Review

The front and rear of collective cell migration

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Abstract

Collective cell migration has a key role during morphogenesis, during wound healing and tissue renewal in the adult, and it is involved in cancer spreading. In addition to displaying a coordinated migratory behavior, collectively migrating cells move more efficiently than if they migrated separately, which indicates that cellular interplay occurs during collective cell migration. Over the last years, evidence has accumulated confirming the importance of such intercellular communication and exploring the molecular mechanisms involved. These mechanisms are based both on direct physical interactions which coordinate the cellular responses and on the cell collective behavior that generates an environment optimal for efficient directed migration. These studies have described how leader cells at the front of cell groups drive migration and have highlighted the importance of following cells, which communicate between them and with the leaders to improve the efficiency of collective movement.

The development of multicellular organisms involves morphogenetic movements where large groups of cells migrate in a coordinated manner to contribute to the formation of tissues and organs (Box 1)[1]. Collective migration also occurs in the adult during wound healing, tissue renewal and angiogenesis and has been involved in tumor spreading [2] (Box 1). Elucidation of the molecular mechanisms underlying collective migration is thus fundamental not only for our understanding of morphogenetic processes but also for the identification of new therapeutic targets to prevent tumor spreading and metastasis.

During collective migration, multiple cells migrate in the same direction at a similar speed. Moreover, these cells coordinate their response to the environment, ensuring that cells that would otherwise be immobile or migrate in a different direction also follow the global movement. Thus, the major feature of a collectively migrating group of cells is that it migrates more efficiently than if cells were isolated. Although single cells have a higher instant velocity, they undergo a less persistent migration, quickly and frequently changing direction. Such collective behavior involves a physical or chemical crosstalk between individual migrating cells. In the case of cohesive groups such as the fish lateral line primordium or epithelia cell sheets (Box1), direct cell-cell contacts not only maintain the group physical integrity, they also contribute to the coordination of the motile behavior of adjacent cells. However, cranial neural crest cells and neurons in the rostral migratory stream display only transient contacts during migration (Box 1). Nevertheless collective behavior has also been observed in these loosely associated streams of cells, indicating that communication either through diffusible factors or by the local alteration of the extracellular matrix (ECM) can also promote cell coordination.

The molecular mechanisms that control single cell polarization and migration have been extensively studied and the basic mechanisms of single cell migration can be also applied to collective movement (Figure 1a,b). Single cell migration is based on the establishment of a front-to-rear polarity axis, including polarized cytoskeletal rearrangements and the polarized organization of membrane trafficking (Figure 1a). Underlying this front-to-rear functional polarization is a front-to-rear polarization of signaling cascades involving, in particular, the small GTPase proteins of the Rho family. At the front, Rac and Cdc42 induce cytoskeletal rearrangements, including rapid actin polymerization, leading to the formation of membrane protrusions such as filopodia and lamellipodia, and simultaneously promote integrin engagement

with the extracellular matrix[3] (Figure 1a). At the rear, a distinct signaling pathway involving Rho promotes acto-myosin contraction.

The same mechanisms are at play in each individual cell during collective migration of loose cell streams. However, in cohesive cell groups, cellular contacts modify the distribution of the classical features found in isolated migrating cells. The cells located at the front of the group are called leader cells (Box2). These cells sense the microenvironment and dictate the direction and speed of migration of the entire cell cluster (Figure 1c). The definition of leader and follower cells is based only on their relative position within a cell cluster, with leader and follower cells located at the front and back of the cluster, respectively. Because of their position, leader cells are exposed to higher levels of external signals such as chemoattractants and play a major role in extracellular matrix remodeling during migration. Behind leader cells, cell-cell contacts impair the formation of a classical leading edge implying that the mechanisms actually driving the migration of so-called followers must be different from that of the leader cells (Figure 1c). These followers must therefore rely on strong cellular interactions to collectively polarize. In a migrating single cell, signaling at the rear can modulate the speed and the direction of migration [4-6].

Similarly in groups of cells, follower cells can also influence the behavior of the leaders to modulate the collective movement.

In this review, we discuss our current understanding of the mechanisms underlying the collective behavior of migrating cells. We describe the fundamental role of the leader cells in sensing the microenvironment and dictating the direction and speed of movement to the cell group. We then discuss the often-overlooked role of followers, highlighting how interactions between followers and also between followers and leader cells affect individual cell behavior to maintain group integrity and to promote efficient directed collective migration.

Leaders sense the microenvironment

Although leader cells are generally localized at the front of the migrating group, cells that are not located at the periphery of the cell group can relocate to the leading front to become leaders[7].

Cells become leaders in response to external cues, which include the extracellular matrix, soluble factors and neighboring cells (Figure 1c).

Interaction of leaders with the extracellular matrix

The interaction between cells and the extracellular matrix occurs mainly through integrins, which transduce both mechanical and chemical signals. Integrin-mediated signaling responds to the composition and the stiffness of the extracellular matrix[8, 9]. Moreover, extracellular matrix fibers control the migration of multicellular streams *in vivo* by providing directional cues[10, 11]. In vitro wound healing assays have revealed that new interactions with the extracellular matrix are induced at the wound edge of the cells. These interactions trigger integrin-mediated signaling, which leads to cytoskeletal rearrangements, structural reorganization and morphological polarization, typical of leader cells [12] (Figure 2).

Depending on cell types and cell substrates, several integrin dimers have been involved in collective migration. In particular, β1 integrins are used by endothelial cells, astrocytes and epithelial cells[13-15]. Engagement of integrins with the extracellular matrix leads to the recruitment and activation of Cdc42 and/or Rac, through adapter proteins associated with GEFs (Guanosine Exchange Factors), such as Scrib and βPIX or Par3 and TIAM-1[16-19] and intracellular kinases such as FAK and Src[20, 21] (Figure 2). Activation of these small GTPases promotes the extension of membrane protrusion a the cell front. Their downstream effectors, such as the Scar/Wave complex, induces the polymerization of actin filaments in the vicinity of the leading edge plasma membrane, creating pushing forces required for membrane protrusion[22]. Small GTPases also promotes the polarization of the microtubule network and the associated vesicular traffic[23], thereby providing the cell front with additional membrane and membrane receptors (Figure 2). The polarized intracellular organization of the leader cells promotes positive feedback loops and contributes to the stabilization of the polarized cell state[23].

Stimulation by soluble chemotactic factors

In vivo, collective migration is frequently promoted by soluble factors such as chemokines or growth factors (Figure 1c). For example, collective migration of endothelial cells

is essentially driven by VEGFs (Vascular Endothelial Growth Factors) but can also be supported by bFGF and other cytokines as well as nitric oxide (NO)[24], which initiates the directional migration of tip cells and blood vessel formation (for reviews[13, 25]). Leader cells are pivotal in sensing environmental soluble factors to promote the chemotaxis of the entire migrating cell group. In the fish lateral line primordium (Box 1), the expression of the receptor Cxcr4b, which interacts with the Sdf1 chemokine, in the leader cells is sufficient to drive collective chemotaxis[26].

Soluble factors promote the collective behavior in two different ways. First, signaling through growth factor receptors or chemokines, like signaling through integrins, induces cell polarization and protrusions. In the lateral line primordium, Sdf1 binding to Cxcr4b promotes actin-driven membrane protrusion via the heterotrimeric G protein subunit Gβ1[27]. Most soluble factors activate Cdc42 and Rac via phosphoinositide-mediated signaling[28, 29] to eventually promote actin polymerization. Like in single chemotactic cells signaling through growth factors or chemokine receptors frequently acts via the polarized recruitment and activation of a PI3K and Rac positive feedback loop leading to actin rearrangements and membrane protrusion (Figure 2b). Moreover, there is an important crosstalk between tyrosine kinase receptor or G-protein coupled receptors and integrin signaling[30, 31]. VEGF and bFGF impact on integrin signaling by regulating integrin expression or FAK phosphorylation[32-34]. This interplay between integrin and chemotactic receptor signaling is highlighted by the fact that collective chemotaxis requires cell adhesion to the extracellular matrix. In *Dictyostelium discoideum* migrating towards cAMP, inhibition of cell-substrate interactions using polyethylene-glycol coated surfaces prevents cell streaming[35]. In this case, the authors had shown that loss of adhesion to the substrate does not directly affect the cytoskeletal dynamics required for cell protrusion and migration but perturbs cell-cell interactions. Conversely, integrin signaling generally potentiates growth factor receptor activity[36, 37]. Expression of the integrin α6β4 in pancreatic cancer cells increases cancer spreading and metastasis by promoting HGF-induced activation of Rac1[38].

Second, chemotactic factors induce intracellular signaling that ultimately controls gene expression and defines the characteristics of leader cells. *D. melanogaster* border cells polarize in response to PVF (PDGF-VEGF-related factor) and EGF (Epithelial growth factor)[39]. The cell of the group that is the most responsive to these growth factors becomes the leader. FGF stimulation

of tracheal cells in *D. melanogaster* leads to the activation of MAPK followed by upregulation of its own receptor reinforcing the leader phenotype[40, 41]. Increasing levels of FGFR signaling also upregulate Delta1 in the leader cells, which interacts with Notch situated on the membrane of followers and inhibits the FGFR-MAPK signaling cascade in these cells[42, 43]. A similar signaling is also at play during vascular sprouting[44, 45] and tumor angiogenesis[46, 47],in vertebrates ensuring the stability of leader cell characteristics.

Interactions between leaders

When large sheets or clusters of cells are migrating, the leader cells are linked together by adhesive structures, including adherens junctions, to form a front line (Figure 1c). Cadherins are the major transmembrane component of adherens junctions. They interact and control the actin and microtubule networks via catenins, such as p120-, α– and β-catenin[48, 49]. Because of their tight association with the actin cytoskeleton, adherens junctions are essential for maintaining the integrity of the migrating cell monolayer or cell group (Figure 3a). Impairing cadherin functions dramatically alters collective cell dynamics[50]. As observed in several systems, cells, mainly leader cells, tend to detach and migrate separately[51-53]. However, in border cells, loss of E-cadherin inhibits the formation of protrusions and blocks migration without any dissociation of the cell cluster [54]. Variation in the adherens junction molecular composition, and in particular the balance between different cadherins may be responsible for these different behaviors[50]. Whereas E-cadherin mediated junctions are reinforced when submitted to pulling forces, P-cadherin is not involved in the adaptation of intercellular tension [50]. Another possible explanation for the inhibition of migration following the loss of E-cadherin[54] is based on the fact that border cells use nurse cells as their substrate. The interaction between these cell types during migration is mediated by E-cadherin, analogous to the use of integrins during migration on extracellular matrix. A similar interaction has been proposed for the migration of primordial germ cells in zebrafish[55].

Cadherin-mediated adherens junctions are also required for cell chemotaxis, suggesting that each cell, even leader cells, cannot interpret the chemotactic gradient without interacting with its neighbors (see below) (Figure 1c). Several studies have shown an antagonistic relationship

between cadherin-mediated junctions and integrin-based adhesions[56-58]. Cadherin-mediated contacts are thus required for the correct polarization of the cells and for directed movement[49]. In fact, the anisotropic distribution of adherens junctions is sufficient to promote cell polarization[56, 59] (Figure 3a). In absence of adherens junctions, integrins are constitutively engaged with the extracellular matrix along the entire cell periphery[51]. The protrusions form in random directions and the persistence of migration is strongly reduced[51]. In the case of migrating chains observed during tracheal or vascular sprouting, only one or two leader cells direct the movement and their cell-cell contacts are mostly located at the rear (Box 2). These limited contacts contribute to cell polarization by limiting the formation of protrusions and promoting cell contractility at the cell rear via contact inhibition of locomotion (see below)[60, 61].

Cadherin-mediated interactions with neighboring non-migrating cells also contribute to the polarization of migrating cell groups. In particular, expression of E-cadherin in nurse cells is required for the polarized movement of border cells across the egg chamber[62]. In contrast, overexpression of E-cadherin in nurse cells inhibits cell migration and increases the polarization of border cells in the direction of the oocyte. These results suggest that the level of cadherin expression is an essential parameter that determines whether cells must migrate on top or in between other, or whether they migrate together.

The maintenance and dynamic control of cell-cell contacts is crucial to prevent too frequent changes in leadership and to keep the cohesion of the migrating leaders and more generally of the migrating group. Adherens junctions undergo a continuous acto-myosin driven retrograde flow along the lateral sides of adjacent cells migrating in a wound healing assay[63] (Figure 3b). The rearward movement of adherens junctions ends near the cell rear with the dissociation of cadherin-mediated interactions and internalization followed by recycling of cadherins towards the leading edge and formation of new junctions at the front of lateral contacts[63]. This dynamic treadmilling of adherens junctions makes intercellular contacts very malleable (Figure 3c), while maintaining the mechanical strength of adherens junctions between adjacent cells during migration. Adherens junctions between leaders are connected to thick actin cables and display a stretched morphology indicating that important forces are exerted between adjacent cells[63, 64]. The cadherin complex tightly associated to the cytoskeleton via catenin adaptors can synchronize the dynamics of the actin retrograde flow in neighboring leader cells. The adherens junction-

mediated interaction between contractile actin cables of adjacent cells can also participate in the formation of an acto-myosin cable connecting laterally all the wound edge cells (Figure 3b). When cellular sheets close a limited hole, such contractile cable can function as a purse-string to promote the convergent migration of the wound edge cells[65].

Transmitting information to the followers

The role of leader cells in leading collective migration has been observed in vitro and is also clearly illustrated during several morphogenetic events as well as during cancer invasion[66-68]. During migration of epithelial sheets, ablating the leader cells or separating them from the followers perturbs the directionality and persistence of migration and the collective behavior, highlighting the instructive role of leader cells[15, 66]. Leader cells not only explore the tissue environment and identify the migration path, they also significantly contribute to the directed migration of the followers.

Paving the way

As leader cells move through the 3D environment, they modify and enlarge the path of migration. Traction exerted on the extracellular matrix through acto-myosin-associated focal adhesions can affect the shape of the matrix fibers. The organization of the matrix fibers, can promote directional guidance and cell streaming[10, 11]. Moreover, matrix metallo-proteases secreted by the leader cells cut and remodel extracellular matrix fibers to facilitate collective movement. For example, FGF-stimulated leader tracheal cells in *Drosophila* secrete MMP2. MMP2 secretion contributes to the inhibition of FGFR-MAPK signaling in followers. In embryos lacking MMP2, the stability of the leaders is compromised and new tip cells emerge from the FGF-stimulated followers, prompting tracheal defects[40, 69]. Moreover, carcinoma invasion is promoted by the migration of stromal fibroblast leaders that generate migratory tracks that exert least resistance to migration[70].

Secretion of extracellular matrix components by leader cells can also drastically change the composition of the matrix, so that the followers migrate on a substrate that is different both in

structure and in nature from the initial substratum met by the leaders. The changes in substrate composition and of the nature of the engaged integrins impact on the migratory behavior of the followers increasing the polarized organization of the cell group[14, 71].

Leaders and followers join their forces

Leader cells can generate most of the traction forces that drag the followers behind [72]. Focal adhesions at the front of leader cells mature and associate with acto-myosin cables to promote the contraction of the cell body. Detailed analysis of traction forces and small GTPase activities showed a clear accumulation of traction forces associated with an elevated RhoA activity at the wound edge of epithelial sheets[73]. These forces are transmitted via longitudinal acto-myosin cables to several rows of followers[73, 74]. In *Drosophila* border cells, analysis of the forces exerted on cadherins shows that the tension decreases from the front of the cluster to the rear[62]. Transmission of forces would thus allow, in principle, leader cells at the edge of a monolayer to drag a relatively passive mass of follower cells. However, this coordinated movement not only involves a mechanical coupling between cells, but also the ability of cells to sense the exerted forces.

The capacity of follower cells to respond to the forces exerted by the preceding cells depends on a process known as mechanosensing[75, 76]. Cells sense the physical properties, in particular the rigidity of their microenvironment through adhesive structures such as focal adhesions and adherens junctions. This mechanosensing is mediated by the force-induced conformational changes of key proteins acting as mechanotransducers. These molecules, like talin in focal adhesion and α-catenin in adherens junctions, are key players in bridging the transmembrane adhesion receptor (integrin and cadherin) to the actin cytoskeleton and are thus submitted to forces exerted between the acto-myosin contractile network and the extracellular environment. Mechanotransducers undergo a conformational change upon stretching, revealing new protein interaction domains and inducing biochemical signaling, which in turn can modulate the strength of adhesion. Cell-cell contact associated Merlin has recently been involved in mechanotranduction during collective migration[77]. Pulling forces exerted by leader cells promote the translocation of Merlin from cell-cell contacts to the cell cytoplasm to support the

polarization of Rac1 activation and lamellipodium formation defining the front side of the following cells[77]. In addition, tension exerted on C-cadherin-mediated junctions leads to the reinforcement of desmosomes[78]. In this case, this reinforcement involves interplay between cadherin and integrin signaling[79]. At the molecular level, a common aspect of mechanosensing relies on protein conformational changes. For example, under tension α-catenin undergoes a conformational change that exposes an intramolecular interaction domain, thereby enabling it to bind to vinculin, which results in increased junctional stability[80, 81]. In a similar way, tension seems to stabilize the binding of α-catenin to actin, thereby linking external mechanical forces to the cytoskeleton[82, 83]. It is also possible that forces alone may directly polarize cells without interfering with signaling pathways.

Followers can also directly participate to pulling forces[26, 84]. It has been shown that stress builds up within the monolayer several cell rows away from the leading edge, which cannot be explained if forces are generated by leader cells alone. The mechanism proposed to explain this observation is based on a long-range transmission of forces across intercellular adhesions resulting in an increased tension or "tug of war" between leaders and follower cells[84-86]. These observations suggest that force generation does not depend solely on leader cells, but followers also exert traction and play an important role in organizing collective cell migration (see below). Overall the role of leaders and followers and their contribution to forces in collective cell migration is still controversial.

Followers, not just following

Most of the research to understand how directionality is achieved in collective cell migration has focused on what happens at the front of a cell cluster; however, recent findings have shown that follower cells are required for efficient migration. The followers are essential for the polarization of the entire cell cluster by controlling the role of the leaders, indirectly influencing their polarization, and also by participating in gradient sensing and chemotaxis.

Discussing the leadership with the followers

As a cell group migrates through a complex microenvironment, the position of leader cells can be challenged. Leaders and followers can exchange places and roles during migration in vitro as well as in vivo[87, 88]. Tip cells in tracheal branches in *D. melanogaster* remain stable during the entire morphogenesis process [89], whereas the leaders of border cells frequently change [90, 91]. Despite these variations, the position of leader cells remains generally stable for several hours or longer[92, 93].

The dynamic control of leadership is the result of a continuous crosstalk between leaders and followers, which has been particularly well studied in *D. melanogaster* border cells. Activation of Rac has been shown to be both necessary and sufficient to induce the leader cell behavior and collective migration indicating that collective guidance results from a higher level of signaling in the leader cells [94, 95]. However, in such a small cell cluster where receptor activation is almost identical in all cells, peripheral cells have an inherent free edge and can intrinsically polarize towards this free side [96]. Additional signals are in this case essential to coordinate the polarization of the cluster, so that Rac activity and cell protrusions are distributed in a clearly polarized manner between the front and the rear of the cell cluster[91, 97]. Cells adjacent to the leader restrict the activation of Rac in the leader cell. Although Rab11 and moesin have been shown to be involved, the exact mechanism which prevents Rac activation in follower cells remains to be clarified but is likely to involve direct cell-cell interactions[98].

Followers polarize leader cells via contact inhibition of locomotion

During collective cell migration leader cells become polarized, with large protrusions in the direction of migration (Box 2). A concept that has recently emerged regarding collective cell migration is that follower cells play an essential role on the movement of the cluster by inducing polarization in the leader cells via the phenomenon of contact inhibition of locomotion (CIL).

CIL is the process by which upon collision between two migrating cells, they halt their forward locomotion by collapsing protrusions at the site of contact and establishing protrusions away from each other[99-103] (Figure 4a).It has been proposed that, during collective cell migration, CIL ensures the absence of protrusions at points of cell-cell contacts between leading cells and followers, and simultaneously promotes the formation and maintenance of protrusions in the

leader cells in a direction away from their contact with follower cells[101, 103]. There is a wide variety of examples where collective cell migration has been observed in vivo (Box 1). In all these examples major protrusions are observed at the leading edge pointing away from the contact with the follower cells (Figure 1), which is the landmark of cell polarization induced by CIL [100-102]. Thus, CIL between leader and follower cells appears to be a fundamental aspect of collective cell migration.

The molecular basis of CIL can be separated into three core cellular mechanisms (Figure 4b). First, cells need to sense the contact with other cells. Second, a signal needs to be transmitted from the surface to inside the cell. Third, these intracellular signals need to drive protrusion collapse at the cell contact and repolarization with new protrusions away from the cell contact. Cell surface molecules involved in CIL include cadherins (N-cadherin; Cadherin-11;[104-106]), Ephrins/Eph receptors (EphA, Ephrin-A;[107-109]), members of the Planar Cell Polarity (PCP) pathway (Frizzled 7, Wnt11, PTK7;[110, 111]), Syndecan4 [112] and PTK7 [113]. Cadherins and Ephrins mediate, respectively, a homophilic or heterophilic interaction between colliding cells via their extracellular domains. The nature of the interaction across neighbor cells for the PCP proteins. Syndecan4 and PTK7 is not completely clear, but evidence suggests that all PCP components are accumulated and activated at the site of cell contact, including the secreted ligand Wnt11, and that Syndecan4 and PTK7 work as co-receptors for the PCP signaling pathways [110, 112, 114-118]. This activation of PCP signaling at the site of contact leads to the recruitment of other PCP proteins, such as Disheveled, Strabismus and Prickled[110], whereas cadherin engagement leads to the recruitment of Par3 at the site of cell-cell contact[119].

Activation of cell surface proteins in turn leads to the activation of signaling pathways inside the cell. Despite their heterogeneity, molecules involved in the initial cell-cell contacts are generally involved in the regulation of the activity of small GTPases such as RhoA, Rac1 and Cdc42 [107, 108, 110, 119-121], via the activity of the exchange factors Trio and Vav2 [108, 119, 122].

It has become clear that upon cell-cell contact, RhoA becomes activated at the site of contact, while Rac1 and Cdc42 are inhibited at the contact but activated at locales away from it. Actin polymerization is, in turn, controlled by the activity of these GTPases and is required for protrusion formation[22]. Indeed, the actin-binding protein calponin2, that works downstream of RhoA and Rac, and changes in actin flow have been shown to be involved in CIL[123,124]. As a

consequence of RhoA and Rac repolarization, microtubules and microfilaments collapse[119, 123] and focal adhesions disassemble at the site of cell-cell contact[125]. This is accompanied by an increase in tension at the cell-cell contact followed by a rapid actomyosin contraction[123], causing protrusion collapse.

New protrusions away from the cell contact could be generated by the localized regulation of small G proteins at the contact site that uncouples the front and the back of a cell[126]. Another possibility is that a chemical or a mechanical signal is transmitted from the region of cell-cell contact to the other end of the cell. It has been shown that the mechanical force generated by pulling a cell is sufficient to promote the formation of cell protrusions at the opposite end[78]. Although experimental evidence strongly supports the notion that CIL plays a key role in collective cell migration and mathematical models have been developed that support this concept[121, 127-129], a systematic study of the molecular basis of CIL in collective cell migration is still lacking.

An intriguing idea based on the notion that cells are polarized at the edge of a cluster via CIL is that an equivalent polarization should be expected at the back and front of the cluster as in both regions cells have a free edge and are in contact with neighbor cells. Indeed, it has been shown that back and front cells are equally polarized away from the contact in border cells and neural crest cells [91, 94, 104, 130]. The morphology and general behavior is similar in edge cells at the back or front of the cluster: both produce protrusions away from the cell-cell contact exhibiting polarized Rac1 activity; however the size and stability of protrusions and Rac1 activity tend to be higher at the front as they sense higher levels of chemoattractants. The result of this asymmetry is that the whole cluster moves forward regardless of the polarity of the cells at the back of the cluster.

Followers contribute to chemotaxis

It has been found that, in every in vivo system in which collective cell migration has been studied, chemotaxis is an important component in determining directionality. In order to maintain a gradient during chemotaxis, a "source and sink" for the chemical are required[131]; however in recent years a more dynamic version of the gradient has emerged, in which cells can generate

their own gradient. This seems to be the case during collective cell migration, where follower cells in a cluster play an essential role in generating this gradient, so that some cells respond more efficiently to chemoattractants when they are part of a cluster than as single cells, suggesting again that leaders need followers to respond to external signals[104, 132].

This notion is supported by studies on the migration of the lateral line of zebrafish embryos (Figure 5a). The chemoattractant Sdf1 is expressed in cells prefiguring the track on which lateral line primordium migrates, but Sdf1 is expressed uniformly and not as a gradient [133, 134]. While the Sdf1 receptor Cxcr4b is expressed throughout the primordium, a second receptor Cxcr7 is expressed only at the rear[93]. It has been shown that Cxcr7b binds Sdf1, functioning as a sink and thereby generating a gradient across the primordium[135-138]. A similar mechanism for a self-generated chemotactic gradient has recently being shown for the migration of melanoma cell (Figure 5c)[139]. Lysophosphatidic acid (LPA) functions as a strong attractant for melanoma cells, which at the same time break down LPA, generating a gradient with low LPA in the tumor and high LPA outside. This self-generated gradient around the melanomas prompts the tumor cells to migrate away from the tumor and out into the surrounding skin and blood vessels[139].

A different mechanism of self-generated chemoattractant gradient formation is found in the migrating neural crest cell in *Xenopus laevis* embryos (Figure 5b). The neural crest is able to respond to the chemoattractant Sdf1[140, 141], which is expressed by a group of epithelial cells, called placodes, which are initially adjacent to the neural crest. Neural crest cells are attracted towards the Sdf1 produced by the placodes, but upon contact between the two cell types, CIL drives the placodal cells to move away from the neural crest. This drives the placodal cells further ahead of the neural crest cells, while maintaining the attraction of the neural crest towards the Sdf1 produced by the placodes[125]. This mechanism of dynamic attraction and repulsion ensures effective directional migration of both cell types.

Taken together, these examples illustrate that a common mechanism driving collective cell migration is the generation of a chemotactic gradient, and that this gradient is formed by the action of the follower cells in the cluster. This last example of interaction between two distinct populations of cells to generate directional migration is not uncommon. Potentially similar mechanisms are observed in the interactions between the ureteric bud and the metanephric mesenchyme mediated by GDNF (Glial cell-Derived Neurotrophic Factor)[142], between the

anterior and posterior regions of the primordium lateral line mediated by FGF[143] and between stroma fibroblasts and tumor cells[144, 145] (for a discussion see[146]).

Conclusions and perspective

Collective cell migration is essential for morphogenetic movements as well as for tumor spreading. Collective migration is more than just the coordinated behavior of a group of cells, as it improves the migratory capacities of each individual cell to induce a movement that is faster and more directed. At the front of migrating cell groups, leader cells play a pivotal role in driving collective movement. Despite their crucial role in controlling collective migration and therefore their implication in tumor spreading, the mechanisms leading to the emergence of leader cells and the molecular specificities of these cells remain unclear. Deciphering these signals will help us better understand how invasive cells can arise from non-migrating tissues. The leaders integrate signals coming not only from their physical microenvironment but also from the messages sent by neighboring cells. This is where the so-called followers are in fact active participant in the control of the migration speed and direction. Over the last years, several reports have shown the variety of information that can be transmitted by the followers through direct contact, exchange of soluble factors, and also through the modification of the microenvironment. This suggests that the behavior of leader cells in a group of migrating cells is in large part the result of what is occurring at the rear and that deciphering the intercellular signals exchanged within the cell group may point out new ways to promote or inhibit collective migration.

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Glossary terms:

Adherens junctions: molecular complexes allowing intercellular interaction. They involve the homophilic interaction of classical cadherins and a large complex of cytosolic proteins, such as catenins, bridging cadherins to the cytoskeleton, including actin stress fibers.

Chemotaxis: process by which cells undergo directed locomotion along a chemical gradient.

Filopodia: Finger-like membrane projection frequently found at the leading edge of migrating cells. These membrane protrusions are formed by the polymerization of actin bundles and can, in particular, be induced by the small GTPase Cdc42.

Focal adhesions: molecular complexes allowing cell adhesion to the extracellular matrix. They involve the transmembrane protein integrins, and a large complex of cytosolic proteins bridging integrins to the cytoskeleton, including actin stress fibers.

Integrin: Familly of transmembrane proteins involved in cell interaction with protein fibers of the extracellular matrix (ECM). α and β integrins form heterodimers whose conformation and affinity for the ECM is regulated by inside-out signaling. Upon engagement with the ECM, integrin dimers induce intracellular (outside-in) signaling.

Lamellipodia: thin sheet-like membrane extension frequently found at the front of migrating cells. The formation of lamellipodia involves the polymerization of a branched actin meshwork and the formation of transient adhesions with the cell substrate.

Nurse cells: cells which contribute to the development of the oocyte of invertebrate organisms. In *Drosophila melanogaster*, 15 nurse cells are included in the egg chamber provides the nutrients, RNA and proteins required for the oocyte growth.

Desmosome: cell-cell adhesion complex typically found in epithelial cells. Desmosomes involve specific members of the cadherin family of transmembrane adhesion proteins and are connected to keratin filaments.

Online summary:

- During collective migration, cells not only migrate in a coordinated manner, they also migrate faster and in a more directed way than individual cells. Coordination and efficiency of collective migration rely on cellular interactions through soluble and contact-mediated signals.
- Leader cells, generally localized at the front of the migrating group, present specific molecular features and morphological characteristics, which are reinforced by the soluble and contact-mediated signals present in their microenvironment..
- Leader cells facilitate the directed migration of followers directly by generating pulling forces and indirectly by modifying the extracellular matrix composition and structure.
- Intercellular contacts between collectively migrating cells involving several sets of membrane proteins, induce a local inhibition of locomotion through the regulation of Rho GTPases. Contact inhibition of locomotion is an essential event promoting the coordinated polarization of collectively migrating cells.
- Several lines of evidence have shown that the followers actively participate to the collective movement by communicating with one another and with the leaders, by generating forces, by contributing to generation of chemotactic gradients.

Display items

Box 1 | **Collective cell migration in vivo**

Cells that undergo collective migration are commonly found in vivo, in the pharyngeal endoderm [147], cranial placodes[148], nephric ducts[149], and during mammary branching morphogenesis[150], hear regeneration[151] and angiogenesis [152]. Four well-studied in vivo models are:

Border cell in *Drosophila melanogaster* (see the figure, part a): The ovary is composed of ovarioles and the egg chamber. In the egg chamber contains one oocyte and several support cells, which are surrounded by epithelial follicle cells. During oogenesis, anterior polar cells (purple) recruit neighbor cells to form the border cell cluster and start their migration. One or two leading cells extend protrusions in response to the chemoattractants PVF1, Spitz and Karen, secreted by the oocyte[153-156], and Gurken, localized at the dorsal-anterior corner of the oocyte[153, 155, 156]. DE-cadherin (pink) plays distinct roles during border cell migration, and it functions in a positive loop with Rac1[62].

Lateral line in zebrafish (see the figure, part b): The primordium of the zebrafish lateral line is formed by a cluster of more than 100 cells that migrate from the head to the tail of the embryos, where it forms series of rosette-like mechanosensory organs[157]. The primordium migrates as a compact epithelial cluster with large polarized protrusions at the front. The direction of migration is determined by a self-generated gradient of Sdf1 (see also Fig 5a)[136, 137, 157].

Neural crest in *Xenopus laevis* (see the figure, part c): cephalic neural crests are formed in the dorsal part of the neural tube and migrate ventrally contributing to many head structures [141]. The cephalic neural crest undergoes an epithelial to mesenchyme transition (during which E-cadherin disappears) but still migrates as a cohesive cluster of cells that influence each other behavior [104]. The cluster configuration is maintained mainly by chemotaxis between neighbor cells, instead of cell adhesion (chemoattractant C3a, blue circles[158]).

Cancer invasion (see the figure, part d): during metastasis cancer cells spread from one organ to another (e.g. melanomas from the skin of a hand can spread to different organs on the body). This spreading can involve collective cell migration; which is usually found in epithelial cancers; although leader cells can acquire a more mesenchymal phenotype with cell generating

protrusions and activating Rac1. Often cell clusters migrate following some physical clues, such as collage fibers, myofibrils, basal lamina from muscles, nerves or blood vessels [2].

Box 2 | **The place of leader cells during collective migration**

Because of their localization at the front, leader cells (shown in green, see the figure, panels a-f) have a contact-free edge that can form and extend protrusions, unlike lateral and rear sides that are involved in cell-cell contacts. Moreover, followers are all equivalent when the cells migrate in unlimited sheets or chains (that is, without a defined rear) (see the figure, **panels a-c**), whereas the followers that are located at the rear of limited cell groups (see the figure, **panels d-f**) have a rear side which is not involved in cell-cell contact. These different examples of collective cell migration configurations also illustrate the fact that the number of leader cells varies depending on the context, and in particular with the geometry of the migrating group. The collective migration of cell monolayers, for example during fibroblast or astrocyte wound healing in vitro, and dorsal closure in *Drosophila melanogaster* is driven by a first row of leader cells (as represented in **part a**) or by clustered leader cells localized at the front edge, as is the case during epithelial wound healing in vitro] (as represented in **part b**). Similarly limited numbers of leader cells have been identified in broad fronts of invasive cancer [26, 159]. One or two leader cells, also called tip-cells (**part c)**, are sufficient to guide the collective migration of strands of cells (for example endothelial cells during angiogenesis or tracheal development) or cell groups as observed during cancer invasion [72, 160] (panel d). During the lateral line primordium migration in zebrafish, a group of 10 to 20 leader cells guide the primordium along a path of Sdf1 secreting stromal cells (**panel e)**, whereas a single cell leads the migration of the small cluster of *D. melanogaster* border cells (**panel f**).

Figure 1 | Polarization in single cells and in collectively migrating cells.

a | Front-to-rear polarization during single cell migration

During single cell migration the main engine for movement seems to be at the front of the cell, where active membrane protrusion occurs and the cell adheres to the ECM. As the lamellipodia extends, integrin-based nascent adhesions form to eventually mature in focal adhesions, on which longitudinal acto-myosin cables are anchored. Mature focal adhesions are maintained during migration until they reach the retracting edge of the cells, where they are disassembled. For clarity, only a few nascent and mature adhesions are shown. Locomotive forces applied to focal adhesions drive movement of the cell on or through the substratum. While events at the cell leading edge provide essential forces for forward movement, the cell rear also actively participates to cell displacement by controlling the detachment from the ECM and the contraction of the cell body. The microtubule network and intracellular membrane compartments also organize in a polarized manner along the direction of migration.

b | Polarization in a group of collectively migrating cells

Reflecting this front-rear orientation, leader cells are clearly polarized : they show an elongated morphology, polarized along the direction of migration[89, 161, 162]. This polarization has been observed in vitro during wound healing and in vivo during the migration of Xenopus laevis head mesoderm, lateral line in zebrafish, border cells in Drosophila melanogaster, cephalic neural crest and others[101]. However, observations in vitro show that some wound-edge Mammalian epithelial cells elongate more than others and some epithelia leader cells can spread perpendicularly to the direction of migration[7, 15]. The increased spreading of leader cells associated with a mesenchymal phenotype reflects a transient loss or reorientation of epithelial baso-apical polarity in favor of a front-rear polarity[89]. However, leaders retain some epithelial characteristics, remaining attached to their neighbors. Leaders often display dynamic actin-based protrusive structures. Although not limited to leader cells, finger-like filopodia and ruffling lamellipodia form at the front edge of the epithelial monolayer[7] as well as at the leading edge of tip cells initiating vascular sprouts in mammals and tracheal branches in *D. melanogaster*. The morphology of leader cells is associated with the expression of specific genes which promote cytoskeleton remodeling and cell migration[159, 163, 164].

c | Polarizing environmental cues. In a cohesive cell group, the leader cells are submitted to polarized environmental cues. While the cell rear is engaged in intercellular contacts, the cell front interacts with the extracellular matrix or with non-migrating cells of the tissue. Adherens junctions by locally inhibiting the formation and maintenance of focal adhesions [56, 57] restrict the localization of focal adhesion to the cell front.

Figure 2 | **Polarization of leader cells by integrin-induced signaling. a** | During in vitro wound-induced cell migration, integrin signaling leads to the recruitment at the leading edge plasma membrane of the polarity protein Scrib which interacts with the Rac and Cdc42 GEF βPIX [17]. Phosphorylation of βPIX by Src promotes its Cdc42 GEF activity[165]. Cdc42 contributes to the reorganization of the microtubule network[23, 166]. Cdc42 activates the polarity complex formed of Par6 and aPKC which in turn induces microtubules anchoring and centrosome and Golgi positioning in front of the nucleus[167]. MRCK also contributes to the Cdc42-dependent retrograde flow of actin fibers leading to the rearward nuclear movement[168]. Reorganization of membrane traffic towards the leading edge is likely to participate to the formation of membrane protrusion, the development of new adhesions and the reinforcement of polarity signaling[16, 169]. **b** | Via its SH3 domain, leading edge βPIX also recruits Rac and PAK[170, 171]. Rac can be activated by PI3K as several Rac GEFs are activated by PIP3. Alternatively, the Rac GEF Tiam1, possibly associated with Par3 and PKCzeta, also promotes Rac activation, cell migration and tumor invasion[18, 19, 38]. Active Rac control actin-driven protrusion[22] and microtubule elongation through PAK[172]. The schematics represent a cell migrating in a flat 2D rigid substrate. Similar integrin-mediated signaling cascade is likely to occur in a 3D environment although the contribution of integrin signaling varies with the substrate rigidity. In addition to variation in intracellular signaling, the geometry of the 3D environment profoundly impact on the cell protrusion morphology.

Figure 3 | **Role and dynamics of adherens junctions between leader cells. a** | The anisotropic distribution of adherens junctions in leader cells induces the anisotropic distribution of focal adhesions. This induces a polarized integrin-mediated signaling which leads to the orientation of

the cytoskeleton, the centrosome and the Golgi apparatus towards the cell front and participates to the coordination of the migration direction between leader cells. **b** | Lateral adherens junctions are tightly linked to transverse acto-myosin cables and contribute to a multicellular actin network. These transverse actin cables move together in a retrograde flow (black arrows), which may coordinate the actin dynamics in neighboring cells and contributes to cadherin-dependent positioning of the nucleus [56, 173]. **c** | The retrograde flow of adherens junctions allows the dynamic rearrangement of cell-cell contacts when cells move around obstacles (such as cells or matrix fibers). Cadherin recycling which compensates the retrograde flow of junctions leads to cadherin accumulation at the leading edge. This pool of cadherin can immediately be recruited to sites of new cell contacts during cell collision and thus contribute to contact inhibition of locomotion.

Figure 4 | **Contact inhibition of locomotion (CIL). a** | CIL is the process by which a cell change the direction of migration upon collision with another cell (**a**,top right), which lead to cell polarization, with protrusion being inhibited at the cell contact and new protrusion produced away from the contact. If cell density increases a similar phenomenon of inhibition of protrusion at the cell contact takes place, but at a larger scale, where only the cells exposed to the free edge become polarized and produce protrusions away from the cluster (**a**, left). Cell contact during CIL is indicated with a red square. **b** | Molecular mechanism of CIL. Cell surface molecules interact at the site of cell-cell contact (red square), which leads to the recruitment of several other proteins and finally RhoA activation and Rac inhibition at the contact. This is translated into microtubule catastrophe, disassemble of focal adhesions (white circles) and actomyosin contractility at the contact, leading to protrusions collapse. At the opposite end of the cell activation of Rac leads to microtubule and microfilament polymerization and stabilization of focal adhesions (red circles), with the consequent formation of protrusion.

Figure 5 | **Self-generated chemoattractants gradients during collective cell migration. a** | Anterior and posterior domains of the zebrafish lateral line primordium express the Sdf1 receptor Cxcr4 (green), whereas only posterior cells express the scavenger Sdf1 receptor Cxcr7 (red). Initially (top frame) Sdf1 (blue, blue circles) is expressed uniformly along the primordium. Binding of Sdf1 to the Cxcr7 receptor works as a sink for Sdf1 generating a sharp gradient of Sdf1 (middle panel). Cells move towards higher level of Sdf1 present in the anterior end of the primordium (bottom panel). **b** | Neural crest (grey cluster) chemotax towards Sdf1 (blue

background) secreted by placode cells (pink cluster) (top panel). When neural crest reaches the placode (middle panel) they trigger a CIL response (red line) forcing the placode to move away, and the neural crest cells then follow (bottom panel). **c** | Melanoma cells can chemotax towards LPA (blue background) which is distributed homogenously in the extracellular medium (top panel). Melanoma cells degrade LPA generating a gradient with concentrations low next to the cells and higher away from them (middle panel). Melanoma cells sense this LPA gradient and move accordingly (bottom panel).

Biographies:

Roberto Mayor:

Roberto Mayor is professor of Cell and Developmental Neurobiology at the Cell and Developmental Biology Department, University College London. He obtained his PhD in 1990 from the University of Chile working on preimplantation mouse development. After that he started his postdoctoral training at the National Institute for Medical Research in London, UK working on Xenopus laevis in the laboratory of Dr. Michael Sargent, where he showed that the Snail genes are expressed and play an important role on neural crest development in the frog. He moved to Chile in 1993 as a group leader where he pursued studied on neural crest induction. In 2004 he got an independent leader position at University College London, where he started his current work on neural crest migration, demonstrating that neural crest exhibit collective cell migration and contact inhibition of locomotion .

Sandrine Etienne-Manneville:

Sandrine Etienne-Manneville is Directrice de Recherche at the CNRS and professor at Ecole Polytechnique (Palaiseau, France). She studied cell Biology and biochemistry at the Ecole Normale Supérieure in Paris and obtained her PhD in Immunology in 1998, working on the regulation of leukocyte infiltration in the central nervous system. During her four years of postdoctoral training in the laboratory of Prof A.Hall at the MRC-LMCB in London, she deciphered Cdc42-mediated polarity pathways during astrocyte directed migration. In 2003 she obtained a CNRS position at the Curie Institute (Paris, France). In 2006, she moved to the Pasteur

Institute (Paris, France) as a group leader to pursue her study of astrocyte polarization and migration. Her research team focuses on the molecular mechanisms controlling polarization and migration of normal and tumoral astrocytes.

ToC:

The front and rear of collective cell migration

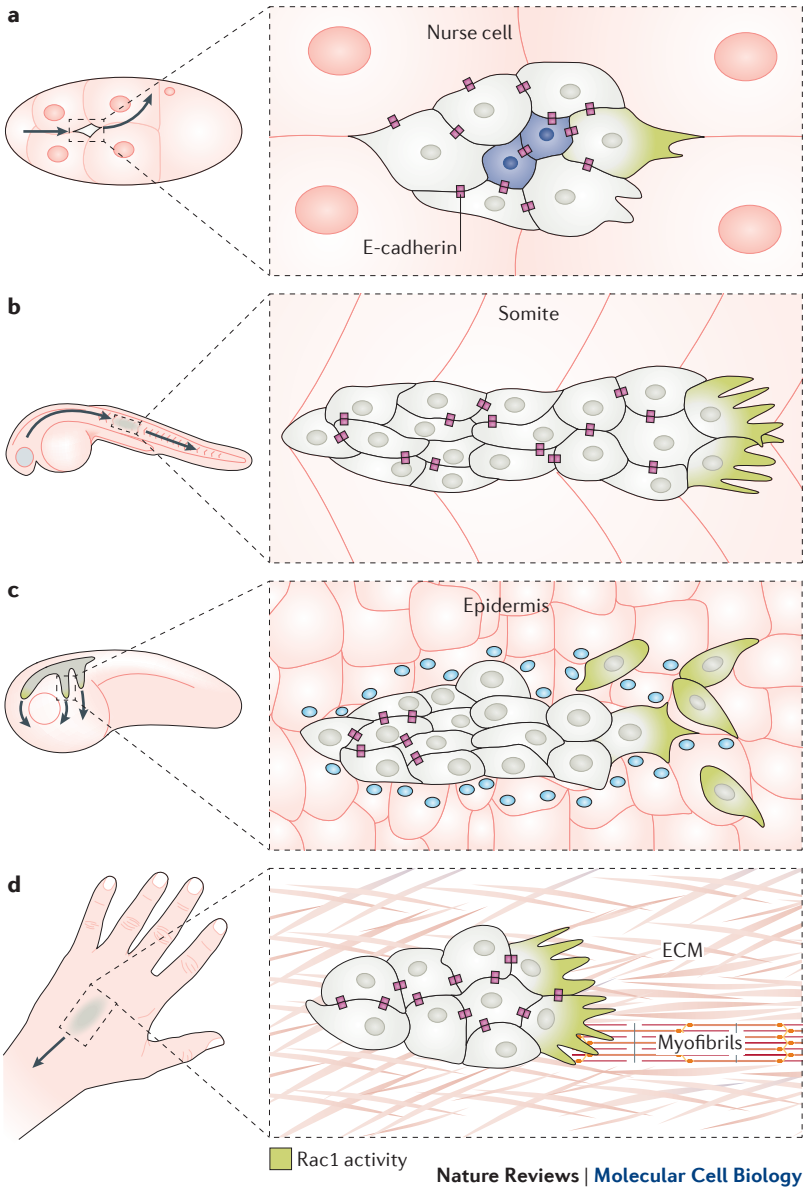
Roberto Mayor and Sandrine Etienne-Manneville

Collective cell migration has a crucial role during morphogenesis, wound healing and tissue renewal, and it is involved in cancer spreading. Recent studies highlight the importance of intercellular communication in this process: migration is driven by leader cells at the front, and follower cells communicate between them and with the leaders to improve the efficiency of collective movement.

Subject categories:

Biological sciences / Cell biology / Cell migration / Collective cell migration
[URI /631/80/84/2334]
Biological sciences / Cell biology / Cell migration
[URI /631/80/84]
Biological sciences / Cell biology / Cell adhesion
[URI /631/80/79]

Box 1


a
Nurse cell
E-cadherin
b
Somite
c
Epidermis
d
ECM
Myofibrils
Rac1 activity
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Box 2

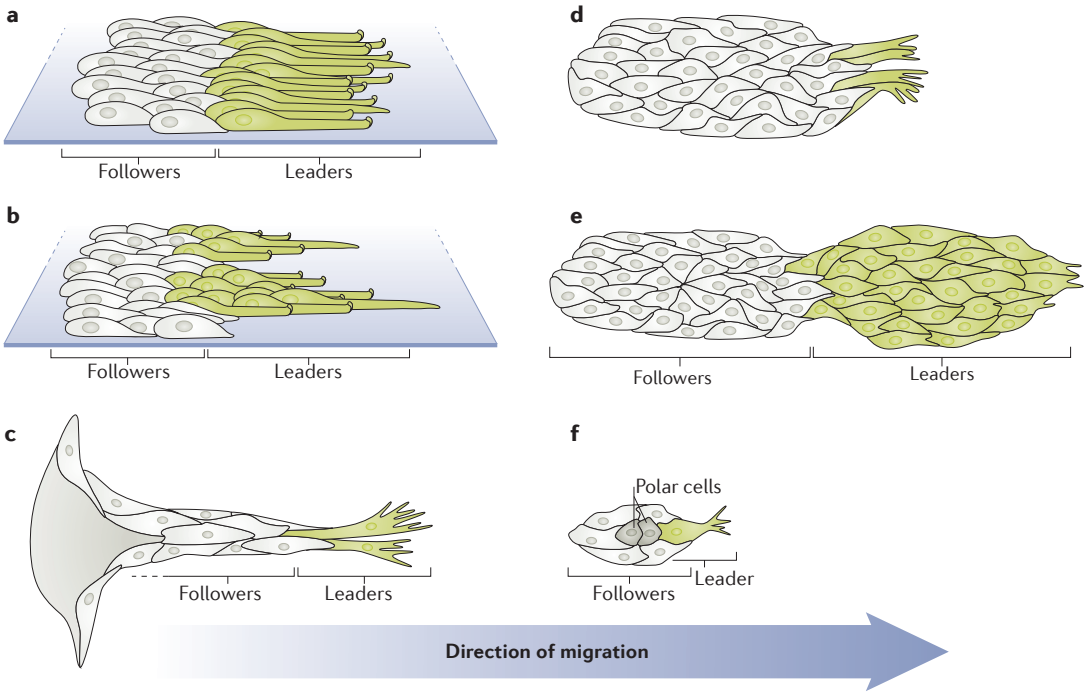
a
Followers
Leaders
b
Followers
Leaders
c
Followers
Leaders
d
e
Followers
Leaders
f
Polar cells
Leader
Followers
Direction of migration

Fig 1

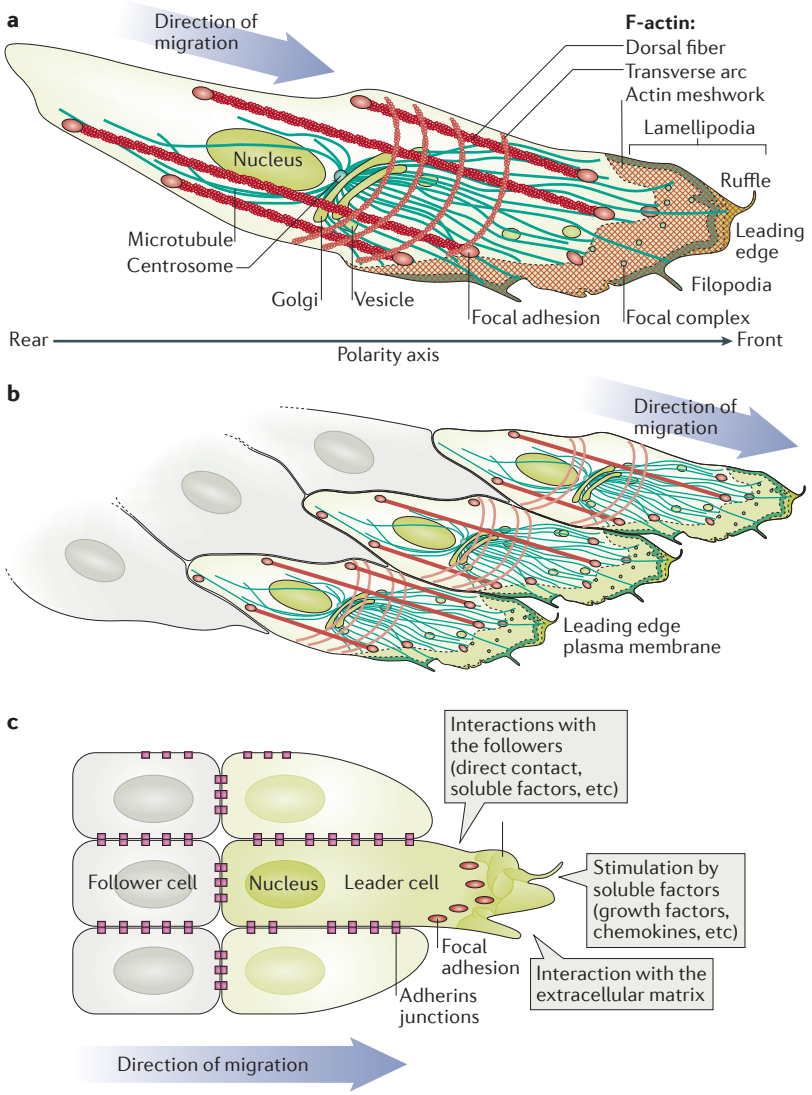

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Fig 2

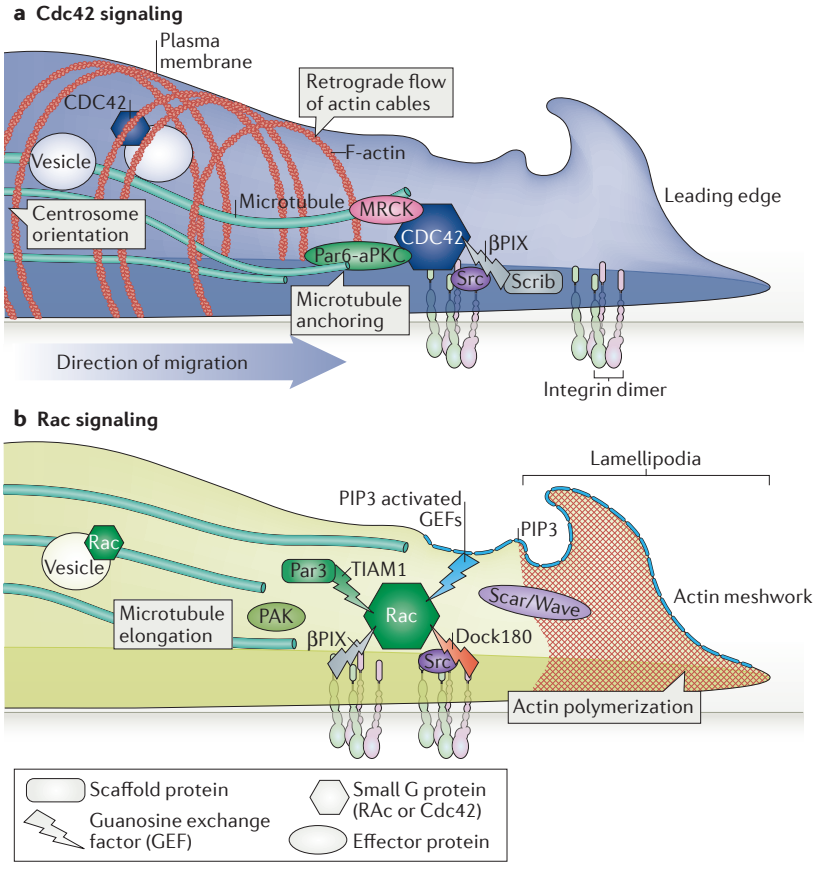

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Fig 3

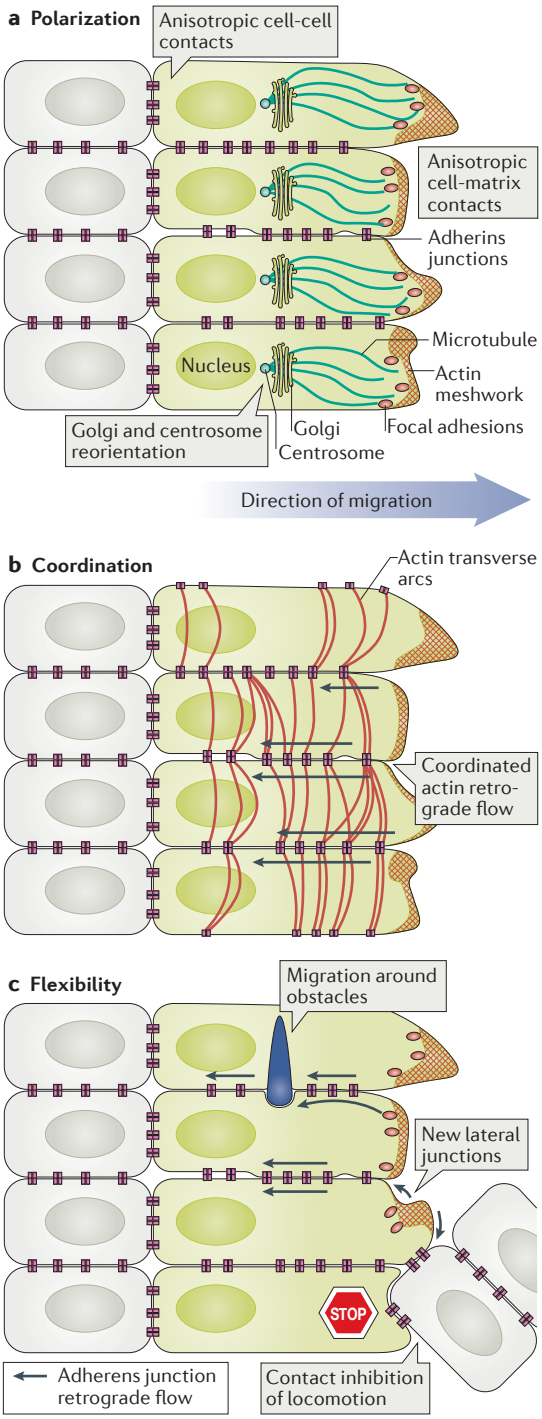
a Polarization
Anisotropic cell-cell contacts
Anisotropic cell-matrix contacts
Adherins junctions
Microtubule
Actin meshwork
Nucleus
Golgi and centrosome reorientation
Golgi
Centrosome
Focal adhesions
Direction of migration
b Coordination
Actin transverse arcs
Coordinated actin retro-grade flow
c Flexibility
Migration around obstacles
New lateral junctions
STOP
Adherens junction retrograde flow
Contact inhibition of locomotion

Fig 4

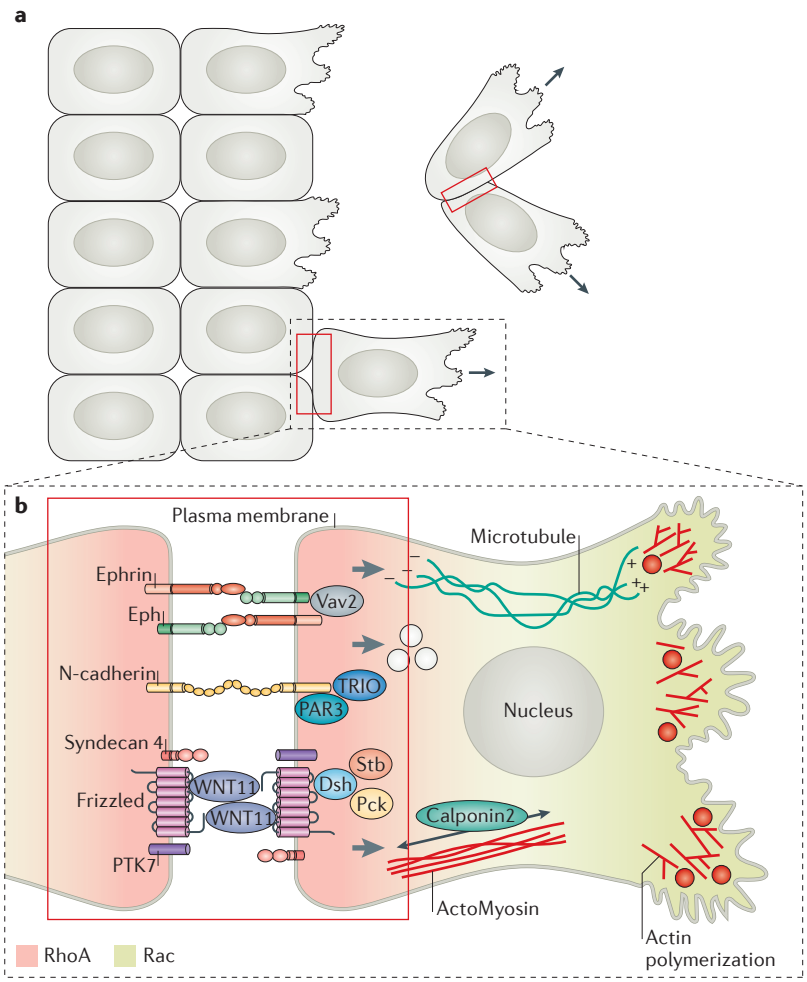

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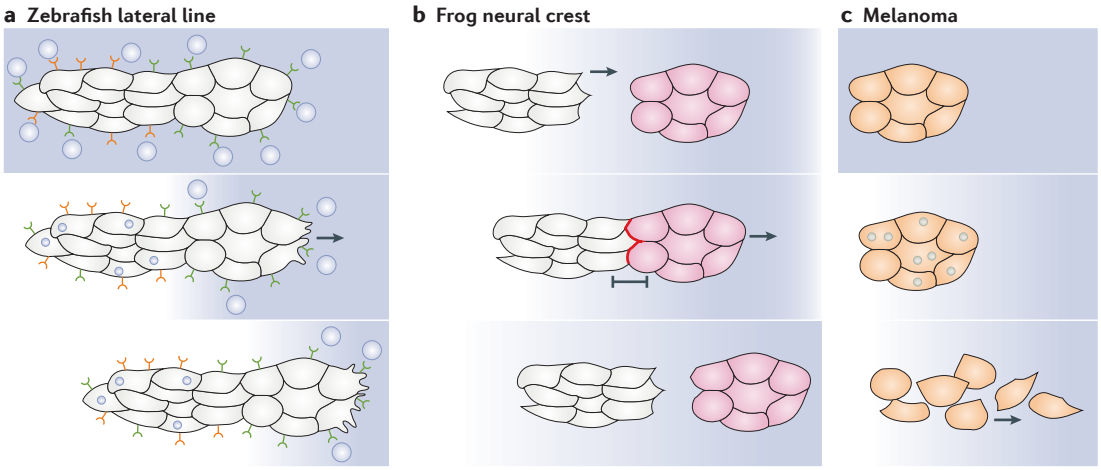
Fig 5



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