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REVIEW

Self-organized cell migration across scales – from single cell movement to tissue formation

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ABSTRACT

Self-organization is a key feature of many biological and developmental processes, including cell migration. Although cell migration has traditionally been viewed as a biological response to extrinsic signals, advances within the past two decades have highlighted the importance of intrinsic self-organizing properties to direct cell migration on multiple scales. In this Review, we will explore self-organizing mechanisms that lay the foundation for both single and collective cell migration. Based on *in vitro* and *in vivo* examples, we will discuss theoretical concepts that underlie the persistent migration of single cells in the absence of directional guidance cues, and the formation of an autonomous cell collective that drives coordinated migration. Finally, we highlight the general implications of self-organizing principles guiding cell migration for biological and medical research.

KEY WORDS: Cell migration, Self-organization, Symmetry breaking

Introduction

The ability of cells to migrate is an underlying principle that governs various biological processes ranging from development to physiology and disease. During embryonic development, for example, proper tissue arrangement, which lays the foundation for the future body architecture, crucially depends on large-scale migration events (Scarpa and Mayor, 2016; Solnica-Krezel and Sepich, 2012; Weijer, 2009). Similarly, an active immune response requires immune cells to migrate over large distances within an organism to fight off pathogens and reach sites of inflammation (Hampton and Chtanova, 2019; Krummel et al., 2016). Mechanisms regulating cell migration can also be repurposed by aberrant cells in the context of disease. Most noticeably, one cause of cancer progression is cells spreading from the primary tumor to populate secondary sites in the form of metastases (Chambers et al., 2002; Friedl and Wolf, 2003). In that regard, understanding the molecular regulation of cell migration will not only improve our understanding of how complex life is formed, but also our ability to tackle disease.

Cells can have different modes of migration (see Box 1). Importantly, they can switch between states in a highly dynamic and adaptable manner, as seen in both single cells and cell collectives. Despite this complexity, migrating cells share one main characteristic, i.e. a front-rear polarity along the axis of migration (de Pascalis and Etienne-Manneville, 2017; Mayor and Etienne-Manneville, 2016). Cell polarity and migration have long been thought to be initiated and guided exclusively through external signals (Roca-Cusachs et al., 2013; Schier, 2003; Wang and Knaut, 2014), yet in recent years the intrinsic ability of cells to establish and guide their own migration gained considerable attention (Carmona-Fontaine et al., 2008; Haas and Gilmour, 2006; Harris et al., 2012; Tweedy et al., 2016b).

The general concept of self-organization was first discussed towards the end of the 18th century by philosopher Immanuel Kant, who described an organism as a unit in which the origin of each part depends on the existence of the other parts (Kant, 1790). Today, self-organization has been defined as the emergence of order in a system based solely on the collective interaction of its individual components. In this way, each component on its own would not be able to account for the properties of the entire system. To achieve self-sustainability, individual components are functionally linked to each other in cause-and-effect relationships, and the entire system is built on a network of feedback loops, rather than linear connections (Karsenti, 2008; Sthijns et al., 2019).

Throughout the past two centuries, self-organizing systems have been described in various areas of science, ranging from social behavior in insects and mammals (Bonabeau et al., 1997; Couzin and Krause, 2003) to spontaneous folding of proteins (Gerstman and Chapagain, 2005; Molkenthin et al., 2020; Phillips, 2009) and self-assembly of nanoparticles (Kotov, 2017; Ponsinet et al., 2017). The first evidence of self-organization in tissue formation dates back to 1910, as Ross Harrison observed the directional outgrowth of *in vitro* cultured nerve fibers in the absence of external guidance signals. However, the self-organizing properties of this system were not clearly recognized at the time (Harrison, 1910). It was only in the 1970s that the autonomous migration of a single cell *in vitro* was appreciated as a self-organizing system (Albrecht-Buehler, 1979; Allan and Wilkinson, 1978; Gail and Boone, 1970; Potel and Mackay, 1979), and only in the 21st century that the importance of self-organizing aspects for cell migration was formally described *in vivo* (Carmona-Fontaine et al., 2008; Haas and Gilmour, 2006; Harris et al., 2012). At first glance, external regulation of cell migration, e.g. through a pre-shaped gradient that the cell merely needs to follow (Haeger et al., 2015), as opposed to self-organization, seems to be a more efficient mechanism and therefore the preferable mode of movement. However, research in recent decades has revealed the limitations and fragility of pre-defined external guidance cues, such as the sensitivity to fluctuations in chemokine levels (Fuller et al., 2010) or the challenge of guiding migration over long distances through a complex environment (Tweedy et al., 2016a). Self-organized migration, on the other hand, presents multiple advantages, including the surprising simplicity of the system, reduction of the network to a handful of key players, and a resilience to environmental fluctuations and genetic defects.

In this Review, we will explore how different levels of cell migration are self-organized, from the polarization of a single cell to the coordinated movement of a cell collective. We aim to highlight emerging concepts of self-organized regulation of cell migration *in vitro* and *in vivo*, focusing mainly on the developing embryo. In addition, we will shed light on the importance and advantages of self-sustainable systems in physiology and disease. It is important to

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Box 1. Different modes of cell migration

Mesenchymal migration

Generally considered the most prevalent mode of migration, mesenchymal migration is characterized by an elongated cell shape and actin polymerization at the leading edge, causing the extension of actin-rich protrusions. Integrin-mediated focal adhesions tether the frontal actin cortex to the substrate, while adhesive contacts in the rear detach. Rear contraction causes a retrograde actin flow that imposes a traction force, thereby pulling the cell forward (de Pascalis and Etienne-Manneville, 2017).

Amoeboid migration

A process characterized by a round cell shape and increased cellular contractility. Several different types of amoeboid migration have been characterized.

Adhesion dependent (e.g. primordial germ cells)

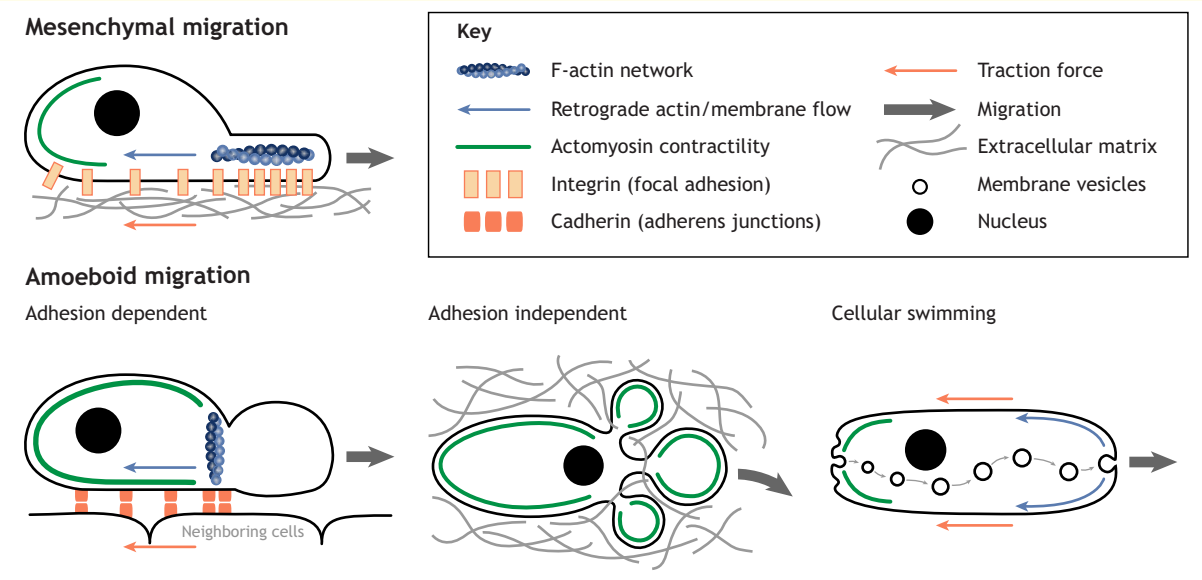
Breaks in the cortex cause the extension of hydrostatic, actin-depleted blebs. Cadherin-mediated adhesion occurs through the actin cortex at the base of the bleb. The high contractility in the cell triggers a retrograde actin flow that generates sufficient traction force to pull the cell forwards (Paluch and Raz, 2013).

Adhesion independent (e.g. leukocytes)

Amoeboid cells can use their ability to easily change shape to move through a three-dimensional environment by extending into gaps in the surrounding structures (e.g. extracellular matrix) (Yamada and Sixt, 2019). Using their nucleus as a mechanical sensor for gaps in the environment, they choose the path of least resistance while still following a global guidance cue (Renkawitz et al., 2019).

Cellular swimming (e.g. macrophages)

High contractility at the rear of a floating cell causes a rearward membrane flow that is further supported by a polarized vesicle trafficking from back to front. The membrane flow exhibits a rearward traction force against the surrounding medium that propels the cell forwards (O'Neill et al., 2018).



note that most aspects of self-organized cell migration discussed below have been studied in different systems and organisms. Hence, many open questions remain regarding the general applicability of these observations, the connection between individual aspects and the differences between systems.

Migration as a single cell

The migration of an individual cell can be subdivided into three steps: symmetry breakage, establishment of a front-rear axis and initiation of movement. These steps have long been thought to be governed by external cues such as chemoattractant gradients (Swaney et al., 2010; Wang and Knaut, 2014) or substrate rigidity (Angelini et al., 2010; Roca-Cusachs et al., 2013). However, studies in the past few decades have highlighted the ability of individual cells to initiate each of these steps autonomously and migrate independently of external signals (Sasaki et al., 2007; Takagi et al., 2008). Here, we will explore the intrinsic regulatory networks that allow single cells to self-organize to accomplish efficient and persistent migration.

Step 1: breaking symmetry

In order to migrate, an apolar cell first has to break symmetry and become polarized – with a distinct front and rear. Symmetry breaking is often triggered by an external stimulus, yet it can also occur spontaneously (Raynaud et al., 2016; Verkhovsky et al., 1999; Wedlich-Soldner and Li, 2003). Independent of its cause, symmetry breaking is governed by an underlying excitable network of intertwined feedback loops (Lindner et al., 2004; Nishikawa et al., 2014). An apolar cell resides in a quiescent state, characterized by continuous small oscillations between active and inactive states of the biochemical networks that regulate cell polarity, at a level that is not sufficient to excite the system. However, sufficiently large perturbations within the network can elevate the system above the activation threshold to an excited state where the cell becomes primed and polarized for migration. Owing to the self-amplifying nature of the feedback system, even small perturbations such as stochastic noise within the cell are, in principle, sufficient to trigger the active state, which can then be stabilized through the feedback network over a long period of time (Huang et al., 2013).

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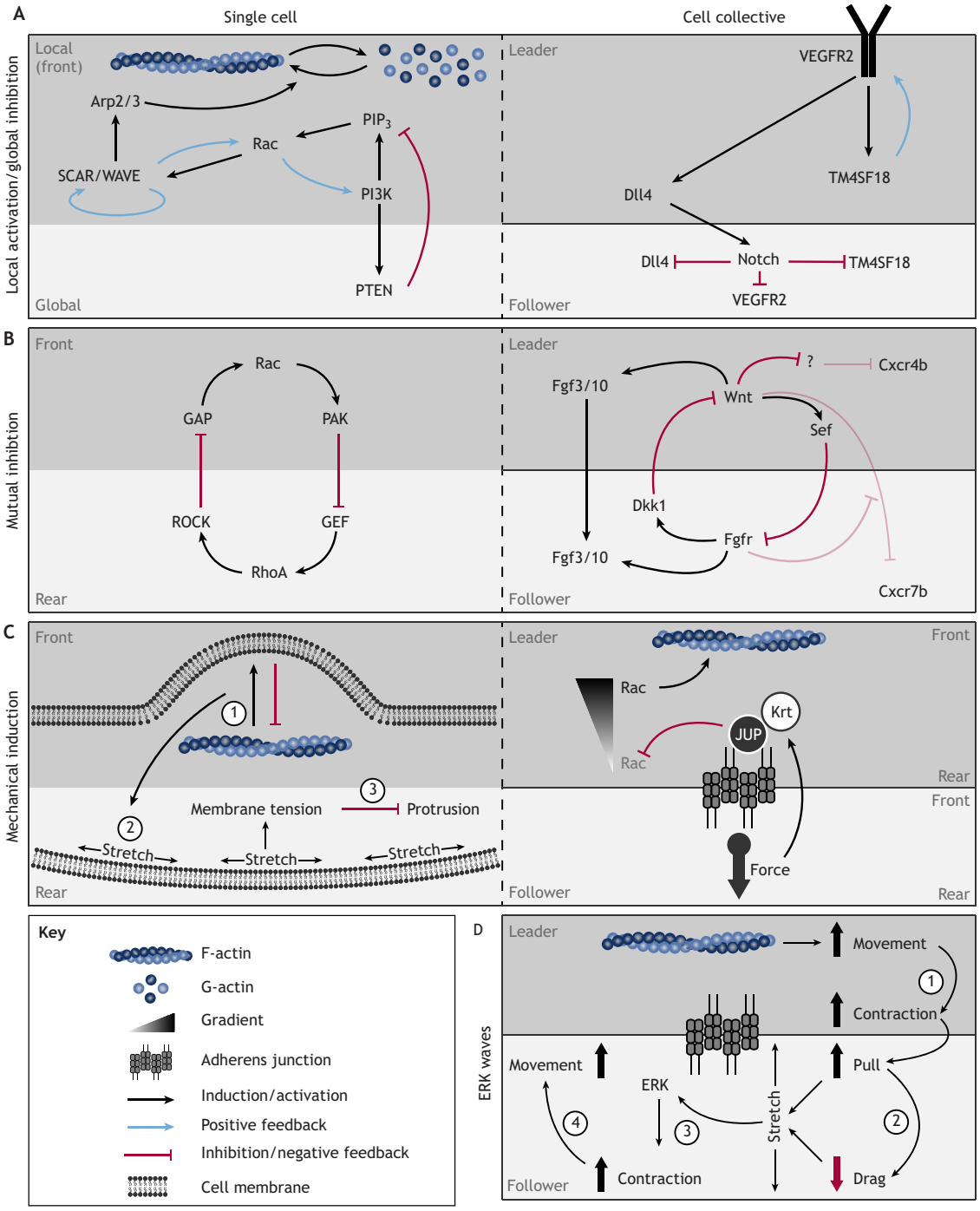

Fig. 1. Self-organized symmetry breaking and establishment of a front-rear axis. (A) Local activation and global inhibition. Single cell (left): the local-excitation-global-inhibition (LEGI) model allows for locally restricted formation of actin-rich protrusions. One example is based on two oscillating systems connected through Rac, centered around synthesis and degradation of PIP_3 on one side, and around actin polymerization and disassembly on the other side. Cell collective (right): lateral inhibition selects leader cells during angiogenesis through local activation of VEGFR2 signaling. The downstream target Dll4 is presented on the membrane and induces Notch signaling in neighboring cells (follower), suppressing VEGFR2 expression and leader fate. (B) Mutual inhibition. Single cell (left): mutual inhibition between Rac and RhoA, mediated by ROCK and PAK, respectively, maintains distinct front and rear domains. Cell collective (right): in the zebrafish lateral line primordium, Wnt and Fgf signaling define leader and follower domains, respectively. Wnt signaling indirectly induces leader-specific Cxcr4b expression and inhibits Fgfr (via Sef) and Cxcr7b expression. Fgf signaling in the follower domain blocks Wnt signaling via the Wnt inhibitor Dkk1, which allows expression of Cxcr7b. (C) Mechanical induction. Single cell (left): lamellipodia formation triggers local extension (1) and global stretching (2) of the plasma membrane. The concomitant increase in membrane tension prevents ectopic formation of additional protrusions (3). Cell collective (right): the drag force in follower cells of epithelial cell sheets, arising through stochastic fluctuations in cell-matrix interactions, or when the rear of leader cells is pulled forward, is transmitted through adherens junctions to the leader cells. Pulling forces at the rear recruit junctional plakoglobin (JUP) and keratin (Krt), which inhibit Rac activity, leading to a Rac activity gradient and subsequent protrusion formation in the front. (D) ERK waves. Forward migration of a leader cell in an epithelial cell sheet (1) pulls the front of the first follower cell forwards and induces its stretching along the migration axis, as the rear remains attached to the second follower cell (2). The cell deformation induces ERK signaling in the first follower cell, which triggers contraction of the rear (3) and forward migration (4). This induces a stretch in the next cell, triggering the propagation of the ERK wave through the tissue.

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Symmetry breaking in the front

At the front of a cell, symmetry breaking is mediated through Rac activity, which connects two independently oscillating systems (Fig. 1A, left). Although still controversial with regard to its general importance (see below), the first network is based on the oscillation of PIP_3 levels. Formation of PIP_3 is mediated by PI3K signaling, while degradation is controlled by PTEN (Comer and Parent, 2002; Insall and Weiner, 2001; Matsuoka and Ueda, 2018). PIP_3 triggers Rac signaling (Huang et al., 2013), but the subtle oscillations in PIP_3 levels in a quiescent state are not sufficient for Rac activation. The second oscillating system is based on actin polymerization. Rac enhances SCAR/WAVE complex activity, which induces actin polymerization while also sustaining Rac activity, thus resulting in a positive-feedback loop (Devreotes and Horwitz, 2015). In the quiescent state, actin is blind to Rac signaling and oscillates between constant polymerization and depolymerization by recruiting its own negative regulator coronin (Brieher et al., 2006; Cai et al., 2007). Local stochastic perturbations in the PIP_3 signaling network, however, can drive Rac activity above the threshold and thus trigger a chain of positive-feedback loops leading to actin polymerization and the establishment of a stable actin network on one side of the cell that allows for cell polarization (Fukushima et al., 2019; Huang et al., 2013).

While PIP_3 signaling has been investigated in much detail, some studies question the necessity of this pathway in cell polarization. For example, experiments in *Dictyostelium discoideum* that block PI3K signaling and thereby PIP_3 production reported no impairment of cell polarization and only minor defects in chemotaxis in the presence of an external chemokine gradient (Hoeller and Kay, 2007; van Haastert et al., 2007; Veltman et al., 2014). This suggests the existence of alternative signaling pathways that can mediate symmetry breaking and polarization, e.g. PIP_5 kinase-dependent signaling, as has recently been described (Fets et al., 2014). Further research will be needed to fully understand the importance of PIP_3 signaling in cell migration, and to identify and characterize possible alternative pathways across different cell types.

Whether mediated by PIP_3 signaling or by alternative pathways, the probability of these spontaneous breaks in symmetry ultimately depends on the threshold level. Artificially decreasing or increasing the threshold by manipulating individual components of the excitable system has been shown to increase or reduce spontaneous initiation of cell migration, respectively (Miao et al., 2017). However, it remains to be determined how the threshold level is regulated *in vivo*.

Symmetry breaking in the rear

Symmetry breaking in the front of the cell was previously considered to be the only entry point into cell polarization, but in recent years the initiation of polarization has also been observed in the back of various cell types (Mseka et al., 2007; Yam et al., 2007). Although this indicates the presence of an excitable system capable of breaking symmetry at the rear of the cell, its molecular mechanisms are not as well understood.

According to current knowledge, an excitable system in the rear of the cell is most likely centered around a positive-feedback mechanism between actomyosin contractility and retrograde actin flow (Yam et al., 2007). Through recruiting its own inhibitor, myosin light chain phosphatase (MLCP), myosin II is thought to oscillate between active and inactive states. Although myosin II activity is known to display oscillating properties in tissue morphogenesis (He et al., 2010; Qin et al., 2018; Zhang et al., 2020), it has yet to be confirmed in the context of self-organized cell migration. In support of an excitable system centered around actomyosin contractility, recent studies have reported that fluctuations in actin dynamics and cell adhesion can induce spontaneous symmetry breaking in the rear (Barnhart et al., 2015).

Global symmetry breaking

The aforementioned molecular networks of spontaneous symmetry breaking in a specific domain mainly lead to the formation of an actin-rich lamellipodium as the defining protrusion of the front that pulls the cell forward (discussed further below). Nevertheless, various cell types are known to migrate based on the formation of blebs, rather than actin-rich protrusions, following an amoeboid-like cell migration behavior (Paluch and Raz, 2013) (see Box 1). The decision to migrate using lamellipodia or blebs depends on the balance between actin polymerization and contractility, with the latter favoring cell blebbing (Bergert et al., 2012). Blebs are caused by local breaks within the actin cortex that allow the membrane to detach and, with sufficient intracellular pressure, extend to form a spherical actin-deficient protrusion (Paluch and Raz, 2013). Owing to a general instability of the cell cortex, breaks can occur stochastically in both space and time, yet are subsequently repaired, resulting in dynamic blebbing around the cell periphery. However, mathematical models over the past decade have proposed that, at a crucial threshold of global cell contractility, a stochastic break in the cell cortex can lead to spontaneous symmetry breaking and polarization of the cell (Callan-Jones and Voituriez, 2013; Hawkins et al., 2011; Recho et al., 2013). Recently, this has been confirmed experimentally in both isolated zebrafish germ layer progenitor cells and human fibroblasts (Liu et al., 2015; Ruprecht et al., 2015). In culture, these cells undergo random blebbing behavior. Upon increase of global contractility, however, the cortical break underlying the bleb causes a local drop in contractility that leads to a steep contractility gradient. This gradient triggers a cortical actin flow that amplifies and stabilizes a single bleb, causing the transition to a polarized state (Liu et al., 2015; Ruprecht et al., 2015). Therefore, at a crucial point of global contractility, stochastic fluctuations in cortical stability can lead to spontaneous symmetry breaking. Importantly, contractility-mediated symmetry breaking acts on a global level and is tightly coupled with the formation of a front-rear polarity axis. Therefore, this bypasses the additional step of establishing a front-rear polarity axis further discussed below.

Step 2: establishing and maintaining front-rear polarity

Once symmetry is broken either at the front or the rear, the signal needs to be propagated to the other pole at the opposite end of the cell to establish a front-rear axis. Cell polarity can be generated through several self-organized signaling networks that are based on: (1) local excitation and global inhibition; (2) mutual inhibition; and/or (3) mechanical forces.

Local excitation and global inhibition

Key to self-organized cell polarity is that the pole at which polarity was initiated maintains its distinct state. This can be achieved through the local-excitation global-inhibition (LEGI) model. LEGI is based on a signal that induces both a short-range activator and a long-range inhibitor (Iglesias and Devreotes, 2012; Meinhardt, 1999). The activator acts fast and locally, and triggers additional positive-feedback mechanisms in close proximity to stabilize the established domain. The inhibitor, on the other hand, diffuses throughout the cell, where it inhibits the formation of ectopic ‘front-like’ domains (Xiong et al., 2010). Therefore, the LEGI model is crucial for maintaining and restricting an established domain but is incapable of inducing the opposing one without any additional signaling networks. The aforementioned PIP_3/PI3K/PTEN network that acts in the front of the cell, is an excellent example of LEGI, with PI3K being the local

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activator and PTEN, after being released from the membrane and diffusing through the cell upon PIP_3 formation, acting as a global inhibitor (Fig. 1A, left) (Gerhardt et al., 2014).

Mutual inhibition

As mentioned above, each domain represses its own activity at the other side of the cell via globally acting feedback mechanisms. In addition, each domain locally blocks the activity of the opposing pole in a phenomenon called mutual inhibition. One well studied example of mutual inhibition in the context of a migrating cell is the opposing activities of Rac and RhoA (Fig. 1B, left). In a simplistic view, Rac is a key regulator of actin polymerization at the front of the cell, where it inhibits RhoA activity. RhoA, on the other hand, is a central mediator of actomyosin contractility at the rear where it blocks Rac activity and thus formation of actin-rich protrusions (Byrne et al., 2016).

It remains to be determined how universal the Rac/RhoA antagonism is, and whether there are other factors that have similar opposing activities. For example, in mouse embryonic fibroblasts (MEFs), RhoA activity has been observed in leading protrusions, questioning the concept of mutual inhibition and a rear-specific role of RhoA (Pertz et al., 2006). Expression of a dominant-negative Rac abolished the activation of RhoA in these protrusions but maintained a protrusive phenotype. Although the antagonism between RhoA and Rac, as well as the function of Rac in protrusion formation is therefore still intact, the maintenance of distinct Rac and RhoA domains is likely not sufficient to establish a front-rear axis.

Mechanical forces

In addition to the biochemical networks governing cell polarity, the accompanying changes in cell shape trigger mechanical forces that support and stabilize the polarized cell (Fig. 1C, left). During formation of a protrusion, the plasma membrane has to extend. Like stretching an elastic band, the growing protrusion causes the plasma membrane to unwrinkle, putting the entire cell under immense tension. The increasing membrane tension surpasses the force imposed by actin filaments and antagonizes actin polymerization, thereby constraining any further extension of the leading edge, and blocks formation of secondary protrusions (Houk et al., 2012). Furthermore, an increase in membrane tension supports the retraction of the rear to release strain. Therefore, the interplay between actin polymerization and membrane tension stabilizes the leading edge and supports induction of the rear (Diz-Muñoz et al., 2013).

Step 3: persistent migration

During biological processes that are characterized by major cell migration events, such as embryonic development (Aman and Piotrowski, 2010) or immune response (Hampton and Chtanova, 2019), cells need to move long distances and populate new territories. Yet, in many cases, the absence of external guidance cues begs the question, how can these cells achieve directional migration? At first glance, persistent directionality of cell migration appears incompatible with the stochastic formation of protrusions around the cell periphery. Theoretically, this uncontrolled initiation of movement would lead to an unbiased random migration behavior that is highly inefficient (Viswanathan et al., 1999). However, the observed migratory behavior of single cells *in vitro* in the absence of external guidance cues is notably different and characterized by two phases. First, a cell undergoes a diffusive phase during which it tumbles around its own position, unable to move forward. Eventually, it transitions into a second phase of directional persistence and the cell is able to move along a relatively straight trajectory for an extended period of time (Harris et al., 2012; Maiuri et al., 2015). Such cell behavior is inconsistent with purely random cell movement, and recent data support the involvement of self-organized regulatory networks that reinforce a stable front-rear axis (Begemann et al., 2019; Theisen et al., 2012).

Maintaining directional persistence in the front

The cell front is a highly dynamic structure, undergoing constant cycles of extension and retraction. Upon retraction, the cell has the possibility to redefine its front-rear axis and polarize in a different direction. Therefore, the key to persistent, self-organized migration is to ensure the new lamellipodium is formed in close proximity to the previous one.

Work in *in vitro* cultured fibroblasts has indicated that directional persistence at the front can be regulated through mechanochemical communication between old and new lamellipodia, which is based on the sensing of membrane curvature (Begemann et al., 2019). This effect is mediated by I-BAR domain-containing proteins that bind to regions of negative membrane curvature, such as the inside of a lamellipodium (Millard et al., 2005; Saarikangas et al., 2009). Through signaling to Rac and the Scar/WAVE complex, I-BAR proteins can initiate actin polymerization and hence protrusion extension in mammalian cells (Begemann et al., 2019; Miki et al., 2000). Upwards bending of a lamellipodium upon retraction leads to a change in membrane curvature, which induces the accumulation of I-BAR proteins and local activation of actin polymerization. This causes formation of a nascent lamellipodium directly underneath the retracting one, and thus maintains the same direction of migration (Begemann et al., 2019) (Fig. 2, right).

Despite evidence from mammalian cells for a role of I-BAR proteins in the mechanical regulation of cell migration, it is still unclear how universal this mechanism is. For example, work in *Dictyostelium* indicated that I-BAR proteins are not necessary for protrusion formation and chemotaxis, even in shallow chemokine gradients (Veltman et al., 2011). Whether I-BAR proteins are dispensable, not only for protrusion formation in general but for persistent migration of *Dictyostelium*, in particular in the absence of external guidance cues, has to be further investigated. Nonetheless, these insights indicate that the above-described mechanism might be cell-type specific, and that there could be alternative pathways maintaining directional persistence that have yet to be uncovered.

Maintaining directional persistence in the rear

Like the cell front, the rear is dynamic, as it maintains a constant balance between focal adhesion and retraction. In certain cell types, such as keratinocytes or fibroblasts, after retraction of the lamellipodium, cell polarity is re-initiated with establishment of the rear, rather than the front (Cramer, 2010; Mseka et al., 2007; Rid et al., 2005). Therefore, a full retraction of the rear reverts the cell to a naïve state in which it can initiate polarization in any new direction. However, maintaining a constant level of focal adhesion in the rear while the cell moves forward can stabilize the front-rear axis. This is achieved through a treadmilling mechanism that involves continuous extension of integrin-based focal adhesion sites in the front-facing part of the cell rear and integrin removal in the back (Theisen et al., 2012). In addition, this treadmilling mechanism applies a drag force on the cell front, which has been shown to promote protrusion formation in the opposite direction. Thus, the cell rear can stabilize the directional persistence in the front (Fig. 2, left).

Maintaining directional persistence globally

In addition to local stabilizing mechanisms, persistence in cell migration is also mediated through global feedback loops, which connect front and rear domains. As discussed above, local breaks in

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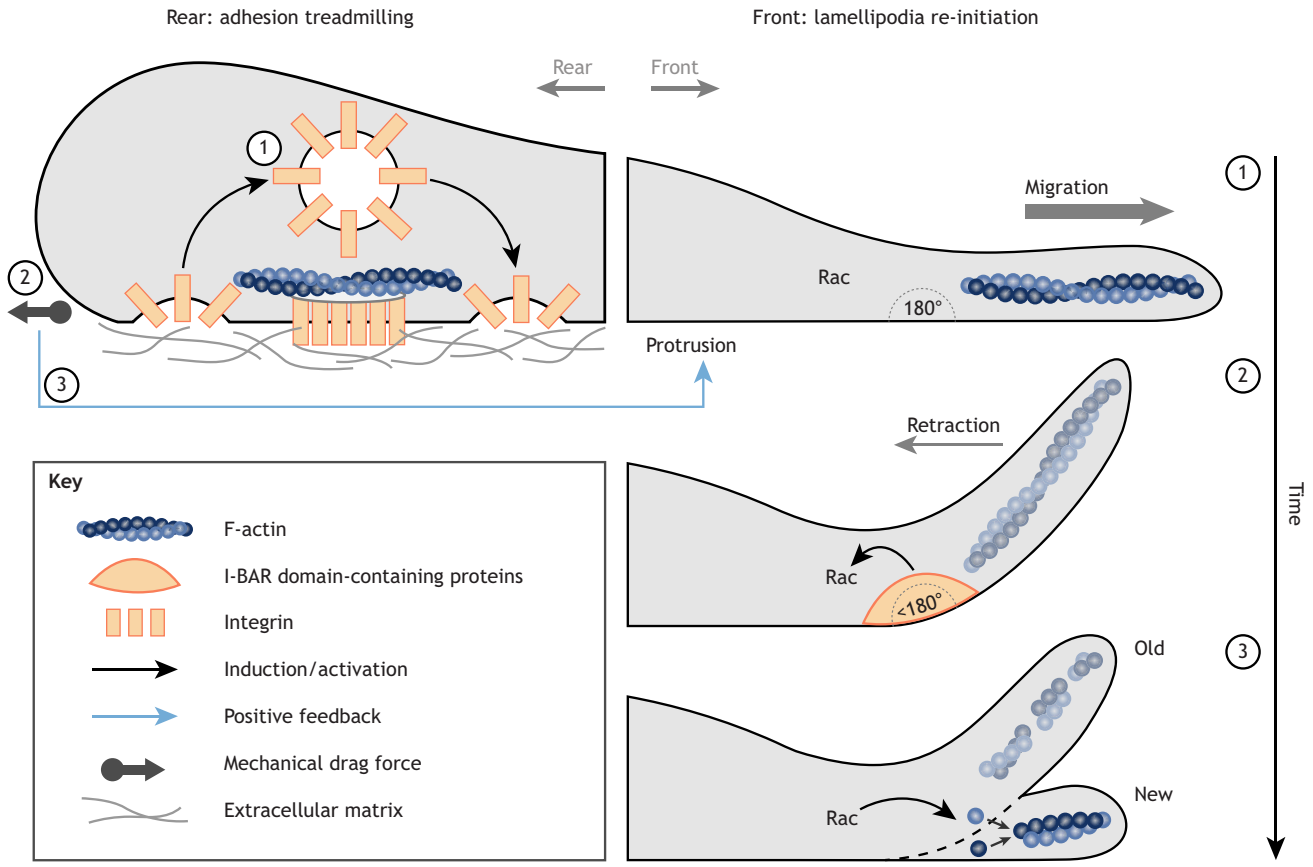

Fig. 2. Directional persistence in self-organized single cell migration. Persistence at the front of an individually migrating cell (right) is maintained through mechanochemical connections between the retracting (old) and the nascent (new) lamellipodium (1-3). Membrane deformations upon retraction are sensed by I-BAR domain-containing proteins and transmitted to Rac signaling (2), inducing actin polymerization and lamellipodia formation at the site of the old lamellipodium (3). Persistence at the rear (left) is maintained through the treadmilling of integrin adhesion sites (1), which prevents complete retraction of the rear. Through this continuous adhesion to the ECM (extracellular matrix), a drag force is constantly applied to the cell rear (2), which stabilizes protrusion formation at the opposite end of the cell (3).

the actin cortex in combination with high global contractility can lead to spontaneous symmetry breaking and the establishment of front-rear polarity (Paluch and Raz, 2013). The local disruption of the actin cortex at the future cell front leads to a local drop of contractility, which initiates the establishment of a front domain and causes actin flows from the region of low to high contractility. Thereby, actin-binding proteins, such as the contractility regulator myosin II, are transported to the opposite end of the cell: the rear domain. This relocalization of molecules connected to actomyosin contractility reinforces cortical flows, leading to a positive-feedback loop that stabilizes the front-rear axis and promotes protrusion (Liu et al., 2015; Ruprecht et al., 2015). Indeed, cell migration assays in various cell types, following different modes of migration, including bleb and lamellipodia based, have demonstrated a causality between cortical flow and migratory persistence, as well as cell migration speed (Maiuri et al., 2015; Yolland et al., 2019).

Migration as a cell collective

Within an organism, cells often do not migrate individually, but rather move in a collective manner to shape tissues or populate new areas (Scarpa and Mayor, 2016; Weijer, 2009). As previously described (Shellard and Mayor, 2019), there are two modes of collective cell migration. In one mode, cells within the collective polarize individually but depend on communication and interaction with other cells in the cluster for efficient migration, e.g. as seen in axial mesendoderm in zebrafish (Dumortier et al., 2012). In the other mode, the cell collective moves as a supracellular unit characterized by an overarching polarity with distinct leader and follower cells, which are dependent on each other and are not able to migrate persistently on their own, as described for the folding of epithelial sheets during ventral furrow formation in *Drosophila* (He et al., 2014). Most cases of collective cell migration, however, seem to combine aspects of both individual and supracellular behavior to different extents, e.g. as observed during the morphogenesis of the *Drosophila* follicular epithelium (Barlan et al., 2017) or *Xenopus* neural crest (NC) migration (Carmona-Fontaine et al., 2008; Shellard et al., 2018). Thus, mechanisms of collective migration should be seen more as a spectrum rather than a dichotomy. In the following section, we explore how the supracellular organization of a migrating cell collective is established, and which mechanisms of single cell migration also apply to a cell collective.

Step 1: defining leaders

Like a single cell, a cell collective usually requires front-rear organization for persistent migration yet faces the particular challenge of establishing an overarching polarity across the entire tissue. This is achieved by defining distinct leader and follower cells with specific morphologies and functions. However, in most cases, these two cell types are highly dynamic, with leader cells being transformed into trailing cells, and vice versa (Richardson et al., 2016; Zhang et al., 2019). Leader cells are typically characterized by mesenchymal properties and extend lamellipodia to the front of the

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collective. Follower cells, on the other hand, show increased contractility and actomyosin activity, and thus resemble the rear of a single cell (Mayor and Etienne-Manneville, 2016). Whether follower cells are completely non-migratory and are simply being pulled forward by the leaders or whether they actively contribute force to the cluster migration likely depends on the degree of supracellular organization in the cluster and is still an area of ongoing research. Nonetheless, the establishment of front-rear polarity in a cell collective follows similar principles to those for individual cells and can be achieved through different mechanisms: (1) local activation and global inhibition (or, here, lateral inhibition); (2) mutual inhibition; and/or (3) mechanical forces.

Lateral inhibition

Lateral inhibition is a commonly used mechanism to make cells distinct from their neighbors (Appel et al., 2001; Sato et al., 2016; Sharma et al., 2019; Simpson, 1990; Xia et al., 2019). Lateral inhibition is based on competition between cells, in which a newly defined leader cell prevents the neighboring follower cells from obtaining the same fate (Sjöqvist and Andersson, 2019). This mechanism is employed to define individual leader cells, rather than a leader domain. Therefore, it is commonly used during branching morphogenesis, as seen in angiogenesis or *Drosophila* tracheal branching, in which a single leader cell needs to be established *de novo* within an existing tissue to induce a new branch site (Jakobsson et al., 2010; Llimargas, 1999).

At the molecular level, lateral inhibition is based on Notch signaling. In the example of angiogenic sprouting, a prospective leader cell is triggered through activation of vascular endothelial growth factor receptor (VEGFR) signaling that induces a series of cell-intrinsic feedback loops mediated by the atypical tetraspanin TM4SF18 (Page et al., 2019) to amplify its activity. VEGFR signaling induces the expression and thereby presentation of the Notch ligand Dll4 at the cell surface. Ligand binding to neighboring cells triggers the Notch signaling pathway in these cells, which inhibits VEGFR expression and thereby keeps the neighbors in a follower state (Jakobsson et al., 2010; Page et al., 2019; Tammela et al., 2011) (Fig. 1A, right).

Mutual inhibition

During lateral inhibition, leader cells suppress neighboring cells from adopting the same fate, which leads to the establishment of a single leader cell. As such, lateral inhibition is unable to establish larger leader and follower domains characteristic for supracellular collectives. Mutual inhibition, on the other hand, applies the same concept in both directions and has been observed to establish larger leader and follower domains, as described in the lateral line primordium (LLP) of zebrafish larvae (Aman and Piotrowski, 2008). Here, mutual inhibition is mediated through Wnt (leader) and Fgf (follower) signaling (Fig. 1B, right). Wnt signaling in the leading domain induces Fgf ligand while suppressing Fgf receptor expression. Therefore, Fgf ligands diffuse to the Fgf receptor-expressing follower domain. Fgf binding induces the expression of Wnt inhibitor Dkk1, which in turn suppresses leader properties in the follower domain. Moreover, the antagonistic Wnt/Fgf signaling axis establishes the differential expression of two Cxcl12a-responsive G-protein-coupled receptors that are important for guided cell migration. As discussed further below, the chemokine receptor Cxcr4b is restricted to leader cells. A still unknown inhibitor blocks expression of Cxcr4b in the follower domain, while in the leader domain this inhibitor is counteracted by Wnt signaling. The scavenger receptor Cxcr7b, on the other hand, is specific to the follower cells, as it is directly inhibited by Wnt signaling in the leader domain (Aman and Piotrowski, 2008; Lecaudey et al., 2008; Valentin et al., 2007). The resulting interlinked non-autonomous cell signaling network ensures the persistence of distinct cellular properties at the front versus the rear in a migrating cell collective.

Mechanical induction

Similar to individual cell migration, biochemical signaling pathways act in concert with mechanical forces to regulate front-rear polarity in cell collectives. The presence of a cell collective adds an additional layer of complexity, as the force is transmitted between cells and can span several cell diameters. Therefore, the application of a dragging force by one cell onto a neighbor can be propagated through the cell collective and induce lamellipodia formation in the opposite direction in even more distant cells (Theisen et al., 2012; Weber et al., 2012).

This phenomenon has been observed in wound healing assays and shown to induce leader cell formation. According to this model, spontaneous fluctuations within the cell cytoskeleton or structural inconsistencies in the substrate lead to alteration of cell-matrix tension. These changes in tension in turn apply a pulling force to neighboring cells (Vishwakarma et al., 2018), which induces polarization of the cells with the leading edge being directed in the opposite direction. Through cadherin-based adherens junctions between cells across the entire tissue, this tension can be propagated from one cell to the next until it reaches the edge. Therefore, cells at the edge polarize and extend actin-rich protrusions away from the cluster, forming the leading domain. Although the molecular mechanism of how polarization is achieved remains largely unknown, it has been observed that pulling forces at the rear of the cell recruit plakoglobin to its adherens junctions. Plakoglobin mediates binding of keratin intermediate filaments, both of which have been implicated in the subsequent cell polarization (Weber et al., 2012). Plakoglobin has previously been suggested to inhibit the activity of actin polymerization factors Rac and Arp2/3 (Todorović et al., 2010). By blocking Rac and Arp2/3 activity specifically in the rear, plakoglobin can establish a gradient of polymerized actin filaments within the cell, which could thus explain how polarization is achieved (Fig. 1C, right).

In addition to this model, recent studies proposed a mechanism for epithelial wound healing in which polarity is established through a global interplay between ERK/MAPK signaling and tissue mechanics (Aoki et al., 2017; Boocock et al., 2020; Hino et al., 2020) (Fig. 1D). Although the described mechanochemical mechanisms are of importance in mediating the connection between ERK signaling and mechanical forces, in this system unidirectional ERK waves propagating from the wound site through the tissue establish tissue polarity and induce cell migration in the opposite direction.

Step 2: stabilizing leader and follower domains

Once leader cells are defined, the cell collective needs to establish and maintain polarity, with distinct leader and follower domains. Each domain has a distinct role in eventually moving the entire collective forward. A combination of mechanical and biochemical communication between individual cells and between domains ensures that every cell takes on the appropriate role at the right time and in the right place, thereby allowing migration as one unit.

Mesenchymal characteristics in the leader domain

Front-rear polarity has to be maintained throughout migration in order for it to be persistent. Most importantly, leader cell character has to be stabilized within and restricted to the front domain to prevent spreading of the cell cluster. How this is achieved remains the subject

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of intense research. Different mechanisms have been described in different systems, although it is currently unclear whether each system has evolved its own way of maintaining the leader domain or whether a combination of mechanisms acts together.

One of the key mechanisms of collective cell migration is contact inhibition of locomotion (CIL), which will be discussed in more detail below. The underlying basis for CIL is that cell-cell contact inhibits protrusion formation at the site of collision (Mayor and Carmona-Fontaine, 2010). Therefore, only cells with at least one free side can acquire mesenchymal character, thus restricting this fate to the outer cells of a collective.

Although CIL partially explains why outer but not inner cells can adopt mesenchymal character, how these characteristics remain restricted to cells at the front of the cluster and inhibited in lateral cells is still largely unclear. Studies in wound healing assays have observed the presence of supracellular actomyosin cables and coinciding RhoA activity, both in the rear and the sides of coherently migrating cell clusters (Reffay et al., 2014). As RhoA activity is known to prevent lamellipodia formation (Byrne et al., 2016), this is a possible explanation for the lack of lamellipodia formation in these regions. However, how these actomyosin cables are established in the first place and whether there is a feedback connection between these cables and lamellipodia formation in the front, and thus a possibility for self-organization, is still unclear.

Contractility in the follower domain

How the rear cells contribute to active forward migration of a collective is still largely unknown. The rear of a single cell is characterized by increased actin-myosin contractility. A similar feature, but organized in a supracellular manner, has been observed in *Xenopus* NC cells (Shellard et al., 2018). Connected through N-cadherin junctions between cells, a supracellular actomyosin cable spans the entire width of the cell cluster along the back. That way, all rear cells contract simultaneously, mimicking one giant cell. This contractility gradient triggers a retrograde flow of peripheral cells from front to back (Shellard et al., 2018), conceptually similar to retrograde membrane flow in single cell migration (see Box 1). To maintain the shape of the cluster, cells in the rear are pushed to intercalate between their frontal neighbors, making their way through the entire tissue until reaching and replacing the leader cells in the front (Shellard et al., 2018). Whether a similar process is in place in other systems or whether there are alternative mechanisms providing contractility in the rear requires further investigation.

Furthermore, a molecular connection between leader and follower domains, that would indicate a self-organization in NC migration, remains to be identified. However, artificial induction of contractility on one side has been shown to induce a leader domain on the opposite site, hinting towards the existence of feedback loops between the two domains (Shellard et al., 2018).

Step 3: providing direction

As discussed above, a single cell that is placed in an environment without external guidance signals can, due to self-organized mechanisms, maintain a certain degree of directional persistence. In a migrating collective, the sheer number of migrating cells provides an additional layer of directionality control, which has been shown to support persistent migration even in the absence of a chemokine gradient. Using two example mechanisms described in different systems, we will explore how cell collectives can maintain directional persistence and what advantages these mechanisms bring to the developing organism.

Contact inhibition of locomotion

Contact inhibition of locomotion (CIL) is a phenomenon in which, upon cell-cell contact, the formation of protrusions is inhibited at the site of collision. As stated above, this assists in polarizing supracellular systems by restricting lamellipodia formation to the edge of the cell cluster. However, CIL, in combination with additional features, such as mutual attraction, has also been shown – through computational modelling approaches – to increase directional persistence (Carmona-Fontaine et al., 2008, 2011) (Fig. 3A).

The outcome of CIL largely depends on cell density. In a dense cluster, all inner cells are tightly attached to neighbors and therefore inhibited to form protrusions at all sides, with the exception of cells at the cluster edge. In collectives with more loosely attached cells that are individually polarized and migratory (as shown in Fig. 3A), colliding cells can re-polarize and extend a new protrusion in the opposing direction until they encounter another cell (Mayor and Carmona-Fontaine, 2010; Roycroft and Mayor, 2016; Stramer and Mayor, 2017). Thus, cells are constantly repelled from the point of highest cell density, which biases their migration towards unpopulated regions. Migration solely based on CIL therefore leads to cell dispersion until the cell density is too low and collision events are not frequent enough to provide any directional bias, e.g. as seen in Cajal-Retzius cells (Villar-Cerviño et al., 2013). Thus, to keep the collective coherent, additional layers of control are required, e.g. physical borders, that can restrict cell dispersion. On a molecular level, though, NC cells have, for example, been shown to additionally express the chemoattractant C3a and the corresponding receptor C3aR, which cause the cells to attract each other. This mutual attraction after repulsion balances out the CIL-caused dispersion of cells and allows for a more efficient and persistent migration (Fig. 3A) (Carmona-Fontaine et al., 2011).

Two different mechanisms have been described to be responsible for CIL. In *Xenopus* neural crest migration (Fig. 3B, left), the planar cell polarity (PCP) pathway is activated locally at the site of collision, leading to a local activation of RhoA. Owing to the accompanying RhoA-induced increase in contractility, a new cell rear is established. As described above, this is sufficient to induce a new cell front at the opposite end, leading to repolarization of the cell (Carmona-Fontaine et al., 2008). An alternative mechanism has been described in head mesoderm of *Drosophila* embryos (Fig. 3B, right), in which CIL depends on transient adhesion between the two colliding cells and subsequent remodeling of the cytoskeleton. Two colliding cells form cadherin-mediated adherens junctions that connect the actin networks of both cells through interacting with the cytoskeleton. This brief fixation blocks retrograde actin flow in both cells and therefore causes the collapse of the destabilized lamellipodium. Furthermore, the collision event disrupts growth of microtubules supporting the leading edge, which have been shown to depolymerize when encountering an immobile object, in this case the colliding cell (Janson et al., 2003; Laan et al., 2008). The role of the microtubules network during CIL is not fully understood; however, it has previously been suggested to mediate the release of the adhesive contact between the two cells (Stramer et al., 2010). Once the adhesion is released, the cortical tension suddenly decreases, which leads to spontaneous contraction of both cells at the collision site and subsequent cell polarization in the opposite direction (Davis et al., 2015).

Self-generated chemokine gradients

One of the most common external guidance cues for directional cell migration is a chemokine gradient. Traditionally, chemokine gradients were thought to be formed through a localized source, as

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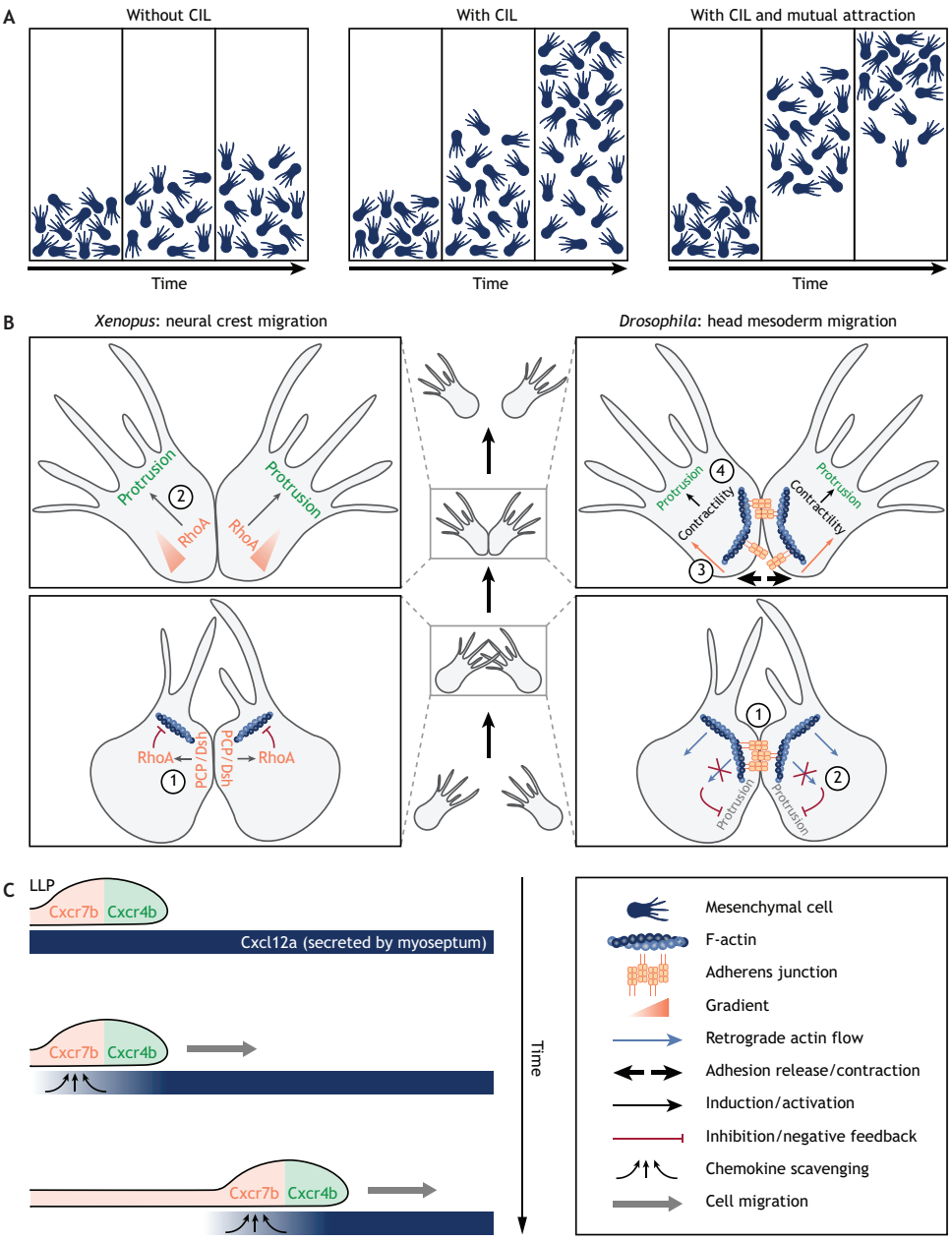

Fig. 3. Directional persistence in self-organized collective cell migration. (A) Collective migration through contact inhibition of locomotion (CIL). Without CIL (left), cells migrate randomly with very little net displacement. With CIL (middle), cells are repelled from regions of highest cell density and spread out. The presence of physical boundaries adds an additional level of directionality by preventing uncontrolled radial dispersion of cells. CIL in combination with mutual attraction between cells (right) allows for collective migration with a directional bias. Adapted from computational modeling of Carmona-Fontaine et al. (2011), where it was published under a CC-BY 4.0 license. (B) In *Xenopus* neural crest cells (left), CIL relies on PCP signaling. Accumulation of PCP factors at the collision site activates RhoA signaling (1) and therefore local cell contractility. The newly established RhoA gradient induces actin polymerization and extension of nascent protrusions away from the site of collision (2). In *Drosophila* head mesoderm (right), CIL is mediated by tethering of the actin cytoskeletons of the colliding cells through adherens junctions at the site of collision (1). This induces an abrupt stop of actin retrograde flow and the collapse of the lamellipodium (2). Release of the adhesion causes spontaneous contractility at the collision site (3), defining the new cell rear, and inducing actin polymerization and protrusion formation at the opposite side of the cell (4). (C) Self-generated gradients. In the environment of a uniformly expressed chemokine, cell collectives can self-generate a chemokine gradient. In the zebrafish lateral line primordium (LLP), the scavenger receptor Cxcr7b removes the uniformly expressed chemokine Cxcl12a in the rear of the tissue. This forms a Cxcl12a gradient that is sensed by Cxcr4b in the front of the tissue, allowing for directed cell migration.

described in primordial germ cell (Doitsidou et al., 2002) or neutrophil migration (de Oliveira et al., 2013). However, recent studies have discovered multiple examples in which a cell collective has the capability to self-generate a gradient from a uniform chemokine distribution, making a pre-formed gradient unnecessary. By breaking down the chemokine itself, the migrating tissue continuously generates a local concentration gradient and can thereby guide its own migration (Tweedy et al., 2016b). This phenomenon was first described in *Dictyostelium* (Sucgang et al., 1997; Tweedy et al., 2016a) and in recent years has been observed *in vivo* for the migration of the LLP in the developing zebrafish larva (Donà et al., 2013; Valentin et al., 2007; Venkiteswaran et al., 2013).

The LLP arises as a cluster of approximately 100-150 cells (Agarwala et al., 2015) behind the developing ear and migrates along the underlying myoseptum on either side of the zebrafish larvae towards the tail tip. Its migration is driven by the chemokine Cxcl12a and its corresponding receptor Cxcr4b. Cxcl12a is secreted by cells of the myoseptum and initially equally distributed along the path of migration (Dalle Nogare and Chitnis, 2017). To generate the guidance cue in the first place, the scavenger receptor Cxcr7b is expressed in the follower domain of the LLP (as discussed above). Cxcr7b binds, internalizes and thereby removes Cxcl12a from the environment, specifically in the rear region. This generates a locally confined yet steep gradient across the LLP. Meanwhile, the front of the LLP expresses Cxcr4b, which reads the self-generated gradient and drives the migration of the LLP towards higher concentrations of the chemokine. Unlike a stationary sink, the Cxcr7b-expressing cells migrate along the gradient together with the entire LLP tissue. Thus, as the tissue migrates, a local gradient is continuously formed and provides a self-generated guidance signal (Donà et al., 2013; Haas and Gilmour, 2006; Valentin et al., 2007; Venkiteswaran et al., 2013) (Fig. 3C).

A self-generating gradient is simple, yet extremely robust, as it does not depend on a pre-defined, precisely shaped gradient. The

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establishment of a pre-existing gradient, which usually requires a complex molecular network to define the source, sink, counter gradients, diffusion rates and more is unnecessary. Furthermore, a self-generated gradient provides a higher robustness in navigating through complex environments and allows for guidance over a long distances, whereas pre-existing long-range gradients are usually very shallow and, hence, more difficult to follow (Tweedy and Insall, 2020; Tweedy et al., 2019 preprint). Finally, the self-organizing system can handle a wide range of ligand concentrations and compensate for severe ligand overexpression through receptor turnover, making it a robust and reliable system for a developing organism (Lau et al., 2020; Wong and Gilmour, 2020; Wong et al., 2020).

Conclusions and perspectives

Self-organization is an underlying concept driving both individual and collective cell migration. Within a single cell, individual molecular regulators engage in a complex network of feedback loops that allow symmetry breaking and self-sustained migration. In a collective, each cell forms an individual component of the whole. The interaction between these individuals, whether through physical contact, mechanical forces or cell signaling, establishes an overarching order that allows for collective migration.

In this Review, we have presented general concepts and selected examples that demonstrate the importance of self-organized cell migration during embryonic development. Understanding how self-organization is achieved by migrating cells also has important implications for medical research. Immune cells are some of the most migratory cells in the body and are known to employ mechanisms of self-organization for their movement. Immature T cells, for example, constantly search for antigens and target cells. To this end, they migrate long distances without any directional cues. *In vivo* studies have shown that T cells, in the brain in particular, undergo a Lévy walk: a type of migration that comprises the two phases of motility (diffusive and persistent) we have described above. This mode of migration is mediated through self-organized intrinsic directionality. This enables T cells to reside in a specific location long enough to recognize a potential antigen and to transmit an activating signal, while still covering a sufficiently large area during their search (Harris et al., 2012; Krummel et al., 2016).

This is just one example of the importance of self-organized cell migration for our health and immune response. On the other hand, the advantages of self-organization are also exploited by pathogens and diseases. As such, basic research of cell migration has significantly contributed to understanding the mechanisms of tumorigenesis and metastasis in cancer, and provides valuable input on potential treatment therapies (Chambers et al., 2002; Friedl and Wolf, 2003; Wang et al., 2004). During metastasis, streams of cancer cells delaminate from the primary tumor, migrate away and populate secondary areas within the organism, thereby causing the disease to spread (Friedl and Gilmour, 2009). As cancer cells migrate through different tissue types and organs lacking consistent guidance signals, cancer cells exploit self-organizing mechanisms to drive their own migration. Recent advances in understanding the process of metastasis have shown that it acts via a self-generated gradient of lysophosphatidic acid (LPA), a chemokine of unknown origin, that is present in the tumor environment and broken down by tumor cells (Muinonen-Martin et al., 2014; Susanto et al., 2017). Cancer cells are able to follow this local self-generated gradient and spread out. Preventing cancer cells from egressing from the tumor in the first place would be an efficient treatment to stop the progression of the disease. Therefore, understanding how self-organization provides robust guidance to migrating cancer cells is essential to develop new therapies.

Although self-organized migration is often beneficial for a particular biological system, the underlying complex networks are difficult to decipher or even recognize from a researcher's perspective. Dissecting self-organization requires the combination of biology and physics, including computational modeling and experimental research, cross-disciplinary areas that have only gained significant popularity within the past two decades. As a consequence, the concept of self-organization has long been overlooked in our understanding of cell migration. For many migration events, both in development and disease, initiation and guidance cues remain unclear. Whether self-organizing mechanisms are much more widely used during cell migration events than currently recognized is yet to be determined. Future research in this area could provide exciting answers to many long-standing questions.

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Competing interests

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