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Collective Cell Migration: A Mechanistic Perspective

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Collective cell migration is fundamental to gaining insights into various important biological processes such as wound healing and cancer metastasis. In particular, recent in vitro studies and in silico simulations suggest that mechanics can explain the social behavior of multicellular clusters to a large extent with minimal knowledge of various cellular signaling pathways. These results suggest that a mechanistic perspective is necessary for a comprehensive and holistic understanding of collective cell migration, and this review aims to provide a broad overview of such a perspective.

Cell migration plays a pivotal role in regulating numerous biological processes under both physiological as well as pathological conditions. Single cells need to interact with their environment to move. This is done by employing a number of different cellular structures such as lamellipodia, filopodia, and podosomes. Although the typical steps involved in the migration of an individual cell over a substrate have already been well studied and characterized (57, 58) (FIGURE 1*A*), recent advances in imaging and molecular biology techniques have enabled us to better probe and visualize these events. Experimental and theoretical analyses strongly suggest that the migratory behavior of single cells in the absence of any external physical or chemical stimuli can be well described by a persistent random walk model (25). Spontaneous lamellipodium formation can randomly polarize a cell and allow them to migrate in a specific direction. Since the persistence of migration under such conditions is very low, they are able to migrate only short distances. However, for cells to be functional in several processes, they need to be able to migrate persistently in a specific direction over long distances. Breaking this "symmetry" (i.e., equal probability to migrate in all directions) requires either external chemical gradients from soluble (chemotaxis) and substrate-bound [haptotaxis (19, 20, 37)] ligands or physical (30) cues within the extracellular environment that introduce a bias such that cells are forced to prefer a particular direction to migrate. For example, leukocytes are strongly attracted to inflammatory chemokines secreted by various cells. The role of chemical cues in guiding and regulating cell migration has been extensively studied and still remains a topic of intense research. However, the role of mechanical and physical cues within the cellular microenvironment in governing cell migration has only recently garnered much attention (35, 70). The application of microfabrication and soft lithography techniques have further boosted our ability to better interrogate the role of micromechanical cues in regulating cell migration (36, 63).

However, it is well recognized that, in several biological processes [e.g., gastrulation (32, 75), wound healing (40) and cancer metastasis (22)], cells do not migrate individually but rather collectively either as clusters, chains, or sheets. Collective migration serves to keep the tissue intact during remodeling, allows mobile cells to carry other cells that are otherwise immobile, and ensures appropriate distribution of cells within a tissue. It has also been observed that the overall migration pattern of such cohesive groups tends to be vastly different from the migration characteristics of the individual cells constituting such groups, and it is rather characterized by the type and strength of their reciprocal interaction (28, 34). Such a behavior has prompted a comparison of collective cell behavior with the wider phenomenon observed in nature known as collective or emergent behavior. Collective behavior or emergent phenomena refers to distinct migratory or movement patterns over length scales that are typically larger than the individual elements constituting a system (69). It is a common occurrence in various physical (e.g., rods or discs on a vibrating table) (8), biochemical (filaments of actin and microtubules migrating over immobilized myosin and dynein motors) (55, 59), inert (e.g., sedimenting colloids), (56) and living (schools of fish and flocks of birds) systems (43, 44) and spans across sizes over several orders of magnitude (from microscopic to macroscopic systems). In these systems, the migration characteristics of the isolated constituents become less relevant, and the manner in which each constituent influences and/or responds to the motility of its neighbors becomes the more important and defining feature. In fact, Vicsek

et al. showed for the first time that emergence of collective behavior in a system can be described by a simple kinematic model (Vicsek model and its variations) (12, 68) in which the direction of a given particle within the system is determined by the average of all the particles within its neighborhood (FIGURE 1, B AND C). In such a system, the transition to an "ordered" collective behavior phase was observed to be dependent on the density of the particles. Several studies have indeed confirmed that the density of particles constituting a system remains one of the most important physical parameters governing the emergence of collective behavior (55). In many systems, it is observed that there exists a critical density that triggers the emergence of collective behavior. Other important physical parameters governing collective behavior include boundary restrictions (physical constraints), feedback loop systems (that allow constituents to sense and respond to their neighbors), and presence of "leaders." For example, in sedimenting colloids, the correlation length of swirls is dependent on the boundaries of the chamber (56). Furthermore, in locusts, it has been observed that the swarming behavior is lost when they were subjected to abdominal denervation [which prevents them from sensing the presence of a neighbor (6)]. However, it is important to observe that, in contrast to "local spatial coupling" that is characteristic of these systems, multicellular assemblies typically demonstrate physical adhesion between neighbors that provides strong "mechanical coupling" as well as a means for biochemical signaling. Notwithstanding this difference, collective cell migration shows several characteristics that closely mimic other physical and biochemical systems. Indeed, a large body of accumulating evidence suggests that coordination among cell clusters during migration is regulated by various chemical (7, 37) and physical cues (60) within the cellular and extracellular environment. In fact, the multiscale tuning model for cell migration proposed by Friedl et al. (24) and recently further expanded by Ashby et al. (5) suggests how a complex interplay between cell-autonomous migration behavior, external chemokine gradients, cohesive forces regulating cell-cell interaction, and adhesive mechano-chemical features of the extracellular matrix determine the final migratory behavior of single as well as clusters of cells.

This review aims to give a broad overview of our current understanding of collective cell behavior,

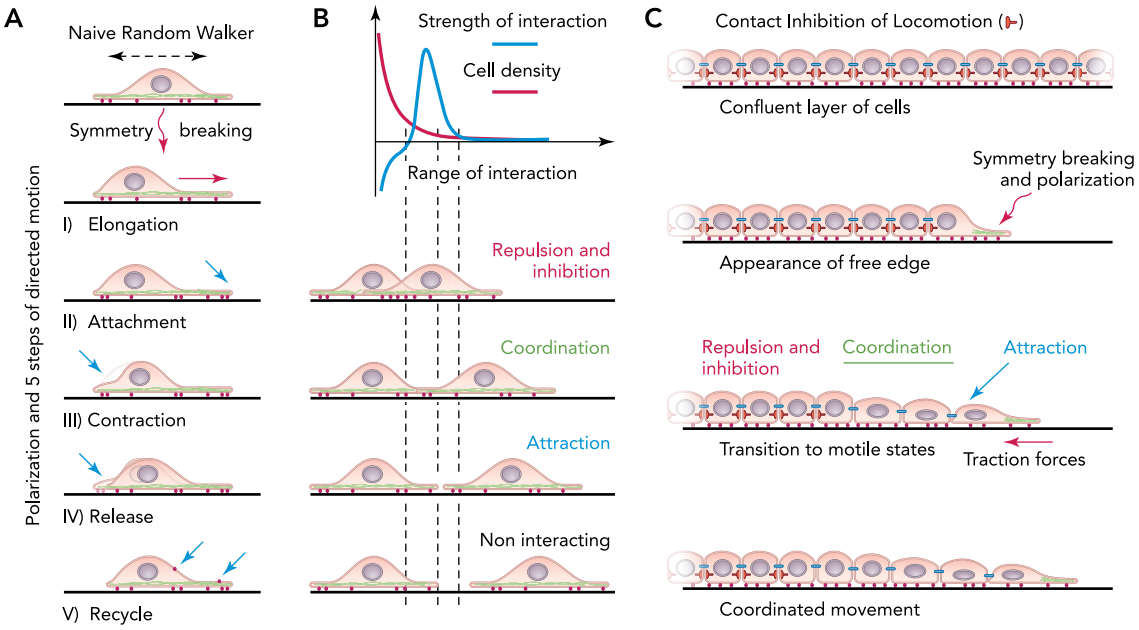

FIGURE 1. **Overview of migration in single cells and a monolayer of cells**
A: cartoon illustrating the mechanism of single cell migration. Symmetry-breaking events lead to cell polarization and directed migration. Cell polarization in this review refers to the asymmetric distribution of the migratory machinery. I to V show the steps required during migration of single cell. I, actin-rich protrusions (lamellipodia) elongate the leading edge of the cell; II, adhesion proteins attach the newly formed protrusion to the substratum; III, acto-myosin contraction leads to the retraction of the rear of the cell and its progression to the front; IV, adhesive proteins release from the substratum and allow progression of movement; V, migratory machinery is recycled to get ready for *step I*. *B*: Vicsek model is adopted to illustrate the strength of interaction between cells as a function of density and the distance separating them. At high densities and small separation distances, cells repel and inhibit each other. Critical density and optimal separation distance induce coordinated migration. At very low densities and long separation distances, they do not sense their neighbors. *C*: cells in a highly dense monolayer are subject to contact inhibition of locomotion (CIL). All cells are symmetrically inhibited, resulting in little migration. Availability of free space to migrate breaks the symmetry and polarizes the cells at the edge toward the free space. This overcomes the inhibitory signals and promotes cell motility. Motile cells at the front exert attractive forces on their neighbors, which, in turn, coordinate their movement.

with a specific focus on physical and mechanical cues and the use of microfabricated technologies, to understand how various micromechanical cues within the cellular microenvironment regulate collective cell behavior.

Methods to Control and Quantify Collective Cell Behavior In Vitro

Methods to probe collective cell migration in vitro can be roughly categorized by the relative directionality of the migration (arrow heads, FIGURE 2*A*). Inward migration closing the gap is referred to as "void," and it mimics the closure of a wound. Conversely, outward migration of a sheet of cells expanding and invading its surroundings is defined as "nest" (5). An alternative categorization (presented below) considers the way symmetry breaking is set up. Removal of cells from a confluent sheet is referred to as "cell depletion assays," and other methods where confined cells are exposed to a free space by removal of a barrier are called "cell exclusion assays" (5).

Cell Depletion Assays

The scratch wound assay. Cells are scratched away from the monolayer using a needle (FIGURE 2*B*). Alternatively, stencils of PDMS are pressed against the cells to cause their death. Scratch wound assay has been and still remains one of the most common in vitro experiments to study collective cell migration (76). Notwithstanding the simplicity of the technique, the method has inherent disadvantages, such as inability to precisely control the size and shape of the wound, damage to cells at the leading front, and disruption of ECM on the substrate.

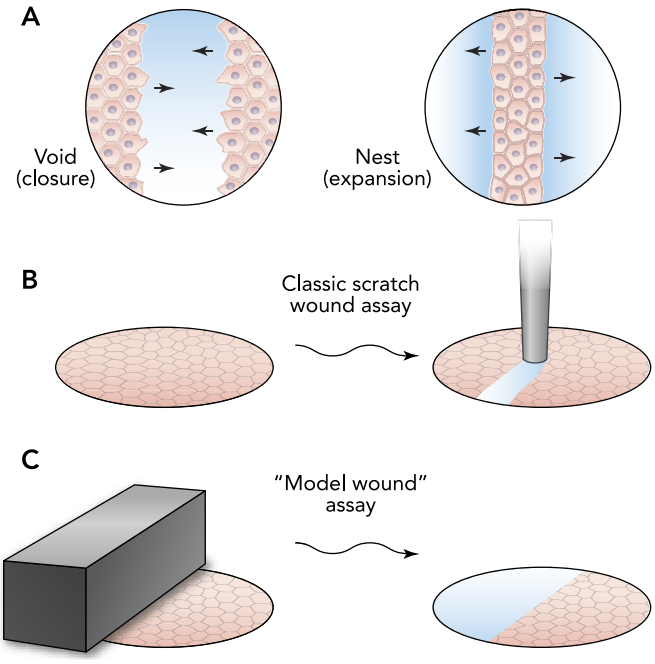

FIGURE 2. **Schematic depicting different assays to study collective cell behavior**
A: "void" and "nest" types of migration assays. *B*: classical scratch wound assay. *C*: model wound assay.

Laser ablation. Lasers are used to ablate regions of defined size and shape within an epithelial cell sheet. This method elegantly overcomes most of the disadvantages of the wounding done with a needle and allows full control of the shape and size of the wound while leaving the substrate undamaged. However, it requires specialized and expensive equipment (77).

Cell Exclusion Assays

Membrane patterning. First introduced by Whitesides et al, the method uses spin coating to fabricate free-standing elastomeric PDMS membranes with circular or square shapes through "holes" to subject cells to well defined physical constraints (49). The membrane is subsequently lifted off to allow cells to spread and migrate collectively from well defined initial geometries.

Micro-stencils. Similar in concept to the membrane patterning method described above, this method uses an alternate method to fabricate free-standing PDMS membranes with rectangular through holes (stencils) (51). Cells are initially confined to these rectangular wells and allowed to reach confluence. Stencils are subsequently peeled off, allowing cell monolayers to migrate collectively into the available free space.

Masking microcontact printed patterns. This method uses microfabricated substrate to first print a defined protein pattern of interest. Subsequently, a PDMS barrier is placed on to mask a portion of the micropattern (67) (FIGURE 2*C*).

Micropillars. Micropillars can be used to create gaps within epithelia that close when the pillars are removed (4). This method allows us to vary the size and shape of the wound with precision and in a high-throughput manner.

Three-dimensional microwells. Microfabricated substrates have also been employed to prepare microwells within soft collagen gels (46). Cells are initially restricted to these wells but subsequently invade the surrounding matrix, providing a model system to study collective cell behavior in 3D.

Measuring Cell-Substrate Traction Forces

Measuring traction forces exerted by cells on the substrate provides rich insight into the physical mechanisms regulating cell migration. In the context of collective cell migration, such information enables us to not only understand the contribution of cells at the leading edge, vis-a-vis cells farther away within the monolayer, but also allows us to characterize the role of cell-cell interactions in

governing the overall migratory behavior of the collective. Two of the most commonly used techniques to quantify substrate traction forces are micro-force sensor arrays and traction force microscopy.

Micro-force sensor arrays. In this technique, cells are allowed to attach on top of soft elastic micropillar arrays made up of PDMS (18, 61). The bending of micropillars in response to forces exerted by the cells can be obtained using particle-tracking algorithms. Since the elastic modulus of PDMS is known, the spring constant of the micropillar can be estimated if its height and diameter are known. For small deflections, the force acting on the micropillar is the product of the deflection and its spring constant (18).

Traction force microscopy. In this method, cells are grown on soft elastic substrates (most commonly polyacrylamide gels) with embedded fluorescent beads (9, 14). Forces exerted by the cells cause the beads to be displaced. The displacement of the beads is used to compute the stress field.

Emergence of Collective Cell Behavior and Its Regulation

As mentioned before, single cells in isolation tend to migrate randomly with little persistence in the absence of any external cues. Even epithelial cells, which typically show a strong directed migration as a monolayer during wound healing, migrate randomly when they are single (54). However, such a transition to an ordered migration is highly dependent on the density of cells (54). Furthermore, change in cell density has also been shown to alter the correlation length (2) as well as material properties of epithelial monolayers (3) probably due to maturation of intercellular contacts. Although this is not surprising considering that density of the constituents of a system appears to be a critical regulator of the emergence of collective behavior across various inert and living systems, it is certainly interesting to understand how cells sense "density" and alter their migration characteristics accordingly.

One of the earliest mechanisms that has been proposed to explain such a social behavior of cell clusters is contact inhibition of locomotion (CIL) (1). CIL was originally reported by Abercrombie et al. in fibroblasts from chicken heart where they observed that cells change their direction of migration when they come into contact with other cells. Such a change in direction of migration was associated with inhibition of protrusions in the direction of the contact and development of new protrusions away from the site of contact. This suggests that CIL is not only a "migration inhibitory" mechanism but also a mechanism that determines as well as alters cell polarization. Taken together, this leads to the important conclusion that cells at the leading edge of a wound are "different" from cells within the cluster, and this allows a monolayer of cells to migrate directionally toward the free space for efficient wound closure or tissue expansion. For a long time, CIL has been only a phenomenological observation, and little was known regarding the molecular mechanisms governing CIL. CIL can be broadly considered as a feedback loop system where cells first need to sense density, i.e., the presence of other cells. Subsequently, they need to convert this sensory signal into a response that involves alteration of the direction of migration and polarization of the cells. Intercellular adhesion proteins and the corresponding signaling molecules have long remained the key suspects in regulating CIL. Although cadherins have been the favorite cell surface receptors implicated in CIL, other potential candidates that have been proposed include atypical cadherins, nectins, ephrins, and notch-delta receptors (41, 64). Indeed, mechanical force applied to cadherins has been shown to be sufficient for polarizing a cell (74). Activation of Rho-GTPases through intercellular adhesion molecules has also been suggested as a possible mechanism that governs the "response" arm of the CIL feedback loop. Recently, working with neural crest cells, Fontaine and Mayor have shown that CIL occurs in vivo and is regulated by the noncanonical Wnt signaling pathway (10). However, simulations in silico suggest that large-scale coordination within multicellular clusters can indeed be largely explained from a mechanical perspective with minimal knowledge of the biochemical signaling across the adhesion (31). Further experiments are necessary to delineate the contribution of individual signaling systems to the overall collective behavior as well as to understand how the mechanical cues and biochemical cues converge to allow for a consensus decision to be made.

Additionally, it has been observed that some cells could behave as "leader" cells (38), being able to drag "followers" in the appropriate direction. Interestingly, leader cells have indeed been identified and proposed to play an important role in directing the overall migration behavior of cell clusters. For example, during wound healing of simple epithelial monolayers, it has been observed that some cells at the leading edge of the wound spread much more than others and attained a mesenchymal phenotype (FIGURE 3*A*) (51). However, availability of free space to migrate does not immediately induce formation of leader cells. They are typically observed ~1 h after the monolayer edge is allowed to migrate. Furthermore, leader cells do not necessarily evolve from the first row of cells at the edge of the monolayer. Rather, they can

even originate far away from the edge and be brought to the front by flows within the epithelium (51). Indeed, cells far away from the edge of a monolayer have been shown to extend cryptic lamellipodia underneath their neighbors (21), suggesting that leader cells are not necessarily restricted to the edge of a monolayer (FIGURE 3*B*). These leader cells drag along a small cluster of follower cells, resulting in the formation of "finger-like" instabilities of the leading front (51). Staining reveals thick actin bundles (51) as well as stretched E-cadherin morphology (48) connecting the leaders to the followers, suggesting a strong mechanical coupling and force transmission within these finger-like structures. Velocity field analysis and mechanical perturbation of leader cells using laser ablation strongly support the role of leader cells in providing local guidance cues to cells following them (53). It was further observed that inhibition or expression of a dominant negative form of Rho

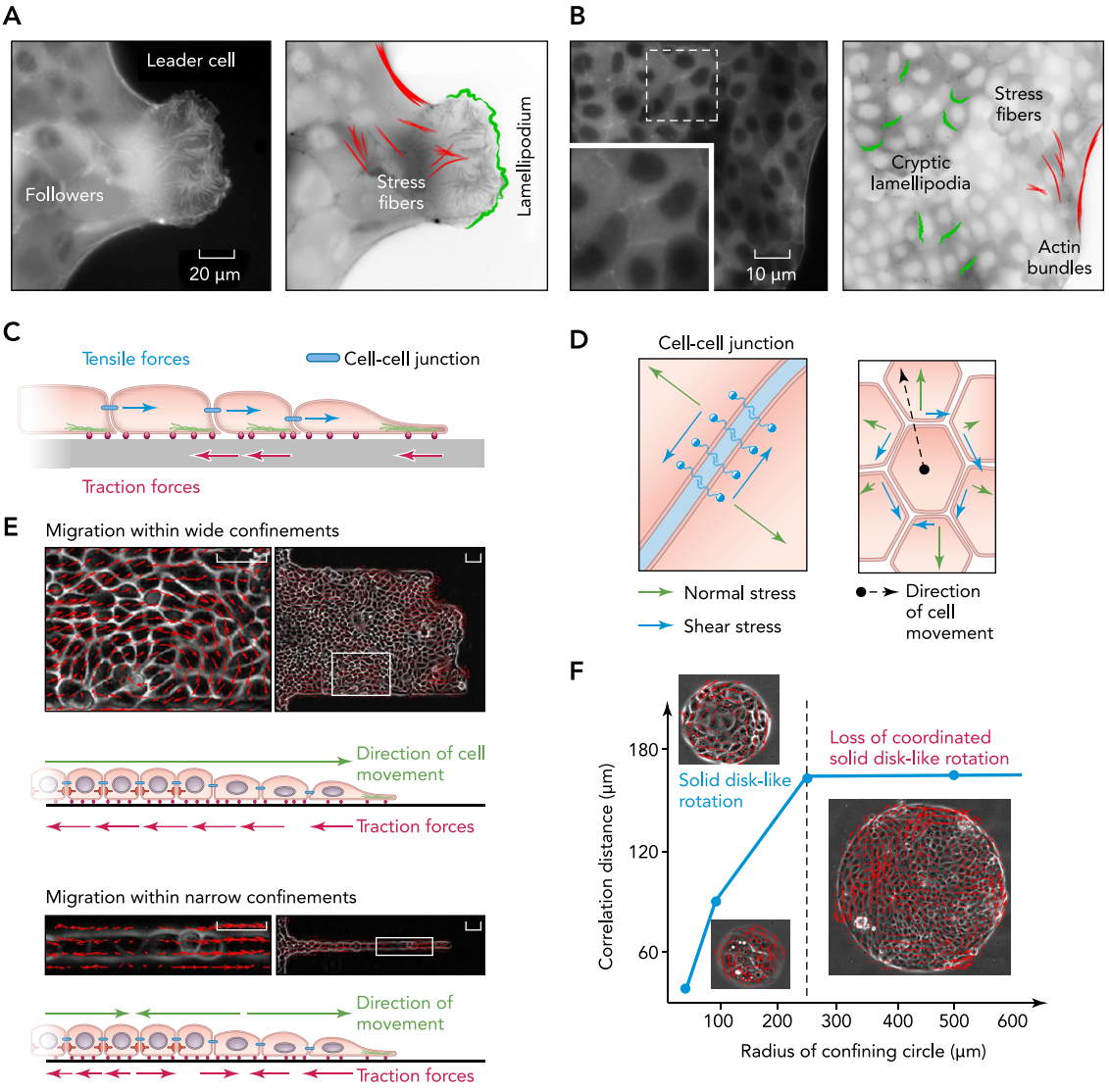

FIGURE 3. **Mechanisms of collective cell migration from a mechanistic perspective**
A: positive (*left*) and negative (*right*) fluorescence image of a migrating monolayer of MDCK cells stably expressing actin-GFP showing finger-like instabilities capped by cells with mesenchymal morphology characteristic of leader cells. *Right* image is superimposed with "cartoonized" structures (red and green) to illustrate the distribution of actin bundles and lamellipodia that is typical of leaders and followers in finger-like protrusions. *B*: cryptic lamellipodia extended by cells far away from the leading edge. *Inset*: magnified view of the region bounded by the dotted box showing cryptic lamellipodia. *Right* image is superimposed with pseudo-colored structures (red and green) to illustrate the distribution of actin bundles and cryptic lamellipodia. *C*: cartoon illustrating the mechanical interaction of cells with the substrate through focal adhesions and with their neighbors through cell-cell junctions. The former results in traction forces, and the latter transmits the tension across the sheet of cells. *D*: cell-cell junctions are subjected to shear and normal stresses during migration as a result of mechanical coupling. Cells tend to migrate along the direction of maximal normal stress and minimal shear stress. *E*: particle image velocimetry (PIV) tracking of MDCK cells migrating within wide channels (~400 μm) showing vortex formation (*top*) and within narrow channels (~20 μm) showing a contraction relaxation mode of migration (*bottom*). Cartoons showing the distribution of traction forces under both conditions are depicted below the respective images (*F*) plot of the correlation distance (of velocities) of MDCK cell clusters as a function of the radius of the confining circular pattern.

A increased the number of leader cells at the leading edge, whereas expression of a constitutively active form of Rho A inhibited the formation of leader cells. On the other hand, activation of Rac in leading cells has been shown to be necessary and sufficient to direct collective migration in border cells of *Drosophila* (73). Recent experiments suggest that such a restricted spatial localization of Rac to the leader cell within a cell cluster in turn is regulated by the small GTPase Rab11 and the actin cytoskeletal regulator moesin (52). The role of leader cells in sensing environmental chemokines and directing collective cell migration in developmental processes in vivo has been demonstrated elegantly by Hass and Gilmour (29). Using the lateral line primordium of the zebra fish as a model system, they showed that the presence of specific receptors on cells was necessary for them to become leaders. Mutants with cells deficient for the specific receptors were unable to migrate properly. More importantly, transplantation of even a small number of wild cells expressing specific receptors could efficiently direct the mutant primordial cell migration. The important role that leader cells play in influencing the behavior of cells following them has also been demonstrated in processes such as cancer metastasis (23, 33) and tracheal tube elongation in *Drosophila* (11).

The concept of active leader cells at the edge of a monolayer dragging a passive mass of follower cells suggests that cell-substrate traction forces should be localized to the leading edge with little or no tractions away from the edge. In vitro experiments using traction force microscopy suggest that, although large traction forces are indeed localized to a few rows of cells at the leading front, cells farther away from the edge also exert significant (although lesser) traction forces (65). There appears to be a continuous buildup of stresses within the monolayer as we move away from the leading edge into the cell sheet that cannot be explained if forces are exerted by leader cells only (FIGURE 3*C*). Rather, the actual mechanism appears to be long-range transmission of forces across intercellular adhesions resulting in a global state of tension or "tug of war" between cells at the leading front and cells at the back. Indeed, recent experiments suggest that the intercellular adhesion sites within a migrating monolayer are subjected to significant stresses (both normal and shear) that are extremely heterogeneous in distribution (FIGURE 3*D*). That cells prefer to migrate along the direction of maximum principal and minimum shear stress (plithotaxis) (60) suggests that an integration of multiple mechanisms governs collective cell migration. The debate on the role of leaders vs. followers and their contribution to collective cell migration is currently inconclusive. However, such a discrepancy could result from the different types of model systems used, and further experiments that combine genetic manipulations with mechanistic interventions across a variety of cell and tissue types could provide a better insight.

Substrate Architecture, Dimensionality, and Rheology

Although it appears that an integration of several mechanisms (e.g., leader cells, large-scale force transmission across intercellular adhesions, and plithotaxis) regulate the overall collective behavior of cellular monolayers, it is necessary to emphasize that such behavior can be further modulated by external cues imposed by the ECM or chemokine gradients. In particular, it is well recognized that micromechanical cues within the extracellular matrix such as topography, physical constraints, porosity, and elasticity have as strong regulatory influence on various cellular processes in general and cell migration in particular (47). Accordingly, several studies have tried to dissect the individual contribution of such cues to cell migration. Although earlier studies have predominantly focused on the response of single cells, there is increasing interest in how such cues within the microenvironment direct and regulate collective cell behavior. As described in a previous section, the use of soft lithography and microfabrication-based approaches such as microcontact printing, removable microstencils, and 3D microwells have provided versatile tools to precisely manipulate the cellular environment on the micrometer scale.

It has been observed that single cells migrate faster on highly confined geometries (e.g., narrow straight lines) in a myosin II-dependent fashion (17). Such a migration in 1D appears to relate closely with the migration of cells on 3D matrices. Recent studies also suggest that confined geometries alter the migration mode of collectively migrating epithelial monolayers (67). Particle image velocimetry analysis reveals that collectively migrating MDCK cell sheets show large scale "vortices" that are typically 100–150 μm in diameter and represent the natural correlation length of these epithelial monolayers (67) (FIGURE 3*E*, TOP). Such large-scale vortices disappear when cells are forced to migrate in highly confined geometries that are smaller than this natural correlation length (67). Instead, cells switch to a contraction-relaxation or caterpillar-like migration on such confined geometries (FIGURE 3*E*, BOTTOM). Interestingly, at high cell densities, transition to a more directed migratory pattern has also been observed in 3T3 fibroblasts that are known to form only transient cell-cell contacts (39). Furthermore, it has been observed that confining endothelial cells to closed

circular geometries allows them to break symmetry and migrate in a synchronized circular fashion (30). Such a coordinated rotation has also been described recently in breast epithelial cells grown in 3D collagen gels and termed as coherent angular motion (CAM) (62). It has been suggested that CAM is necessary for cells to organize into higher-order tissue architecture (such as acini and ducts in the case of breast epithelial cells) and for determining left-right axis asymmetry (72). Furthermore, such a synchronized migratory behavior is lost, disrupted, or altered in the presence of antibodies against E-cadherins, in cancerous cells, and in cells that have undergone epithelial to mesenchymal transition (62), underlining the essential role of cell-cell junction in maintaining the collective behavior.

Recent experimental studies and simulations suggest that such a synchronized rotation can be recaptured in 2D by confining MDCK epithelial cell clusters to circular micropatterns (16). The onset of a solid "disc-like" rotation of the clusters appears to be dependent on three key factors: "crowding" or cell density, "confinement" or size of the micropattern, and "cohesiveness" or intercellular adhesion. Cell clusters undergo a synchronized rotation only when they reach a critical density. At sub-confluence (low density), cell clusters occasionally show collective rotation, which is, however, not persistent and disappears quickly when cells expand to fill up the free space within the micropattern. There also appears to be a critical diameter of the circular micropatterns (~200 μm) that can induce such a collective rotation behavior. When confined to patterns that are larger than this critical length scale (e.g., 500 μm), cell clusters do not show a solid disc-like rotation but rather display locally ordered "streams" or transient vortices of ~300 μm in diameter (FIGURE 3*F*). Interestingly, this critical length scale correlates well with the reported natural correlation length of MDCK monolayers. Furthermore, MDCK cells that stably overexpress the transcription factor Snail-1 (resulting in epithelial to mesenchymal transition as well as repressed E-cadherin expression) lose their ability to persistently undergo such a collective rotation behavior. Indeed, highly malignant breast cancer cell lines (e.g., MCF-7 and MDA-MB-231) have completely lost their ability to demonstrate any collective migration patterns. Such a behavior of cancer cells is highly reminiscent of the previously mentioned behavior of locusts that lose their ability to swarm after abdominal denervation (6). This suggests that normal intercellular adhesion is necessary for cells to not only sense and detect their neighbors but also tune their migratory behavior in response to them. However, it is still unclear whether (under these conditions) there exist specialized leader cells at the edge of the micropattern that trigger such rotation. Future studies using such model experiments should also allow us to dissect the relative contributions of mechanical and biochemical processes.

Conclusions and Future Perspectives

Understanding collective cell behavior has recently gained renewed interest primarily due to several technical advances. First, the advent of better

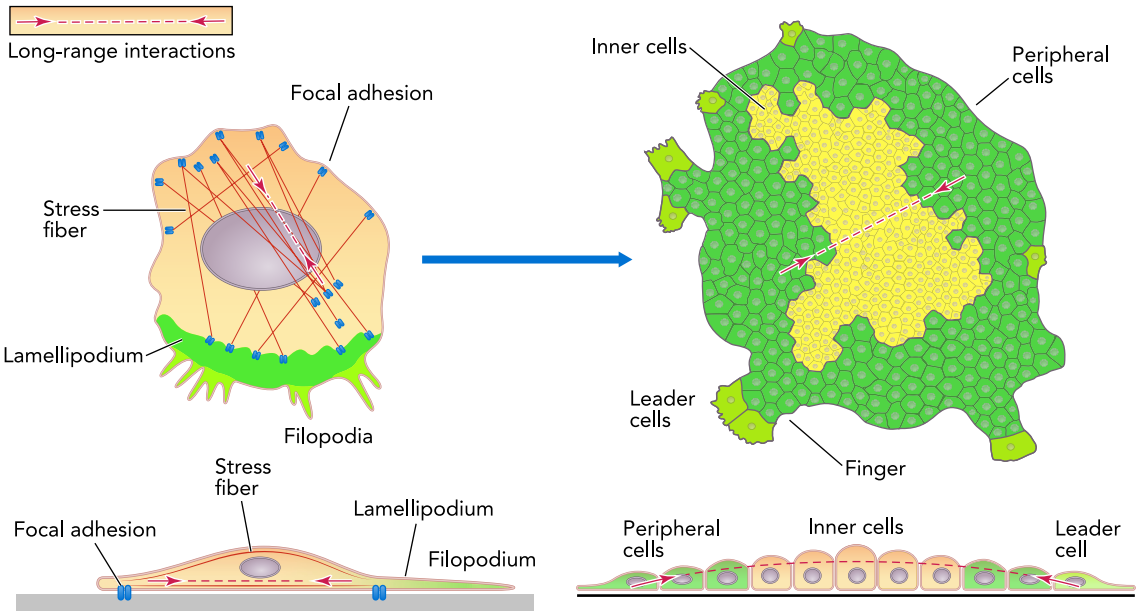

FIGURE 4. **Cartoon suggesting the analogies between a single cell and a large cluster of cells**
A large cell cluster can be considered as a "scaled up" version of a single cell.

microscopy techniques has provided a significant breakthrough in our ability to visualize collective cell migration in vivo with minimal phototoxicity as well as high resolution. Second, the use of various microfabrication approaches has provided us with several in vitro tools to study collective cell migration under well defined initial conditions and with high spatial control that allow for high throughput studies as well as reproducibility across experiments. Third, the ability to quantify the spatio-temporal distribution of forces exerted at the cell-substrate and cell-cell interface within migrating monolayers has provided new mechanistic insights into the regulation of collective cell behavior. Finally, the churning out of vast amounts of data from these novel technologies has prompted theoretical and experimental physicists to draw qualitative and quantitative analogies with other physical systems and to mine this data for unifying principles governing collective behavior.

In many situations, as we previously described, common features can be attributed to collective cell migration such as the formation of leader cells (33), a gradient of cell density within the monolayer from the edge to the center (27), and the emergence of large-scale mechanisms including multicellular movements (51, 67) or cooperative stress (65). First, these findings point to the existence of integrative modes of guidance for collective migration, but the link with molecular processes has remained obscure. Then it is tempting to make an analogy of large cell clusters and single cells as if these large clusters would behave as a scaled up version of individual cells (15, 42). Within the framework of such a comparison, several remarkable analogies can be drawn between single cells and large cell clusters (FIGURE 4). For example, just as lamellipodia on the periphery of a cell are much more spread out and exert large traction forces, cells at the periphery of a large cluster (or equivalently edge of a monolayer) are more spread than those in the center, form finger-like protrusive structures, and exert larger traction forces. Just as filopodia form spontaneously and randomly at the periphery of a cell and probe the local microenvironment, leader cells also form spontaneously and randomly at the periphery of a large cell cluster to sample the microenvironment and act as "local guides" for cells behind them. Furthermore, experimental (45, 55) and theoretical (71) analyses suggest that subcellular active materials, such as actin gels and microtubules (in the presence of motors such as myosin and kinesin), display spiral or vortex-like flow behavior at critical concentrations. These flow patterns at the level of a single cell are highly reminiscent of the large scale vortices or recirculation patterns observed in migrating cellular monolayers (50, 51, 67) and probably result from coupling of the subcellular dynamic events over several cells through the intercellular adhesion. On highly confined geometries, such large-scale vortices are lost, and cell clusters show a "caterpillar-like" contraction-relaxation pattern of migration. A corresponding analogy can be drawn to subcellular actomyosin force dipoles (sarcomere-like units) that integrate their contractility over larger length and longer time scales to provide a large-scale mechanosensing mechanism (13, 26, 66) to sense and respond to micromechanical cues such as substrate stiffness.

To make sure that the "woods" are not missed for the "trees," it is imperative that cell migration is investigated at different scales (e.g., subcellular, cellular, and multicellular levels). Concurrently, drawing analogies with other systems can provide insight into large-scale mechanisms governing collective cell behavior. Such an approach promises a much more comprehensive understanding of different biological processes mediated by cell migration. ■

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References

1. Abercrombie M, Heaysman JE. Observations on the social behaviour of cells in tissue culture. I. Speed of movement of chick heart fibroblasts in relation to their mutual contacts. *Exp Cell Res* 5: 111–131, 1953.

2. Angelini TE, Hannezo E, Trepat X, Fredberg JJ, Weitz DA. Cell migration driven by cooperative substrate deformation patterns. *Phys Rev letters* 104: 168104, 2010.

3. Angelini TE, Hannezo E, Trepat X, Marquez M, Fredberg JJ, Weitz DA. Glass-like dynamics of collective cell migration. *Proc Natl Acad Sci USA* 108: 4714–4719, 2011.

4. Anon E, Serra-Picamal X, Hersen P, Gauthier NC, Sheetz MP, Trepat X, Ladoux B. Cell crawling mediates collective cell migration to close undamaged epithelial gaps. *Proc Natl Acad Sci USA* 109: 10891–10896, 2012.

5. Ashby WJ, Zijlstra A. Established and novel methods of interrogating two-dimensional cell migration. *Integr Biol (Camb)* 4: 1338–1350, 2012.

6. Bazazi S, Buhl J, Hale JJ, Anstey ML, Sword GA, Simpson SJ, Couzin ID. Collective motion and cannibalism in locust migratory bands. *Curr Biol* 18: 735–739, 2008.

7. Bianco A, Poukkula M, Cliffe A, Mathieu J, Luque CM, Fulga TA, Rorth P. Two distinct modes of guidance signalling during collective migration of border cells. *Nature* 448: 362–365, 2007.

8. Blair DL, Neicu T, Kudrolli A. Vortices in vibrated granular rods. *Phys Rev E Stat Nonlin Soft Matter Phys* 67: 031303, 2003.

9. Butler JP, Tolic-Norrelykke IM, Fabry B, Fredberg JJ. Traction fields, moments, and strain energy that cells exert on their surroundings. *Am J Physiol Cell Physiol* 282: C595–C605, 2002.

10. Carmona-Fontaine C, Matthews HK, Kuriyama S, Moreno M, Dunn GA, Parsons M, Stern CD, Mayor R. Contact inhibition of locomotion in vivo controls neural crest directional migration. *Nature* 456: 957–961, 2008.

11. Caussinus E, Colombelli J, Affolter M. Tip-cell migration controls stalk-cell intercalation during *Drosophila* tracheal tube elongation. *Curr Biol* 18: 1727–1734, 2008.

12. Chaté H, Ginelli F, Grégoire G, Peruani F, Raynaud F. Modeling collective motion: variations on the Vicsek model. *Eur Phys J B* 64: 451–456, 2008.

13. De R, Zemel A, Safran SA. Dynamics of cell orientation. *Nat Phys* 3: 655–659, 2007.

14. Dembo M, Wang YL. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophys J* 76: 2307–2316, 1999.

15. Douezan S, Guevorkian K, Naouar R, Dufour S, Cuvelier D, Brochard-Wyart F. Spreading dynamics and wetting transition of cellular aggregates. *Proc Natl Acad Sci USA* 108: 7315–7320, 2011.

16. Doxzen K, Vedula SR, Leong MC, Hirata H, Gov NS, Kabla AJ, Ladoux B, Lim CT. Guidance of collective cell migration by substrate geometry. *Integr Biol* 5: 1026–1035, 2013.

17. Doyle AD, Wang FW, Matsumoto K, Yamada KM. One-dimensional topography underlies three-dimensional fibrillar cell migration. *J Cell Biol* 184: 481–490, 2009.

18. du Roure O, Saez A, Buguin A, Austin RH, Chavrier P, Silberzan P, Ladoux B. Force mapping in epithelial cell migration. *Proc Natl Acad Sci USA* 102: 2390–2395, 2005.

19. Duchek P, Rorth P. Guidance of cell migration by EGF receptor signaling during *Drosophila* oogenesis. *Science* 291: 131–133, 2001.

20. Duchek P, Somogyi K, Jekely G, Beccari S, Rorth P. Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor. *Cell* 107: 17–26, 2001.

21. Farooqui R, Fenteany G. Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. *J Cell Sci* 118: 51–63, 2005.

22. Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. *Nature Rev* 10: 445–457, 2009.

23. Friedl P, Noble PB, Walton PA, Laird DW, Chauvin PJ, Tabah RJ, Black M, Zanker KS. Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants in vitro. *Cancer Res* 55: 4557–4560, 1995.

24. Friedl P, Wolf K. Plasticity of cell migration: a multiscale tuning model. *J Cell Biol* 188: 11–19, 2010.

25. Gail MH, Boone CW. The locomotion of mouse fibroblasts in tissue culture. *Biophys J* 10: 980–993, 1970.

26. Ghassemi S, Meacci G, Liu S, Gondarenko AA, Mathur A, Roca-Cusachs P, Sheetz MP, Hone J. Cells test substrate rigidity by local contractions on submicrometer pillars. *Proc Natl Acad Sci USA* 109: 5328–5333, 2012.

27. Gonzalez-Rodriguez D, Guevorkian K, Douezan S, Brochard-Wyart F. Soft matter models of developing tissues and tumors. *Science* 338: 910–917, 2012.

28. Gov NS. Traction forces during collective cell motion. *Hfsp J* 3: 223–227, 2009.

29. Haas P, Gilmour D. Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev Cell* 10: 673–680, 2006.

30. Huang S, Brangwynne CP, Parker KK, Ingber DE. Symmetry-breaking in mammalian cell cohort migration during tissue pattern formation: role of random-walk persistence. *Cell Motil Cytoskeleton* 61: 201–213, 2005.

31. Kabla AJ. Collective cell migration: leadership, invasion and segregation. *J R Soc Interface* 9: 3268–3278, 2012.

32. Keller R. Cell migration during gastrulation. *Curr Opin Cell Biol* 17: 533–541, 2005.

33. Khalil AA, Friedl P. Determinants of leader cells in collective cell migration. *Integr Biol* 2: 568–574, 2010.

34. Kim JH, Dooling LJ, Asthagiri AR. Intercellular mechanotransduction during multicellular morphodynamics. *J Royal Soc Interface* 7: S341–S350, 2010.

35. Ladoux B, Nicolas A. Physically based principles of cell adhesion mechanosensitivity in tissues. *Rep Prog Phys* 75: 116601, 2012.

36. le Digabel J, Ghibaudo M, Trichet L, Richert A, Ladoux B. Microfabricated substrates as a tool to study cell mechanotransduction. *Med Biol Eng Comput* 48: 965–976, 2010.

37. Lecaudey V, Cakan-Akdogan G, Norton WH, Gilmour D. Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development (Cambridge)* 135: 2695–2705, 2008.

38. Lecaudey V, Gilmour D. Organizing moving groups during morphogenesis. *Curr Opin Cell Biol* 18: 102–107, 2006.

39. Leong MC, Vedula SRK, Lim CT, Ladoux B. Geometrical constraints and physical crowding direct collective migration of fibroblasts. *Commun Integr Biol* 6: e23197, 2013.

40. Martin P. Wound healing: aiming for perfect skin regeneration. *Science* 276: 75–81, 1997.

41. Mayor R, Carmona-Fontaine C. Keeping in touch with contact inhibition of locomotion. *Trends Cell Biol* 20: 319–328, 2010.

42. Mertz AF, Banerjee S, Che Y, German GK, Xu Y, Hyland C, Marchetti MC, Horsley V, Dufresne ER. Scaling of traction forces with the size of cohesive cell colonies. *Phys Rev Lett* 108: 198101, 2012.

43. Miller N, Gerlai R. From schooling to shoaling: patterns of collective motion in zebrafish (*Danio rerio*). *PLos One* 7: e48865, 2012.

44. Nagy M, Akos Z, Biro D, Vicsek T. Hierarchical group dynamics in pigeon flocks. *Nature* 464: 890–893, 2010.

45. Nedelec FJ, Surrey T, Maggs AC, Leibler S. Self-organization of microtubules and motors. *Nature* 389: 305–308, 1997.

46. Nelson CM, Inman JL, Bissell MJ. Three-dimensional lithographically defined organotypic tissue arrays for quantitative analysis of morphogenesis and neoplastic progression. *Nat Protoc* 3: 674–678, 2008.

47. Nelson CM, Tien J. Microstructured extracellular matrices in tissue engineering and development. *Curr Opin Biotechnol* 17: 518–523, 2006.

48. Omelchenko T, Vasiliev JM, Gelfand IM, Feder HH, Bonder EM. Rho-dependent formation of epithelial "leader" cells during wound healing. *Proc Natl Acad Sci USA* 100: 10788–10793, 2003.

49. Ostuni E, Kane R, Chen CS, Ingber DE, Whitesides GM. Patterning mammalian cells using elastomeric membranes. *Langmuir* 16: 7811–7819, 2000.

50. Petitjean L, Reffay M, Grasland-Mongrain E, Poujade M, Ladoux B, Buguin A, Silberzan P. Velocity fields in a collectively migrating epithelium. *Biophys J* 98: 1790–1800, 2010.

51. Poujade M, Grasland-Mongrain E, Hertzog A, Jouanneau J, Chavrier P, Ladoux B, Buguin A, Silberzan P. Collective migration of an epithelial monolayer in response to a model wound. *Proc Natl Acad Sci USA* 104: 15988–15993, 2007.

52. Ramel D, Wang X, Laflamme C, Montell DJ, Emery G. Rab11 regulates cell-cell communication during collective cell movements. *Nat Cell Biol* 15: 317–324, 2013.

53. Reffay M, Petitjean L, Coscoy S, Grasland-Mongrain E, Amblard F, Buguin A, Silberzan P. Orientation and polarity in collectively migrating cell structures: statics and dynamics. *Biophys J* 100: 2566–2575, 2011.

54. Rosen P, Misfeldt DS. Cell density determines epithelial migration in culture. *Proc Natl Acad Sci USA* 77: 4760–4763, 1980.

55. Schaller V, Weber C, Semmrich C, Frey E, Bausch AR. Polar patterns of driven filaments. *Nature* 467: 73–77, 2010.

56. Segrè PN, Herbolzheimer E, Chaikin PM. Long-range correlations in sedimentation. *Phys Rev Lett* 79: 2574–2577, 1997.

57. Sheetz MP, Felsenfeld D, Galbraith CG, Choquet D. Cell migration as a five-step cycle. *Biochem Soc Symp* 65: 233–243, 1999.

58. Sheetz MP, Felsenfeld DP, Galbraith CG. Cell migration: regulation of force on extracellular-matrix-integrin complexes. *Trends Cell Biol* 8: 51–54, 1998.

59. Sumino Y, Nagai KH, Shitaka Y, Tanaka D, Yoshikawa K, Chate H, Oiwa K. Large-scale vortex lattice emerging from collectively moving microtubules. *Nature* 483: 448–452, 2012.

60. Tambe DT, Hardin CC, Angelini TE, Rajendran K, Park CY, Serra-Picamal X, Zhou EHH, Zaman MH, Butler JP, Weitz DA, Fredberg JJ, Trepat X. Collective cell guidance by cooperative intercellular forces. *Nat Mat* 10: 469–475, 2011.

61. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proc Natl Acad Sci USA* 100: 1484–1489, 2003.

62. Tanner K, Mori H, Mroue R, Bruni-Cardoso A, Bissell MJ. Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proc Natl Acad Sci USA* 109: 1973–1978, 2012.

63. Thery M. Micropatterning as a tool to decipher cell morphogenesis and functions. *J Cell Sci* 123: 4201–4213, 2010.

64. Theveneau E, Steventon B, Scarpa E, Garcia S, Trepat X, Streit A, Mayor R. Chase-and-run between adjacent cell populations promotes directional collective migration. *Nat Cell Biol* 15: 763–772, 2013.

65. Trepat X, Wasserman MR, Angelini TE, Millet E, Weitz DA, Butler JP, Fredberg JJ. Physical forces during collective cell migration. *Nature Phys* 5: 426–430, 2009.

66. Trichet L, Le Digabel J, Hawkins RJ, Vedula SR, Gupta M, Ribrault C, Hersen P, Voituriez R, Ladoux B. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. *Proc Natl Acad Sci USA* 109: 6933–6938, 2012.

67. Vedula SRK, Leong MC, Lai TL, Hersen P, Kabla AJ, Lim CT, Ladoux B. Emerging modes of collective cell migration induced by geometrical constraints. *Proc Natl Acad Sci USA* 109: 12974–12979, 2012.

68. Vicsek T, Czirok A, Ben-Jacob E, Cohen II, Shochet O. Novel type of phase transition in a system of self-driven particles. *Phys Rev Lett* 75: 1226–1229, 1995.

69. Vicsek T, Zafeiris A. Collective motion. *Phys Rep* 517: 71–140, 2012.

70. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol* 7: 265–275, 2006.

71. Voituriez R, Joanny JF, Prost J. Generic phase diagram of active polar films. *Phys Rev Lett* 96: 028102, 2006.

72. Wan LQ, Ronaldson K, Park M, Taylor G, Zhang Y, Gimble JM, Vunjak-Novakovic G. Micropatterned mammalian cells exhibit phenotype-specific left-right asymmetry. *Proc Natl Acad Sci USA* 108: 12295–12300, 2011.

73. Wang X, He L, Wu YI, Hahn KM, Montell DJ. Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. *Nat Cell Biol* 12: 591–597, 2010.

74. Weber GF, Bjerke MA, DeSimone DW. A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev Cell* 22: 104–115, 2012.

75. Weijer CJ. Collective cell migration in development. *J Cell Sci* 122: 3215–3223, 2009.

76. Yarrow JC, Perlman ZE, Westwood NJ, Mitchison TJ. A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. *BMC Biotechnol* 4: 21, 2004.

77. Zordan MD, Mill CP, Riese DJ 2nd, Leary JF. A high throughput, interactive imaging, bright-field wound healing assay. *Cytometry A* 79: 227–232, 2011.