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Models of morphogenesis: the mechanisms and mechanics of cell rearrangement

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The directional rearrangement of cells is a key mechanism for reshaping embryos. Despite substantial recent progress in understanding the basic signal transduction pathways that allow cells to orient themselves in space, the extrinsic cues that activate these pathways are just beginning to be understood. Even less-well understood are the physical mechanisms cells use to change position, especially when those cells are epithelial, and how mechanical forces within the embryo affect those movements. Recent studies are providing clues regarding how this fundamental process occurs with such remarkable reliability.

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Abbreviations

A-P	anterior–posterior
CE	convergent extension
Dsh	Dishevelled
ECM	extracellular matrix
FEM	finite element method
FN	fibronectin
PCP	planar cell polarity
PDGF	platelet-derived growth factor
PI3K	phosphoinositide 3-kinase

Introduction: lessons from the deep

Cells engage in a limited repertoire of morphogenetic movements, carefully orchestrated in time and space, to reshape the embryo. One such movement, directional cell rearrangement, often occurs along a preferred axis, resulting in the narrowing (*convergence*) and lengthening (*extension*) of the tissue. In other cases, rearrangement occurs as a byproduct of other concurrent cell movements.

Both non-epithelial, or *deep*, cells and epithelial sheets can rearrange. Deep cells have provided a wealth of information about how convergent extension (CE) occurs. The paradigm for deep cell CE is the dorsal axial mesoderm of

Xenopus (reviewed in [1,2,3••]). At the onset of CE, dorsal cells become polarized mediolaterally and intercalate with their neighbors (Figure 1), extending highly polarized protrusions perpendicular to the axis of extension. As CE occurs, the tissue stiffens, indicating that rearranging cells maintain cohesiveness as a tissue [4].

Other non-epithelial tissues engage in variations on this basic theme (see [3••] for extensive discussion). For example, the deep neural cells of *Xenopus* embryos display 'monopolar' protrusive activity [1], and the loosely organized mesodermal tissues of the zebrafish gastrula migrate as coordinated individuals. Computer-based tracking to plot the trajectories of individual cells has provided impressive visual confirmation that the latter movement is remarkably similar to that in amphibians [5•].

Several papers in the past year have added to our understanding of deep cell rearrangement. In this review, we briefly discuss these recent studies. In addition, we discuss recent advances in our understanding of CE in epithelial cells, a process similar to, but less well understood than, deep cell CE. Finally, we discuss the often overlooked role that cellular and tissue mechanics plays during cell rearrangement.

Planar cell polarity…and more

The signal transduction mechanisms that allow deep cells to rearrange have dominated recent literature. Both the Wnt/Ca^{2+} and the planar cell polarity (PCP) pathways regulate cell polarization during CE, apparently interacting with events in the paraxial mesoderm mediated in part by paraxial protocadherin (PAPC) (reviewed in [2,6,7]). Several recent papers have added to this story. In addition to Slb/Wnt11, Ppt/Wnt5 regulates cell elongation and filopodial orientation in zebrafish deep cells [8•,9•], suggesting that rearranging cells respond to multiple Wnts. Several pathways are likely integrated downstream of Wnts in rearranging cells. For example, Protein kinase C δ affects localization and phosphorylation of Dishevelled (Dsh) in conjunction with a Wnt signal during CE in *Xenopus* [10•]. Dsh, in turn, can activate the Wnt/Ca^{2+} and PCP pathways via its DEP domain, suggesting that the pathways may act together [11••]. Several recent papers show that Prickle, a PCP protein, regulates gastrulation movements [12•–14•]. The ultimate readout of such cell signaling must be alterations in the cytoskeleton, and recent work suggests that Rac and Rho act in parallel pathways downstream of Dsh to activate the JNK pathway and modulate actin assembly,

Figure 1
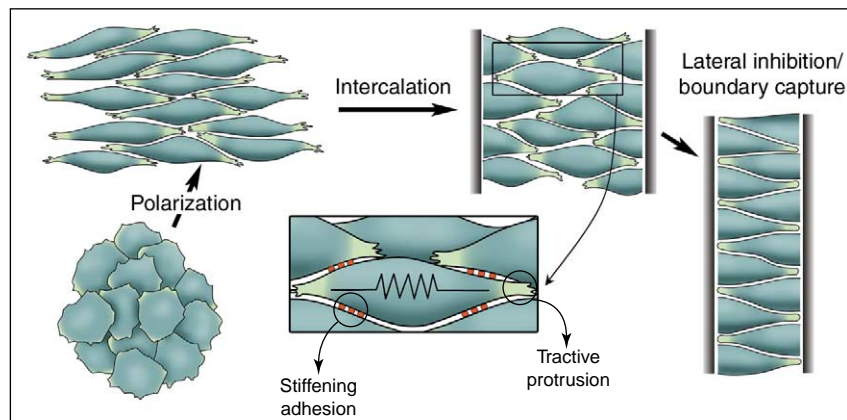
Convergent extension in deep cells. A cartoon depicting the major motile events during convergent extension (CE) of dorsal axial mesoderm in *Xenopus*. Initially, cells show largely random protrusive activity. At the onset of CE, cells orient their protrusions mediolaterally, become bipolar, and intercalate between their neighbors. Cells continue to intercalate until they reach the lateral boundary with presumptive somitic mesoderm, where protrusive activity ceases. Cells in the notochord continue to converge until their mediolateral edges each make contact with the boundary ('boundary capture'). As the cells converge, the tissue extends along the forming A-P axis. Inset: the Keller cell–cell traction model of intercalation. CE must maintain rigidity within the tissue, while individual cells retain the ability to migrate between neighboring cells. 'Stiffening adhesions' (red) are temporary regions of adhesion between cells that allow the tissue to retain structure, but can be easily broken or reformed as cells migrate between each other. 'Tractive protrusions' consist of mediolaterally directed filopodia that adhere to neighboring cells with enough force to pull cells between one other. (Adapted from drawings courtesy of R Keller, with permission.)

respectively [15•,16•]. Such protrusive activity may be modulated by MARCKS, a membrane-bound actin-binding protein, which may act downstream of Rho [17•].

Abrogating Wnt signaling reduces CE but does not abolish it, suggesting that other parallel pathways operate to polarize deep cells. Indeed, platelet-derived growth factor (PDGF) and phosphoinositide 3-kinase (PI3K) are required for filopodium formation and cell polarization during early zebrafish gastrulation [18•]. Given the widespread distribution of PDGF in the embryo, the PDGF/PI3K pathway may be permissive for polarization, whereas Wnt signals stabilize and refine local polarity.

What polarizes deep cells?
Although the emerging story regarding deep cells is gratifying, questions remain. In both *Xenopus* and zebrafish, dorsoventral patterning results in dorsoventral differences in protrusive activity among deep cells (reviewed in [3••,19]); how polarizing cues are spatially organized locally, however, remains unclear. In *Xenopus*, Keller *et al*. have argued that 'boundary capture' occurs as the dorsal mesoderm completes its rearrangement (Figure 1; see [3••]). In this model, the boundary between presumptive notochord cells and somitic mesoderm inhibits protrusive behavior. Intriguingly, an extracellular matrix (ECM) containing fibrillin may constitute this inhibitory barrier [1]. How widespread such mechanisms are is unclear, because many rearranging cells lack an obvious structural boundary. Localized attractive guidance cues may play a role in polarizing monopolar neural

deep cells, based on microsurgical removal or additional of dorsal midline cells in *Xenopus* [20••]. The molecules mediating such attractive cues remain unknown.

How do deep cells rearrange?
Once polarized, cells must change position while remaining rigid enough to produce forces resulting in extension. On the basis of morphological observations [2], Keller *et al*. have proposed a model that balances these key properties of rearranging deep cells [3••]. They propose that rearranging cells use both 'tractive protrusions' (mediolateral protrusions that pull on neighboring cells), and 'stiffening adhesions' (temporary bonds providing rigidity as neighbors intercalate; Figure 1). The stiffening adhesions must remain sufficiently labile to allow for remodeling in response to the tensile forces generated by tractile protrusions.

Although the molecular components permitting such contacts are unknown, cell–cell adhesion molecules must be involved. Indeed, levels of C-cadherin are crucial for normal rearrangement [21], but until recently, integrins were not presumed to be important for deep cell rearrangement. However, Marsden and DeSimone have shown recently that disrupting integrin-based adhesion reduces CE of the dorsal mesoderm and the bipolar orientation of rearranging cells [22••]. Such treatments interfere with C-cadherin-dependent cell sorting, suggesting that the integrin and cadherin systems may interact during CE. Upon closer inspection, deep cells are surrounded by a fibronectin (FN)-containing ECM [22••];

in vivo microscopy suggests that as rearranging deep cells intercalate, they carry attached FN with them (R Keller, personal communication). It is tempting to speculate that FN is required for tractive protrusions, and that stiffening adhesions use cadherins. However, there are many other ways in which these interacting adhesion systems could be deployed.

Epithelial cell rearrangement: additional challenges, additional mechanisms

Epithelial cells can also rearrange, but face the additional challenge of maintaining highly organized junctional connections and apical–basal polarity during rearrangement (for classic examples, see [23]). In many cases, rearrangement result from forces produced by the rearranging cells themselves, such as the lengthening of the neural plate during vertebrate neurulation (reviewed in [24]). A potentially new example of CE is the neuroepithelium of the organ of Corti in the inner ear [25•]. Significantly, both inner ear morphogenesis and neurulation require PCP signaling [24]; recent work adds to this

picture by linking classic mouse neural tube defective mutants to the PCP orthologue, Celsr1/Flamingo [26•].

Although PCP signaling can serve to orient neuroepithelial cells in vertebrates, many other examples of epithelial cell rearrangement have not been linked to PCP signaling, including the sea urchin archenteron (reviewed in [27]), the *Drosophila* germ band [28], hindgut [29], and stigmatophore [30], the *Caenorhabditis elegans* dorsal epidermis [31,32] and intestine [33], and the notochord primordium in ascidians [34] (the notochord employs PCP signaling once its cells have been internalized [35]). Indeed, genetic evidence in *Drosophila* [36••] and in *C. elegans* (T Walston, J Hardin, unpublished) indicates that PCP signaling through Dsh can, at best, play only a minor role in orienting cells in these systems.

What polarizes epithelial cells?

Recent studies suggest at least three other ways that rearranging epithelial cells could become polarized (Figure 2). In the *Drosophila* gut, Upturned, a secreted

Figure 2
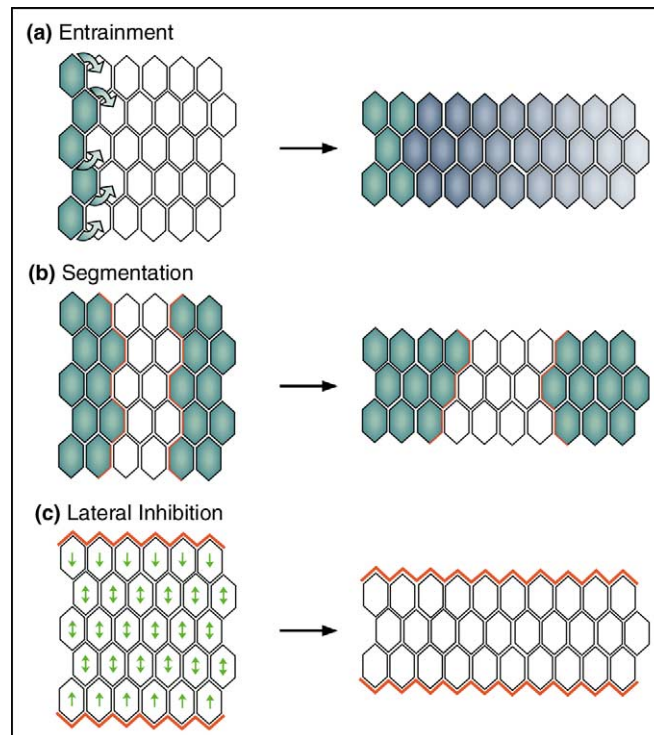
Models for polarization of rearranging epithelial cells. **(a)** Entrainment. In this model, based on work in [37••], cells at one end of the sheet secrete a diffusible factor that is distributed in a gradient across the sheet. The growth-factor gradient leads to polarization and CE of all cells in the sheet. **(b)** Segmentation. In this model, based on germ band extension in *Drosophila* [28,36••], the expression of the pair-rule genes establishes narrow segmental regions along the A-P axis, spanning one, or a few, cell diameters. During CE, cells remain within their segmental compartments and only intercalate with 'like' cells, presumably employing bipolar asymmetries at their A-P and dorsoventral edges. **(c)** Lateral inhibition. This model is suggested by work in *C. elegans* [32]. An array of intercalating cells is bounded on its lateral edges by either a physical or a molecular barrier. As intercalation begins, cells are free to migrate mediolaterally, but cells in contact with the boundary only retain the ability to migrate in a monopolar manner away from the boundary.

protein that acts via the JAK/STAT pathway, is expressed in the small intestine, but acts cell non-autonomously to regulate CE movements in the posterior gut primordium (Figure 2a; [37••]). Significantly, uniform expression of *upturned* or the activated JAK receptor, *hopscotch*, cannot stimulate correct CE, suggesting that a graded distribution of Upturned, and hence STAT activity within responding cells, is required to organize correct movements [37••]. How local production of a secreted protein can result in uniform rearrangements of cells at varying distances from the signal is unknown.

A second mechanism that may polarize rearranging epithelial cells is suggested by work on the anterior–posterior (A-P) patterning system in *Drosophila*, where the pair rule genes *eve* and *runt* transcriptionally regulate germ-band extension (Figure 2b; [28]). Zallen and Wieschaus have recently shown that the A-P system leads to asymmetric protein localization along the A-P edges of cells in the germ band, including myosin II and ectopically expressed Slam protein, along with simultaneous dorsoventral localization of Bazooka/PAR-3. Boundaries at which there are differences in expression of pair rule genes are both necessary and sufficient to induce such bipolarity [36••]. How A-P patterning leads to such subcellular asymmetries is unknown, but could lead to neighbor exchange within the germ band.

A third possibility is that epithelial cells rearrange in response to lateral inhibition (Figure 2c). Currently, there is no clear evidence for such a polarizing mechanism in epithelial cells. However, dorsal epidermal cells in *C. elegans* exhibit greatly reduced protrusive activity at boundaries with lateral epidermal cells, and when their medial tips reach the opposite lateral boundary they undergo a similar reduction in motility [32]. Such observations are suggestive of inhibitory cues, but await direct experimental tests.

How do epithelial cells rearrange

How epithelial cells physically exchange positions is poorly understood. Early models envisioned either contractile elements associated with apical junctional domains or a 'cortical tractor', involving basal protrusions that sweep apically, simultaneously pushing between neighboring cells and shuttling proteins to sites of junctional remodeling (reviewed in [23]). However, protrusions consistent with the cortical tractor model have not been observed. Instead, in the sea urchin archenteron [27], the ascidian notochord primordium [34] and the dorsal epidermis of *C. elegans* [31], basolateral protrusions are present that, like the bipolar protrusions of deep cells, are oriented in the direction of cell movement (Figure 3). In *C. elegans* such protrusions precede the advance of the apical surface [31]. Whether overlying junctions are remodeled passively in response to basolateral events or by generation of apical tension is unknown.

Figure 3
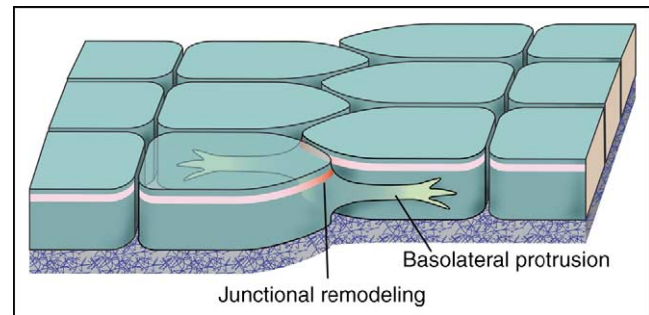
Basolateral protrusive behavior during epithelial cell rearrangement. Although the diagram is based on work in *C. elegans* [31,32], similar protrusions have been seen in other systems [27,34]. Rearranging cells extend basolateral protrusions that initiate intercalation. As intercalation proceeds, apical junctional complexes (pink) must be remodeled (red) as new junctional boundaries are created with neighboring cells.

Regardless, junctional remodeling presumably involves insertion of new junctional material or redeployment of existing junctional particles (Figure 3). Basolateral protrusions have not been observed in the *Drosophila* germ band [36••], suggesting that there may be other uncharacterized mechanisms mediating epithelial cell rearrangement.

How might basolateral protrusions mediate cell neighbor exchange? It seems most likely that such protrusions, if they generate force, do so by exerting traction directly on neighboring cells or the underlying ECM, rather than by pushing. Significantly, in *C. elegans* ECM proteins are not required for dorsal intercalation in the epidermis (reviewed in [38]), suggesting that protrusions, if they exert force, do so directly on neighboring cells.

The apical surfaces of cells may also play an important role in rearrangement. As multicellular tracheal tubes in *Drosophila* are remodeled, cells shift positions to create unicellular tubular elements connected end-to-end. Mutations in the secreted ZP domain protein Piopio and the transmembrane ZP protein Dumpy result in loss of connection between these unicellular elements, suggesting that a luminal complex of Piopio and Dumpy may help to stabilize adherens junctions [39••]. Similar detachment phenotypes result when Rac activity is overstimulated in tracheal cells, and is accompanied by loss of junctional cadherin. Reduction of Rac activity results in excessive cadherin accumulation and stunted elongation [40•], suggesting that careful orchestration of cadherin levels may be required for intercalation. Whether such regulation is a *sine qua non* is unclear because null mutations in the *C. elegans* cadherin, *hmr-1*, do not result in perceptible defects in intercalation of epidermal cells [41].

Mechanics: the forgotten polarizer?

Rearranging cells are usually considered active partners in morphogenesis, but embryonic tissues also respond to externally applied forces. For example, both superficial (epithelial) and deep ectodermal cells rearrange in response to direct mechanical stretching in *Xenopus* explants [42]. Epithelial cell rearrangement is presumed to be a response to external forces as cells seal an opening, such as during epiboly in teleosts [43] and wound closure [44], and during the late phase of elongation of the sea urchin archenteron (reviewed in [27]). In the former, cells detach from the closing free edge, thereby relieving geometric constraints (Figure 4a). In the latter, the tubular archenteron undergoes 'necking' (the Poisson effect), as cells are stretched (Figure 4b).

An inherent challenge related to 'passive' cell rearrangement is how such tissues should be treated mechanically. Precise laser microsurgical manipulation of dorsal closure in *Drosophila* indicates that some non-rearranging tissues behave as elastic sheets that experience viscous drag [45••]. Although individual rearranging cells likely behave viscoelastically [46••], their aggregate behavior is rather more like plastics, which can undergo permanent changes in arrangement. The *Drosophila* germ band is a good example: although just as dramatic as extension, germ band retraction does not involve rearrangement [47]. Similarly, once rearrangement has occurred, ablation of the mesenchymal cells that exert traction on the sea urchin archenteron does not result in appreciable retraction (J Hardin, unpublished). Epithelial tissues have the additional complication that they are clearly *composite* materials — that is, apical and basal ECM presumably affects their bulk mechanical properties.

As our intuition regarding how rearranging cells should behave mechanically is tentative at best, mechanical modeling of such tissues has been performed. One recent attempt to model CE treats the tissue as a lattice whose points are coupled using an equation representing stored energy, which can incorporate differential adhesion [48•]. Although such simulations are capable of producing rearrangement, their relationship to actual cellular processes is unclear. Other simulations involve schemes whereby cells respond to stress by attempting to rearrange their boundaries, and can capture the dynamics of rearrangement events remarkably well [49]. The finite element method (FEM), a well-tested numerical approach to continuum mechanics (reviewed in [50••]), had been used successfully to study aspects of invagination during sea urchin gastrulation (reviewed in [3••,27]). Recent studies by Brodland and colleagues extend the FEM approach to an array of rearranging cells (Figure 4c; [51,52•]). As these models are extended to incorporate the composite nature of cell sheets, they have the potential to clarify which mechanical parameters dominate the behavior of rearranging tissues.

Figure 4
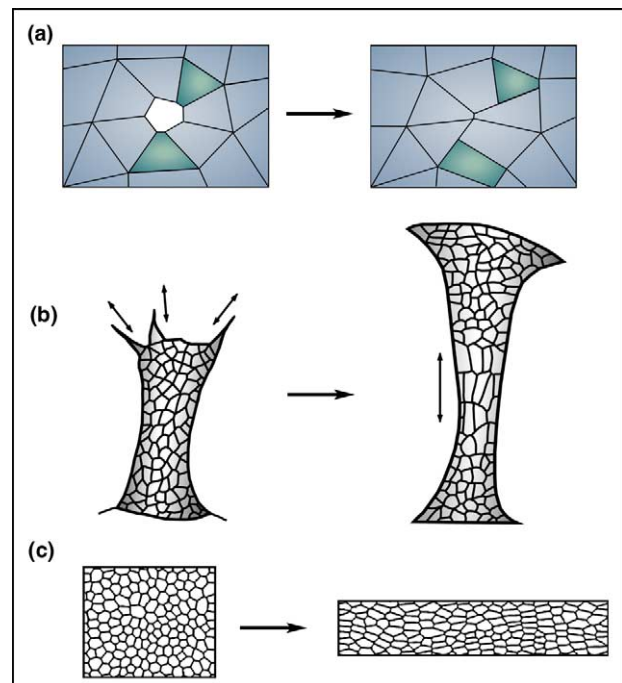
Models of 'passive' cell rearrangement in response to external forces. **(a)** After wounding of an epithelial sheet or during events such as teleost epiboly, cells along the leading edge constrict to seal the opening. During this process, some cells retreat from the leading edge (adapted from [54], with permission). **(b)** Cells in the sea urchin archenteron undergo noticeable stretching where the tube is narrowest in diameter ('necking', or the Poisson effect); cells appear to respond to tension generated by mesenchymal cells at the tip of the archenteron by rearranging. **(c)** A finite element method model shows that in-plane stretching of a two-dimensional sheet of cells initially results in cell elongation, but ultimately cells rearrange to relieve stress generated within the sheet (adapted from [51], with permission).

Conclusions: models, mechanics, and mechanisms united

Ultimately, understanding directed cell rearrangement will require understanding the wider context within which such events take place. Whether this involves the protrusive activity and internal signaling pathways (e.g. PCP signaling) governing deep cell rearrangement, or the patterning and polarizing cues guiding rearranging epithelial cells, our understanding of CE will remain incomplete until our models include both the mechanisms and mechanics of rearrangement. Indeed, mechanical effects are not restricted to reshaping of tissues. In *Drosophila*, externally applied compressive forces designed to stimulate germ band extension are sufficient to induce nuclear localization of Armadillo/β-catenin and *twist* transcription, even in mutants that cannot normally activate *twist* expression [53••]. Such studies suggest that we are only beginning to explore how rearranging tissues affect their surroundings, and that in the future our

understanding of this fundamental morphogenetic event will continue to improve.

Acknowledgements

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