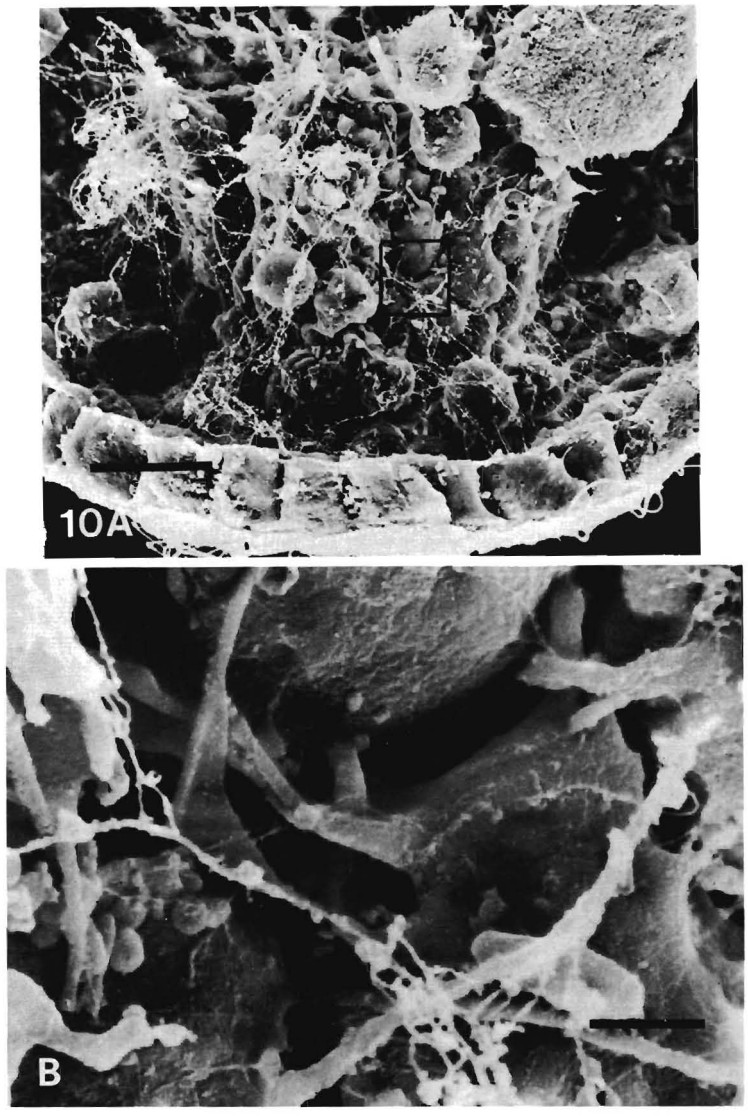

Fig. 10. Slender protrusions at the 1/2 gastrula stage. A. A dense network of overlapping filar processes are extended from the basal surfaces of cells in the archenteron. B. Higher magnification of the enclosed region in A. Cell bodies are rounded, and are studded with overlapping protrusions. Bar, 1 μm.

requiring that the apices of individual endoderm cells change their contacts with it as well. In addition, the apices of all cells in the archenteron and the other epithelial cells of the embryo possess typical apical microfilament bundles, which are clearly revealed both by transmission electron microscopy (TEM) and phallotoxin staining (Ettensohn, 1984*b*; Harris, 1986; J. Hardin, unpublished observations). It is not known what role apical microfilaments might play in archenteron elongation in the

sea-urchin embryo, but their modulation is probably necessary during cell rearrangement.

HYPOTHESES CONCERNING HOW CELL MOTILITY GENERATES EPITHELIAL CELL REARRANGEMENT OF SEA-URCHIN ARCHENTERON CELLS

Any epithelium undergoing cell rearrangement is presented with several challenges. First, a functioning epithelium must serve as a barrier separating the internal from the external environment. Thus rearranging epithelial cells must have a means by which they can change their neighbour relations while maintaining the tight or septate junctional seals at their apical surface. That this is possible is dramatically demonstrated by the enveloping layer during epiboly in *Fundulus*, which undergoes extensive cell rearrangement while maintaining its integrity as a high-resistance permeability barrier (Keller & Trinkaus, 1987). In the case of septate junctions, Fristrom (1982) has proposed a model by which septate junctional domains may be modulated as cells progressively lose their contact with one another, resulting in the appearance of 'tricellular plugs' as the contact domain between cells shrinks to a vertex. The sea-urchin gastrula also possesses such tricellular junctions (Spiegel & Howard, 1983), and it is tempting to suppose that they have a similar function during cell rearrangement in the archenteron. In addition, the apical extensions seen in fractured archenterons (Hardin, 1987*b*) lend credence to the notion of gradually reduced zones of contact between rearranging epithelial cells in the archenteron.

A second issue that must be faced by a rearranging epithelium is the relationship between the apical and basal surfaces of the epithelium. It is generally believed that the basal surfaces of embryonic epithelial cells are the only surfaces that display significant protrusive activity and that if protrusive activity helps to generate cell rearrangement, then it must arise (Kolega, 1986). These notions have led Jacobson *et al.* (1986) to propose the 'cortical tractor' model of epithelial cell rearrangement in the urodele notoplate. In this model, new apical contacts are made by rotation of basal protrusions laterally and finally apically, so that new apical surface is produced by wedging of basolateral membrane onto the apical surface *via* cortical cytoplasmic flow. It is thought that the 'tractoring' of the cortex sweeps junctional components continually up to the apex, maintaining the circumapical junctional arrangement in a state of dynamic equilibrium. Such cortical or amoeboid protrusive activity clearly seems to be important in the case of deep cells (see above). However, in the case of epithelial cells, models that imply a plasticity of the apical–basal boundary of the cell do not seem to be in accord with current thinking in epithelial cell biology, which views the apical–basal boundary as stable, and encompassing not only junctional specializations but sharp differences in membrane proteins, secretory protein traffic and cytoskeletal specializations as well (reviewed by Kolega, 1986).

Alternatively, basal protrusive activity could still be invoked as the driving force for epithelial cell rearrangement, but without altering the apical–basal polarity of the epithelium. In this case, force production would occur on the basal surface of the epithelium, and apical surfaces of epithelial cells would rearrange in response to

forces exerted by their basal ends. A likely candidate for such a mechanism is the cell rearrangement that occurs during insect pupal morphogenesis. The contraction of basal 'feet' probably causes the shape transformations and cell rearrangement associated with insect pupal segment morphogenesis (Locke, 1985).

In the case of rearranging epithelial cells in contact with deep cells, cell rearrangement may simply be a response to extrinsic forces. This suggestion has been made in the case of epiboly of the enveloping layer in *Fundulus* (Trinkaus, 1984*b*), and the superficial layer in *Xenopus* (Keller, 1980). Although the epithelial cells in these cases may not be responsible for force production, it may be misleading to refer to them as strictly 'passive', since they must accommodate massive distortions at the tissue and cellular levels. In the case of *Fundulus* epiboly, 'flowering' of apical membrane occurs as submarginal cells separate from one another, suggesting that the cells of the marginal zone themselves may actually show 'responsive', rather than 'passive', rearrangement.

In addition to the complications introduced by junctional complexes and apical–basal polarity, rearranging epithelial cells also possess a circumapical band of actin filaments. During cell rearrangement or shape change, these microfilament bands must be modulated. It is not clear what role such actin bundles play in force production in epithelia. Tension exerted by apical microfilaments has been suggested as a sufficient mechanism to generate the forces necessary for cell rearrangement in epithelial wound healing (Honda *et al.* 1982), and is presumed to produce invaginations in other epithelia (reviewed by Hilfer & Searles, 1986). The possibility that one function of actin bundles may be to maintain a steady-state tension in an epithelial sheet (Owaribe *et al.* 1981) has led to the 'boundary shortening' model of cell rearrangement (Honda, 1983). In this model, when the shape of cell apices is perturbed in some way (e.g. by wounding the epithelium), the tension generated by the circumapical actin bundles causes the cells to reassume a hexagonal arrangement, accompanied by changes in neighbour relations of the cells. Although not expressly mentioned by Honda, it seems possible that any perturbation introduced at the apical surface, including shape changes initiated at the basal surface, could be accounted for by the boundary shortening model.

None of the foregoing considerations can account for the directionality of cell rearrangement along a preferred axis. Some asymmetry must exist to bias extension in a particular direction. For example, such a bias could manifest itself as an asymmetric distribution of protrusions or cell shape changes, or an anisotropic mechanical response to otherwise isotropic protrusive activity (cf. the discussion of deep cell behaviour above).

The above considerations regarding rearranging epithelia must be brought to bear on the problem of archenteron elongation during sea-urchin gastrulation. The archenteron is a typical monolayered epithelium, with circumapical actin bundles (see above). At least at the outset of secondary invagination, basal surfaces exhibit general pulsatile behaviour (Kinnander & Gustafson, 1960), a feature they share with basal surfaces of *Drosophila* imaginal disc cells (Fristrom, 1976; D. Fristrom, personal communication). It is not known what function, if any, such surface activity

has in cell rearrangement, although this behaviour is similar to that of deep cells in *Xenopus* (see above).

Later, basal surfaces of the archenteron clearly undergo dramatic, coordinated changes in morphology and protrusive activity as the gut rudiment elongates. The oriented lamellipodia that appear at the outset of secondary invagination are particularly intriguing. Their orientation suggests that they are not involved in force production by direct contraction. However, it is possible to imagine that they may be involved in altering the shape of the apical ends of the cells. If the apical surfaces change their shape, then a 'relaxation' phase, perhaps involving some sort of boundary shortening step, could produce the required axial extension. Perhaps the rounded morphology exhibited by the basal surfaces following the extension phase represents the onset of such relaxation during cell rearrangement. While this model is speculative at best, it is at least a step in beginning to unravel the complex processes at work during epithelial cell rearrangement in the archenteron.

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