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Multicellular scale front-to-rear polarity in collective migration

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Abstract

Collective cell migration does not only reflect the migration of cells at a similar speed and in the same direction, it also implies the emergence of new properties observed at the level of the cell group. This collective behaviour relies on interactions between the cells and the establishment of a hierarchy amongst cells with leaders driving the group of followers. Here we make the parallel between the front-to-rear polarity axis in single cell and the front-to-rear multicellular scale polarity of a migrating collective which established through exchange of biochemical and mechanical information from the front to the rear and vice versa. Such multicellular scale polarity gives the migrating group the possibility to better sense and adapt to energy, biochemical and mechanical constrains and facilitate migration over long distances in complex and possibly changing environments.

Collective cell migration is crucial for many physiological processes, from embryonic development to the adult where it participates for instance in wound healing and tissue renewal. Many pathologies have been linked with aberrant collective cell migration, cancer being a primary example [1-4]. Hence there is a need for a better understanding of the mechanisms controlling collective cell migration. During migration of single cells, the establishment of a front-to-rear polarity axis is crucial [5]. The protrusive front points to the direction of migration and is characterized by cytoskeleton-driven membrane protrusion. As the front protrudes, cell migration proceeds depending on the acto-myosin contractility which characterises the rest of the cell, including the cell rear. There, contractility also contributes to the detachment of cell adhesions to promote the translation of the cell. The formation of a front-to-rear axis is induced by polarity signalling which sees as major players the small GTPases proteins of the RHO family. Globally, at the front, CDC42 and RAC promote actin polymerisation and consequentially formation of protrusions such as filopodia or lamellipodia, whereas at the cell rear, Rho and other partners are controlling acto-contractility (For review see [6, 7]). In the case of directed migration, biochemical and mechanical extracellular cues initially induce polarity signalling and the formation of a front-to-rear axis. When cells migrate in a persistent direction, several intracellular feedback loops involving for instance cytoskeletal organisation or membrane trafficking stabilise the front-to-rear polarity and migration direction.

Collective migration includes the migration of cell monolayers, cell groups of various size and shape, connected chains of cells, or more loosely connected cell streams. Model organisms such as *Xenopus laevis*, *Drosophila melanogaster* and Zebrafish, among others, have been crucial for the study and characterisation of collectively migrating cells. In parallel, *in vitro* approaches have been extensively used to elucidate key concepts underlying the establishment and maintenance of collective directed movements. Over the years, these analyses have shown that collective migration does not only characterise cells that move together in a similar direction but also imply that these cells migrate in a more directed manner and sometimes faster than cells migrating individually in similar conditions [6]. This collective behaviour emerges from cell cooperation within the group, allowed by intercellular communication through cell-cell contacts or soluble factors. Our better understanding of the relationships within migrating cell groups have led to the notion that front-to-rear polarity observed in single cell may be extended to a migrating cell group. Such multicellular-scale polarity relies on the establishment of a hierarchy amongst migrating cells. A population of front cells emerges from the migrating collective to serve as leaders which initiate, direct and facilitate the migration of followers, thanks to signalling molecules and transmission of forces through cell-cell contacts [6, 8, 9]. Similar to intracellular signalling observed in single polarised cells, information is exchanged within the cell collective either directly or indirectly in order to influence the behaviour of neighbouring cells, including the leaders, at both mechanical and biochemical levels [10]. As a result, migrating cell clusters, chains and sheets form highly polarised multicellular structures via mechanical and biochemical feedback loops flowing from the front to the back and vice-versa. This collective front-to-rear polarity provides a support not only for the most efficient migration, but also leads to the emergence of new properties such as collective chemotaxis, or durotaxis.

In this review, we first describe front-to-rear polarity within collectively migrating cell group. Subsequently, we focus on the latest advances showing how integration of polarity at a multicellular scale gives rise to the emerging properties of the cell collective, allowing them to sense and adapt to energetic, biochemical and mechanical constrains.

Cell-cell contacts tend to inhibit cell protrusion in a process called contact inhibition of locomotion (CIL), which plays a key role in the establishment of the front of migrating collectives [6, 11]. Upon cell-cell interaction, membrane proteins including for instance cadherins, ephrins and PCP proteins, induce CIL through intracellular signalling and mechanical coupling. Cells located at the edge of the group harbour a free cell edge fundamentally distinct from their other sides engaged in cell-cell interactions. The asymmetric distribution of homophilic cadherin-mediated contacts is sufficient to promote cell polarisation towards the edge of the cell group [12]. Such mechanism restricts CDC42 and RAC activity at the contact free edge of these cells, leads to their polarisation towards the front of the migrating sheet and identify them as leader cells directing the migration of the entire monolayer [13] (Figure 1). Additional signalling can further restrict the number of leader cells. In epithelial sheet only a subpopulation of front cells become leaders and form finger-like structure at the wound edge [14]. Once the migration of leaders is initiated, the formation of contractile actin cables connecting them to the adjacent cells prevents RAC mediated protrusion in neighbouring cells [15, 16]. The mechanical properties of the epithelial monolayer and the initial geometry of the front edge determine the distance between leader cells and thereby the size of the cell population following these leaders [16]. The size of the cell group following one leader depends on the length up to which forces can be transmitted [17].

The situation is more complicated in case of cell clusters where in principle all cells located at the edge of the cluster could become leaders. During border cell migration in the *Drosophila* ovary, a cluster of eight cells delaminate from the epithelium and migrate collectively between nurse cells towards the oocyte. The collective movement is ensured by the expression of DE-cadherin which mediates contact between the border cells and between border cells and nurse cells. Rab11 acts on the whole cluster to promote the formation of a coherent actin-moesin structure that surrounds the cluster and restrict RAC-dependent protrusive activity in the leading cell [18, 19] (Figure 1). Rab11-mediated recycling of CDC42 controls the mechanical coupling within the cell cluster promoting mediated cytoskeletal tension through adherens junctions between adjacent cells [20]. As border cells migration proceeds, increased EGF signalling disrupts the acto-myosin cable surrounding the cell cluster and abolish the restriction of Rac activity to promote protrusion in all cells of the clusters which can then exchange position [21].

During mesenchymal migration of single cells, contraction between the protruding front and the retracting rear is mediated by ventral stress fibres that reach between focal adhesions located at the opposite sides of the cell (Figure 1). Cadherin-mediated contacts relay mechanical forces between the front and the rear of migrating groups (Figure 1). During migration of astrocyte or epithelial monolayer, most of the traction forces are exerted by the leaders with elevated RHOA activity, which pull on the followers [14, 22]. Mechanotransduction at cell-cell contacts and strengthening of adherens junctions at the front and rear of migrating cells can orient the actin cytoskeleton and mediate propagation of tension across the monolayer [9]. Vishwakarma *et al.* suggests a prominent role of forces generated by follower epithelial cells to induce and maintain leaders. Followers are pulling on the edge and by doing so they facilitate polarisation and protrusions formation in leaders [17]. P-cadherin mediated cell-cell contacts in myoblasts promote the concentration of CDC42 at the front edge of leaders and followers and homogenizes the cell polarity axis throughout the monolayer [23]. In migrating endothelial monolayers, VE-cadherin-mediated adherens junctions at the rear of leader cell form protrusive fingers which penetrate the front of the following cells and triggers Rac-driven membrane protrusions [24]. The front-to-rear

polarity of the leaders can thus propagate to followers and contribute to the global polarisation of migrating groups.

In addition to controlling multicellular scale cytoskeletal structures, many molecules involved in cell-cell interactions can recruit polarity complexes to the adhesion sites [11]. The polarised distribution of planar polarity proteins such as receptor protein tyrosine phosphatase Lar at the cell front and the cadherin Fat at the cell rear have recently been shown to sustain polarity signalling between neighbouring cells of the follicular epithelium in the *Drosophila* egg chamber [25, 26]. In this model system, the planar polarisation of the semaphorin, Sema-5c also contributes to the global polarisation of the epithelium. At the leading edge of each cell, the activity of Lar is antagonized mechanism which involves the semaphorin, Sema-5c accumulates at the leading edge of each cell and signal through its receptor Plexin A to suppress protrusions in the cell ahead. It also antagonize Lar activity independently of Plexin A [27]. These recent observations illustrate how collective front-to-rear polarity is reinforced by multiple bi-directional polarity signalling at cell-cell interfaces (Figure 1).

Collective polarization and energy sensing

While in migrating monolayers of astrocytes or epithelial cells leaders are maintained at the front edge for a long period of time, in other systems exchanges between leaders and followers can occur. During migration of cranial neural crests (NCs) in *Xenopus*, chick and Zebrafish embryos, leader cells appear interchangeable. In this case, ablation of leaders does not totally block migration, suggesting a rapid adaptation of followers to the formation of a free edge, generating an asymmetry in cadherin-mediated junctions [28].

During leukocyte single cell migration, the cell front samples the geometry of the migratory path and choose the path of least resistance, suggesting that the cell energy level is critical in migration [29]. Recent work from Reinhart-King's group revealed the influence of cell energetic status in the dynamic polarity of migrating collective [30]. During breast cancer cell invasion, leader cells were shown to require more energy than followers, probably because they are responsible for a large part of the pushing and pulling forces required for migration through the extracellular matrix. Leader cells are thought to remodel the extracellular matrix and the formation of micro tracks for the less invasive follower cells [4, 31]. As collective migration progresses, the energy level of the leader cell decreases until it reaches a threshold level under which leaders cannot invade efficiently (Figure 2). Leader cells are then replaced by followers which become leaders. When put into a challenging environment, for example a denser collagen matrix, leaders' lifetime decreases, suggesting that the leaders-followers switch may be a collective strategy to sustain continued invasion [30]. Cancer cells may have a limited amount of energy to spend and thus exchange between leaders and followers occurs more rapidly. How the bioenergetic status of leaders controls the emergence of new leaders to overtake the failing leaders at the front remain unclear. Cadherin-mediated interaction may serve as sensors of the strength developed by leaders and thereby indicate to the followers when tractions forces are decreasing.

Collective polarization and chemotaxis

Extracellular biochemical cues that control single cell directed migration also contribute to collective migration [15, 16, 32, 33] (Figure 3A). During development, collective chemotaxis must occur over long distance during long period of time in a morphologically changing and growing

embryo. In such context the maintenance of a steep enough chemokine gradient that could be sufficient to drive the cell group by influencing the direction and migration of each individual cell seems unlikely. Converging evidence indicate that the polarisation of the migrating cell groups facilitate long-distance chemotaxis (Figure 3A).

One simple mechanism is that leader cells are initially defined by a specific set of receptors which render them more susceptible to chemokine gradient. This is the case during border cells migration which is initiated by the secretion of specific signals produced by the oocytes (PVF (PDGF- and VEGF-related factor 1) and EGF [34]. The cell expressing the higher level of the receptor tyrosine kinase PVR shows a higher Rac activation and takes the role of leader driving the migration of the whole cluster [1, 35, 36]. Similarly a small population of cranial NCC present a specific transcriptomic signature which allows them to respond to a VEGF gradient and serve as leaders. In turn VEGF stimulation induces a change in gene expression reinforcing the leader cell phenotype [37]. Interestingly followers respond to ectopic expression of VEGF by adjusting their migratory path and their gene expression to become more “leader-like”.

An emergent property of migrating collective is the self-generation of chemokine gradient, allowing the group to migrate in an initially homogenous medium. In the case of NCCs, computer modelling considering VEGF diffusion and uptake and assuming homogenous VEGF production showed that VEGF is progressively consumed by the cells at the front, creating a gradient of VEGF between the front and the rear of the cell group which is likely to improve the chemotactic behaviour [38]. The zebrafish lateral line primordium follows a path marked by a constant concentration of SDF1 (Stromal cell-derived factor 1) from the front to the rear of the lateral side of the animal. Front cells serving as leaders express the CXCR4, which upon SDF1 binding triggers migration. In contrast, followers situated in the second half of the primordium express a combination of CXCR4 and CXCR7. CXCR7 interacts with SDF1 without promoting migratory signals and sequesters the chemoattractant away from CXCR4, thereby generating a gradient of CXCR4 signalling between the front and the rear of the primordium [39, 40]. The self-generation of chemoattractant gradient is also used by melanoma cells [41, 42]. Melanoma cells placed in a medium containing LPA start to migrate after locally breaking down LPA using the lipid phosphatase 3 (LPP3) and creating a substantial outward-facing LPA gradient [41, 42]. This phenomenon is facilitated by high cell density, which increases LPA hydrolysis. This idea is in line with the fact that the “Breslow thickness”, which gives an indication of the tumour cell density, is the most significant factor of poor prognosis for melanoma [43].

Collective polarization and mechanosensing

Both mechanical (substrate rigidity) and geometrical (confinement) characteristics of the cell environment influence the polarisation, the motility and the collective behaviour of migrating collectives.

Single cells can sense and follow gradient of ECM rigidity in a process called durotaxis (Figure 3B). Migrating collective can also undergo durotaxis, but the process appears much more efficient as it can be triggered in conditions where single cells are unable to durotax [44]. Making a parallel with what is observed during single cell durotaxis, it has been proposed that the control at the multicellular scale of the dynamics of cell-ECM adhesion via the focal adhesion molecular clutch, may explain the collective durotaxis [45] (Figure 3B). Transmission of acto-myosin forces across the cell monolayer via cell-cell junctions is essential [44], and may lead to an increased sensitivity to shallow rigidity gradient by integrating focal adhesion mechanical responses through a wider distance. While not analysed in this study, contribution of cell-cell contacts in the

generation of cell polarity signalling is likely to play a key role. Although durotaxis *in vivo* has not been described, a beautiful example showing the importance of substrate stiffness *in vivo*, was described by Barriga *et al.* where they found that NCC in the *Xenopus* embryo undergo collective migration in response to a physiological change in the mesoderm stiffness underneath them. In this case only *via* establishing and maintaining cell-cell contacts, the cluster is able to keep directionality [46].

As single cell migration is influenced by the confinement which controls cell adhesion and spreading [47], force generation and speed of migration, the motile behaviour of confluent epithelial cells are influenced by confinement of the cell monolayer. When plated in domains of limited size epithelial monolayers exhibit collective pulsations as persistent random walkers adapt their motion to that of their neighbours. These results demonstrate that epithelial confinement alone can induce spontaneous collective motility and morphogenesis-like processes [48]. The amplitude and period of collective oscillatory motion of the confined epithelial sheets are dictated by the smallest confinement dimension [49]. At the single cell level the actin flow acts as a sensor of the cell edge curvature to influence migration at the single cell level [50]. It is tempting to speculate that cytoskeletal connection between cells of large cohorts may be used to sense mechanical information on a larger scale and adapt collective migration to the geometry of the environment. Accordingly cell-cell junctions transmit the forces and adapt polarity signalling to generate the collective behaviour observed in confined environment [49].

MDCK cells migrating in strips with a smaller width display an increased speed of migration, pointing to the hypothesis that a geometrical constraint would reduce the uncertainty of the leader cells and provide more directionality to the cluster [51]. Szabò *et al.* showed the importance of confinement during *in vivo* migration of NCCs in *Xenopus* embryos and identified the proteoglycan versican as confining molecule which acts as non-permissive substrate for the NCCs migration [52]. Chemotaxis alone cannot compensate for the lack of versican-induced confinement. More recently Semaphorin 3A was also shown to induce cephalic NC confinement by reducing cell adhesion to the extracellular matrix and Rac-dependent protrusive activity and thereby preventing SDF1-induced migration [53].

Conclusions and perspectives

Collective migration relies on the polarisation of the migrating cell group. Similarly to a single migrating cells, the front of a migrating collective points to the direction of migration and generate most of the forces necessary for the forward motion, while the rear is devoid of stable protrusive activity and instead often display a contractile periphery. Not only the polarity and the cytoskeletal organisation but also the energy level are coordinated at the multicellular. The collective behaviour appears to be mainly insured by cell-cell interactions and accumulating evidence point to the crucial role of direct cell-cell contacts and in particular of cadherin mediated adherens junctions. Cell-cell interactions insure the cohesion of migrating cell groups and they also contribute to the identification of leader cells at the front. Moreover they serve as mechanical bridges that transmit forces between the front and the rear of the cell collective and also as signalling relays which transduce polarity signalling. It is however likely that other structures involved in cell-cell communication, such as gap junctions, are involved. Biochemical and mechanical coordination between cells allows the cell group to sense chemokine gradient, rigidity gradient, geometry of the environment at a multicellular scale. In complex *in vivo* environment, the interplay between contact inhibition of locomotion (CIL) and chemotaxis, or chemotaxis and ECM-cell interactions may also trigger more efficient collective migration. For instance, in human

hepatocarcinoma cells, cell-cell junctions trigger an increased level of chemokine receptors at the plasma membrane which promotes migration [54]. Furthermore, mathematical simulations have suggested that several chemoattractants must act together to define complex and long trajectories. For instance, three chemoattractants are needed to position the trunk NCC into the SG and the DRG [55]. Whether migrating collectives are better at integrating the effects of a large number of factors will need to be investigated.

Aknowlegments

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Figure 1: Front-to-rear polarity in single and collectively migrating cells. This figure illustrates that a parallel can be drawn between the front-to-rear polarity of single migrating cells in which the leading edge is characterized by a higher RAC/CDC42 activity at the front and the inhibition of Rac-mediated protrusion on the lateral and rear sides of the cell (**A**) and what is observed in migrating collectives such as epithelial monolayers (**B**) or *Drosophila* border cells (**C**). In case of collective migration, cell-cell contacts between cells organize the actin cytoskeleton to transmit forces from the front to the rear and limit lateral or rear protrusions. They also contribute to feedback mechanisms improving front-to-rear polarity of the cell group.

Figure 2: Energy levels affect front-to-rear polarity in collectively migrating cells.

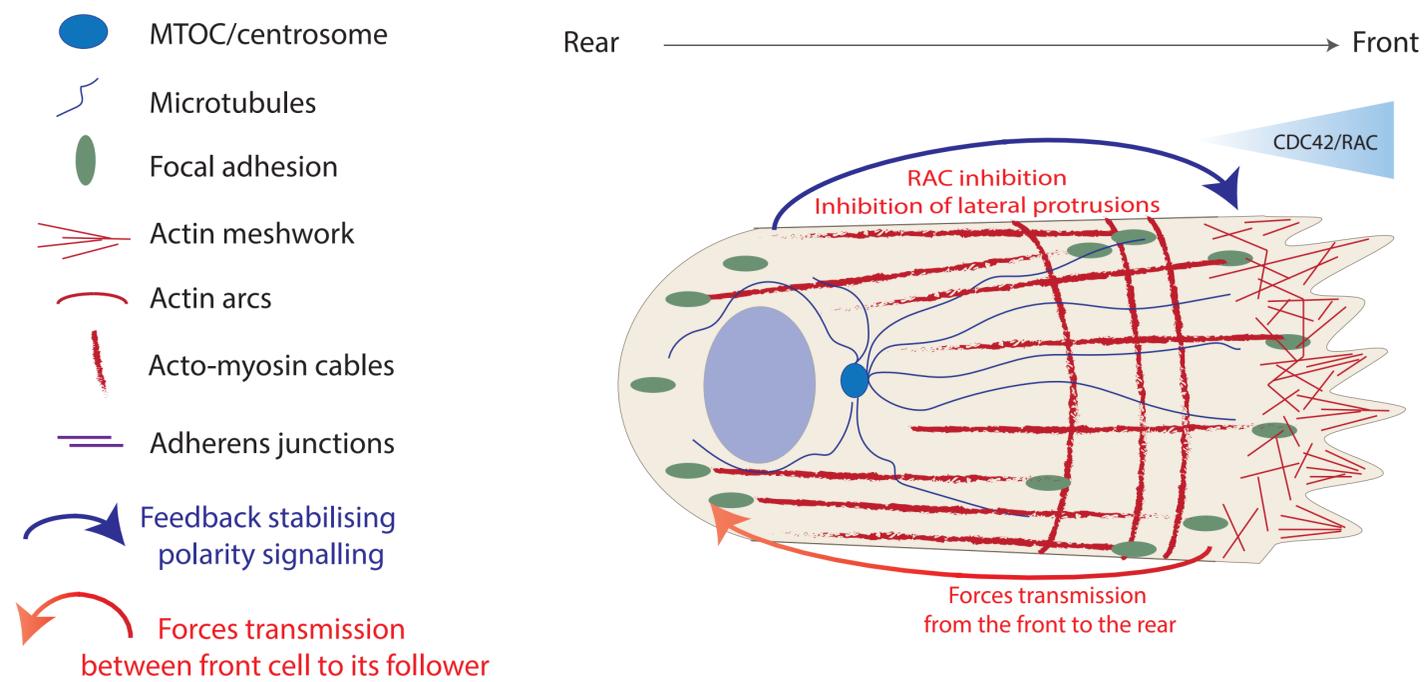
During collective migration in a 3D complex environment, leader cells use more energy than the followers to create the migration path and facilitate the migration of followers. When the energy level of the leaders becomes too low to achieve these tasks, a follower with a higher energy level takes over. The overall front-to-rear polarity and the high speed of migration can thus be maintained.

Figure 3: Similarities between single cell and collective chemotaxis and durotaxis.

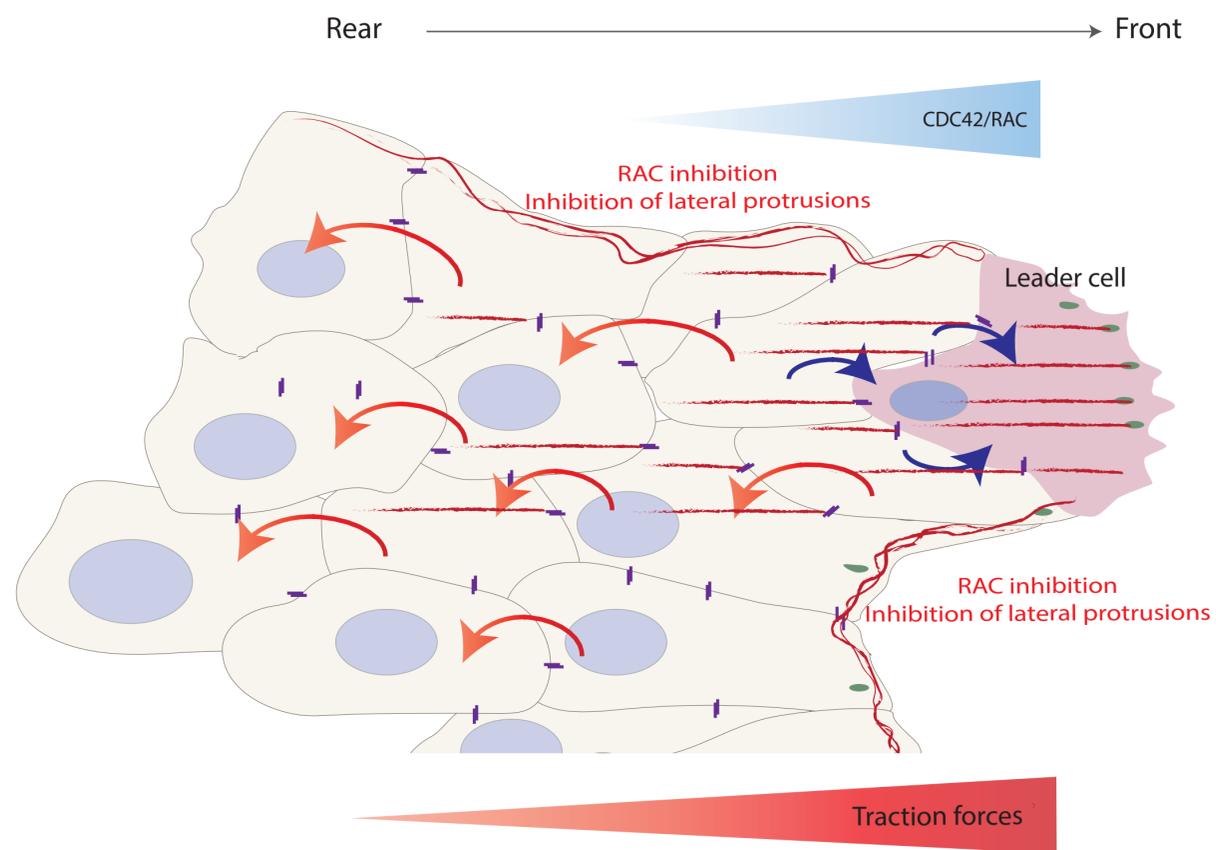
Cell groups like single cells can polarize in response to biochemical (chemotaxis, **A**) and rigidity (durotaxis, **B**) gradients present in the microenvironment. Large cell groups are able to sense and respond to more shallow gradients. Moreover, once polarized cell groups can generate their own chemical gradient, as well as an mechanical easier migratory path. The multi-cellular scale polarization thus allows migrating collective to undergo a more directed migration.

Figure 1

A. Single migrating cell (typical fibroblast cell)



B. Collective migration of a cell sheet (epithelial cell migration)



C. Collective migration of a Drosophila border cells

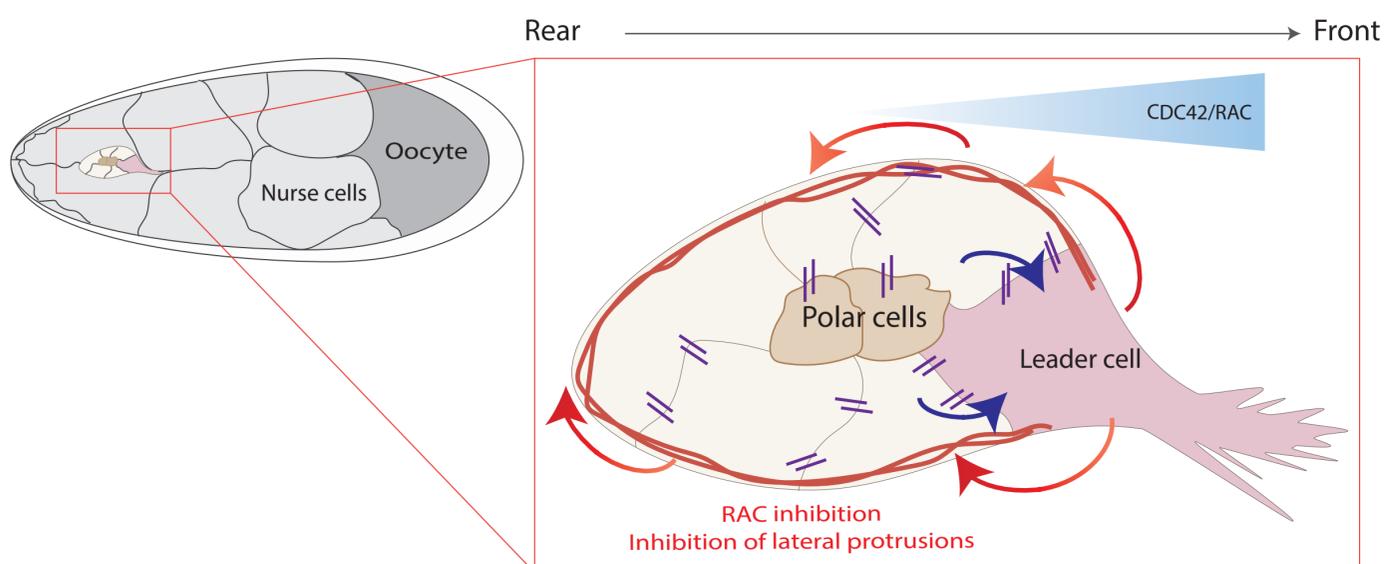
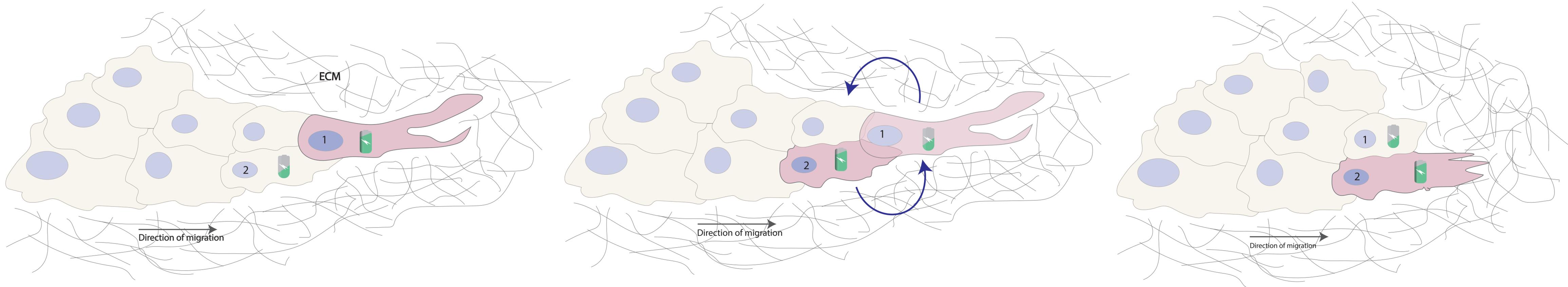


Figure 2



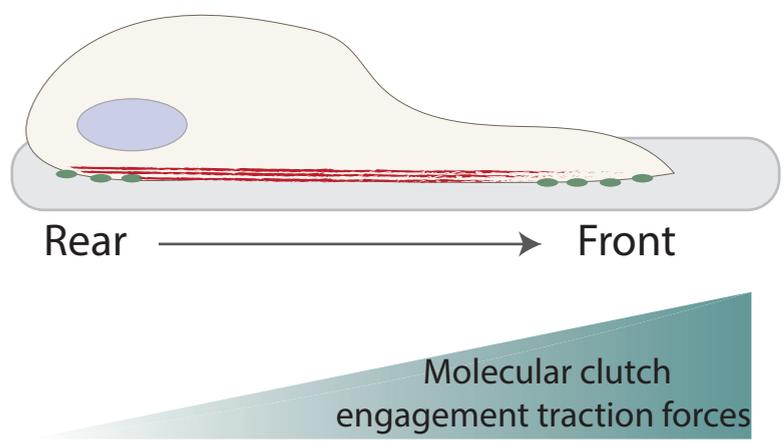
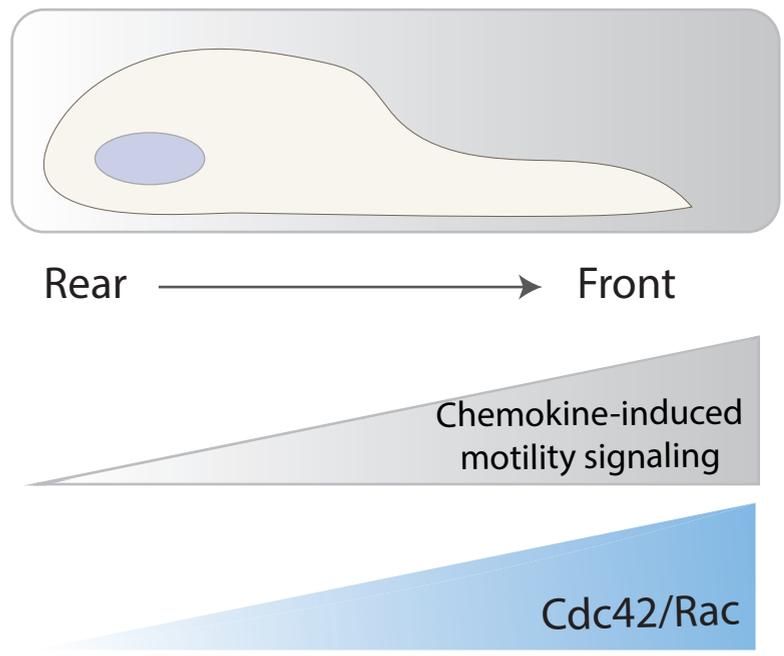
A cell with high energy level become a leader (1).

The leader (1) consumes some of its available energy for migration.
The follower (2) has now higher energy level than the leader.

The follower (2) becomes the new leader.

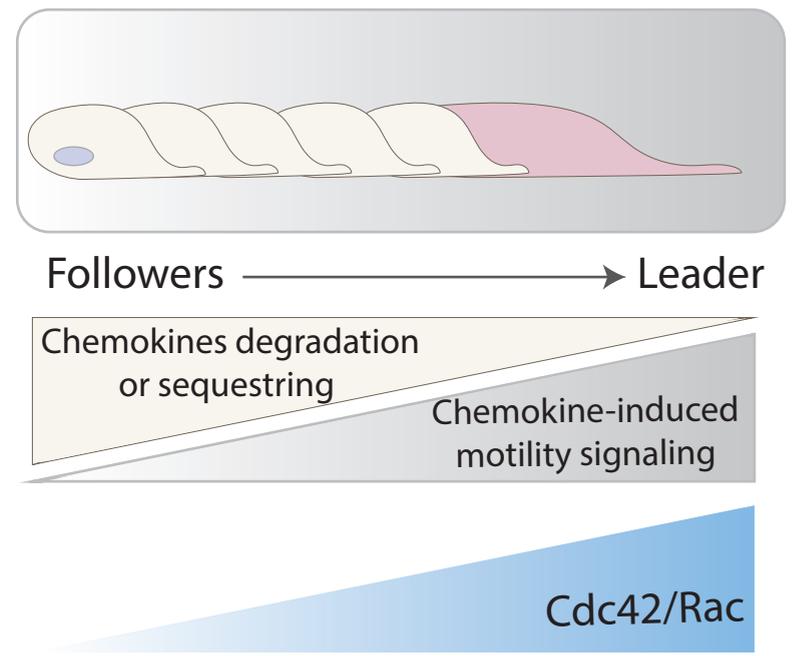
Figure 3

Single cell migration



Collective cell migration

Chemotaxis



Durotaxis

