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Random versus directionally persistent cell migration

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Abstract

Directional migration is an important component of cell motility. Although the basic mechanisms of random cell movement are well characterized, no single model explains the complex regulation of directional migration. Multiple factors operate at each step of cell migration to stabilize lamellipodia and maintain directional migration. Factors such as topography of the extracellular matrix, the cellular polarity machinery, receptor signalling, integrin trafficking and co-receptors, and actin–myosin contraction converge on regulation of the Rho family of GTPases and control of lamellipodial protrusions to promote directional migration.

Introduction

Cell migration is important for embryogenesis, immune surveillance and wound healing. The basic mechanisms of cell motility are relatively well understood. To migrate efficiently, cells must possess an asymmetric morphology with defined leading and trailing edges. Polarized intracellular signalling orients protrusion of the leading edge, integrin-mediated adhesion to the underlying substrate, contraction and detachment at distinct regions of the cell to orchestrate cell motility [1, 2]. This sequence of steps — known as the cell motility cycle — occurs in a wide range of epithelial and mesenchymal cells that migrate in different environments in response to a variety of factors. It is less clear how this basic motility machinery is coupled to a steering mechanism that integrates environmental cues with polarized signalling, adhesion and cytoskeleton remodelling to promote directionally persistent migration.

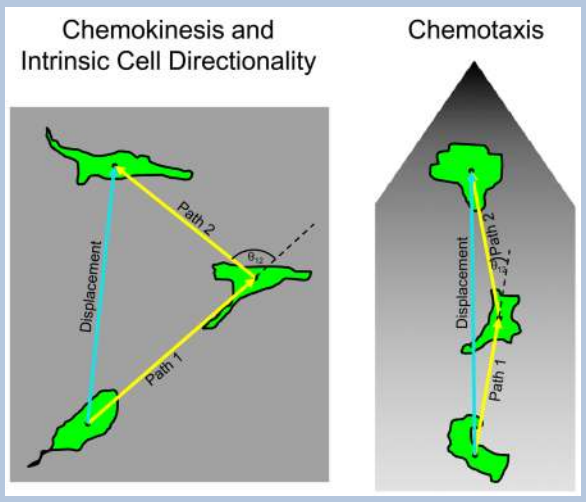
Conceptually, directional cell migration has two sources: intrinsic cell directionality of migration and external regulation. Intrinsic directionality is observed when cells respond to a non-directional motogenic signal [3], such as the uniform application of platelet-derived growth factor (PDGF) [4], that triggers the basic motility machinery in the absence of any external guiding factor (Box 1). Random migration occurs when a cell possesses relatively low intrinsic directionality. If the motogenic stimulus is presented as an external gradient or with another external guidance cue, a steering or compass mechanism coupled to the basic motility machinery responds to the asymmetric environmental factor. The cell then undergoes directed migration [5, 6]. The nature of the asymmetric cue will often define the type of directed migration. Cells undergo chemotaxis in response to soluble cues, haptotaxis in response to graded adhesion in the underlying substrate or other guidance cues anchored within the extracellular matrix (ECM) [7], electrotaxis in response to electric fields [8], and durotaxis in response to mechanical signals in the environment [9].

Box 1

Chemokinesis, chemotaxis and directional migration

Chemokinesis occurs when a factor, applied to the cell either symmetrically or asymmetrically, stimulates cell migration without determining the direction of migration. Chemotaxis occurs when a soluble factor is applied asymmetrically and dictates the

direction of cell migration. The behaviour of a motile cell exposed to these different treatments can be quantified. The example depicts two cells at three time points as they migrate in a uniform concentration (see figure part a) or a gradient (see figure part b) of motogen. At each time point, the migration can be defined by the centre of the cell mass, the distance travelled between positions (path length), the turning angle (θ) and the net displacement. This information can be used to describe the rate and directionality of migration. Directionality is defined as the displacement divided by the total path length of the cell. If a cell is migrating more randomly, directionality decreases and vice versa. It can also be quantified by calculating the mean square displacement [134]. Prior to stimulation of migration or during chemokinesis, these parameters describe intrinsic cell directionality. During chemotaxis, they characterize directed migration. Factors that increase directionality during chemokinesis can promote chemotaxis [13, 14], whereas other factors that decrease directionality can inhibit chemotaxis [14, 64, 68]. More studies will be required to determine whether this relationship is universal.



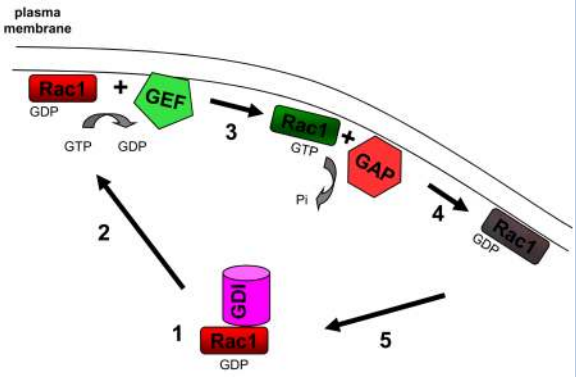
Both intrinsic and externally directed migration can be characterized quantitatively by the velocity and directional persistence of migration [10]. Factors can change the velocity of migration by perturbing the basic mechanism of cell motility. For example, inhibiting the Ena/VASP family of actin-binding proteins slows lamellipodial dynamics and increases the rate of migration [11]. Factors that affect the steering mechanism can alter the degree of directional persistence. For example, when phospholipase A_2 (PLA_2) and phosphatidylinositol 3-kinase (PI3K) isoforms 1 and 2 are deleted from *Dictyostelium discoideum*, the cells migrate at near normal rates but they no longer chemotax effectively [12]. As will be highlighted throughout this Review, agents that increase random intrinsic migration will often diminish directed migration. Conversely, factors that increase directional persistence during intrinsic motility can sometimes promote directed migration [13, 14].

Recent studies on random versus directionally persistent migration during intrinsic motility appear to converge on a fundamental mechanism underlying directional migration. Cells achieve directionally persistent migration by forming and stabilizing actin-rich protrusions or lamellipodia that maintain the orientation of the leading edge [5, 15]. As we will review, multiple factors can influence this process, including the topography of the ECM, cell polarity and cell adhesion. Understanding how these factors are integrated to regulate directional migration remains challenging. It is clear, however, that intracellular signalling, often mediated at the leading edge by the Rho family of small GTPases (Box 2), operates at each step of the cell motility cycle to promote directional migration by regulating leading edge formation.

Box 2

Regulatory proteins and the Rho GTPase cycle of activation

Small GTPases function as molecular switches in which the exchange of GDP for GTP triggers a conformational change that allows binding and activation of downstream effectors to direct cytoskeleton remodelling and adhesion formation [135]. The Rho family of GTPases cycle between GTP-bound (active) and GDP-bound (inactive) states (see the figure). The activity of Rho-family GTPases is regulated by three classes of proteins, guanine nucleotide dissociation inhibitors (GDIs), guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs) [136]. The subcellular localization and protein binding partners of a particular GEF or GAP can specify where Rho GTPase regulation occurs and link their regulation to particular signalling pathways [137]. By utilizing unique combinations of GEFs and GAPs, specific plasma membrane receptors can generate unique activation profiles of the Rho GTPase family with different functional outputs. The inactive GDP-bound GTPase, such as Rac1 (see figure), forms a complex with a GDI in the cytosol (1). The GDI regulates the interaction of the GTPase with intracellular membranes and blocks its binding to downstream effectors. Dissociation of the GDI and delivery to the appropriate intracellular membrane permits binding by a GEF (2). The GEF catalyses the release of the GDP and its replacement by GTP because of the higher concentration of GTP in the cytosol (3). Once active, the GTPase binds and activates downstream effectors by virtue of the conformational shift induced by GTP binding. GAPs bind the active GTPase and accelerate their intrinsic activity to convert GTP to GDP and inactivate the protein (4). The inactive GTPase is bound by the GDI, removed from the membrane, and sequestered in the cytoplasm (5). Members of the Rho family that play a prominent role regulating directed cell migration include Rac1, Cdc42 and RhoA.



This Review will first discuss the link between the stability of protrusions at the leading edge and directional cell migration. We then address how the topography of the ECM contributes to polarization and directional migration. Finally, we examine how the molecular mechanisms that drive each step of the basic motility cycle — polarization, protrusion, integrin dynamics, or contraction and detachment can regulate directionally persistent migration. A recurring theme is that these processes regulate the number and orientation of lamellipodia to regulate directional migration (FIG. 1).

Stable protrusions guide migration

Cells differ in their intrinsic levels of directionally persistent cell migration, a property that can be quantified during chemokinesis [10]. New protrusions are characteristically generated from the pre-existing leading edge, rather than in different directions around the cell [15]. This process

restricting lateral protrusions underlies directional migration in fibroblasts, leukocytes and *D. discoideum* [5, 15]. Some cells can migrate without lamellipodia using bleb-based motility [16] but its role in random versus directional motility is not yet clear. Due to space restrictions, this Review will focus primarily on motility studies of mesenchymal and epithelial cells. For recent reviews of neutrophil and *D. discoideum* directional migration, see Refs 17–19.

Local signalling within a protrusion in response to an external guidance cue can direct the formation of a new protrusion [5] *in vitro* and *in vivo*. For example, the leading edge of neurons migrating within the central nervous system (CNS) consists of multiple extending and retracting branches [20]. Similarly, endothelial tip cells at the growing ends of new blood vessels have several protrusions at their leading edge that direct cell trajectory [21]. In both cases, the direction of migration is determined by the orientation of the most stable branch, which is regulated by external guidance cues and internal signalling [20, 21].

ECM topography guides migration

Cell adhesion can guide the directionality of migration; for example, adhesion to the underlying substratum stabilizes lamellipodial protrusions during chemotaxis and chemokinesis [22, 23]. The topography of the ECM can also provide important regulation of cell motility through physical cues that geometrically constrain adhesion sites to guide directional migration (FIG. 2). During durotaxis, where the pliability of the underlying ECM affects rates of migration, fibroblasts migrate towards a rigid surface or a local region of higher local tension within an elastic polyacrylamide gel [9]. Consequently, when cells probe their physical surroundings, they acquire mechanical information or signals that help determine the direction of migration — e.g., in cell migration toward an increased ECM adhesive gradient during haptotaxis [7].

Classical studies of cells interacting with the fibrillar protein network of fibrin clots established that cells can re-orient the ECM, which in turn can alter mesenchymal cell morphology and migration [24, 25]. In this process termed contact guidance, the physical structure of the surrounding ECM helps control cell shape and migration. Similar effects of ECM topography are found during single-cell mesenchymal migration [26] and embryogenesis [27–29]. In amphibian gastrulation, aligned ECM fibrils facilitate mesodermal cell migration towards the animal pole. [27] Alignment of the fibrillar matrix *in vitro* can control migration, consistent with a role for ECM orientation in promoting the directional migration of these cells *in vivo* [28, 29].

Surface topography influences polarity and migration

'Natural' cell-derived environments contain multiple components, including other molecules besides the oriented ECM fibrils that might affect directionality (e.g., growth factors bound to the ECM). Consequently, a number of bioengineering studies have tested the effects of grooved or etched physical patterns in inorganic substrata. Mesenchymal cells from fish explanted onto parallel grooves in quartz coated solely with denatured type I collagen show cell elongation, polarization and migration along the grooved longitudinal axis [30]. This polarizing effect of topographic patterns has been observed for a wide variety of cell types including oligodendrocytes [31], hippocampal neurons [32] and epithelial cells [33]. Tests of nanotopographic patterns reveal that fibroblasts can respond to a grooved pattern with a depth and width of 35 nm and 100 nm, respectively [34], which is similar to the width of a single collagen fibril (~30–100 nm in width). These studies suggest that cell interactions with physical structures can induce cell responses and signalling independent of chemical factors to promote directional migration.

3D ECM structures promote directional migration

Cell migration and the regulation of directionally persistent migration have been studied primarily *in vitro* on two-dimensional (2D) surfaces. However, three-dimensionality can substantially affect fibroblast cell morphology, signalling and migration [35]. Single fibroblasts migrating in 3D cell-derived matrix often display a spindle-shaped or uni-axial morphology (FIG. 2B) [24, 25]. Mechanical flattening of 3D cell-derived matrices or coating 2D surfaces with solubilized 3D matrix molecules mimic simple 2D substrata with respect to cell morphology, adhesion and random migration. Interestingly, the spindle-shaped uni-axial morphology of cells in 3D can be induced by sandwiching fibroblasts between two 2D elastic polyacrylamide gels coated with collagen [36]. These results indicate that dimensionality, or at least both dorsal and ventral matrix contact, can help regulate the shape and mode of migration of fibroblasts. It should be noted that the cellular response to fibrillar 3D structures may be cell-type specific and dependent on the mode of cell migration (i.e., single cells versus sheets). Further, amoeboid cells undergoing integrin-independent migration may respond only to physical constraints of the ECM rather than to fibrillar ECM structures [37].

1D topography underlies migration on 3D fibrils

The tissue and ECM environments of cells can differ with respect to orientation of the ECM; for example, certain human fibroblasts can produce highly oriented 3D matrices [38] while other matrices show little orientation. Cell migration along highly oriented matrix is highly directional and rapid [39] with many cells 'streaming' one after the other along fibronectin fibrils (A.D.D., unpublished observations). These oriented matrix fibrils can be mimicked by single 1.5 micron lines generated by micro photoablation and coated by matrix. These essentially one-dimensional (1D) fibrils also force cells into a uni-axial morphology with a single lamella [39] (FIG. 2B). Migration along 1D patterns is rapid (>1.5-fold higher than 2D), unidirectional, highly ordered as shown by coordinated protrusion-retraction cycles, and independent of ligand density; these properties match those of cells migrating through oriented 3D cell-derived matrices.

Other similarities between 1D and oriented 3D models not shared by 2D models include distinctive localization of key adhesion components (α_5 and activated β_1 integrin), presence of stabilized Glu-tubulin in an axon-like pattern, rearward-oriented Golgi apparatus and centrosome — both of which point toward the leading edge in 2D wound-healing models (see below) — and sensitivity of cell migration to inhibition of cellular contractility and disruption of microtubules. Consequently, cell association with fibrillar structures appears to provide important physical cues to initiate cell polarization by regulating cell shape and orientation of cellular organelles, resulting in unidirectional cell migration (FIG. 2A).

Nanofibre topography can guide cell migration in vivo

Fibrillar topographical cues in the form of 1D nanofibres can guide axonal growth and glial cell migration *in vivo* [40, 41]. After spinal cord injury, failure of axons to regenerate results in paralysis. This clinical problem is due partially to the inability of axons to traverse scar tissue generated locally by glial cell infiltration into the wound that physically blocks axon regeneration [41]. Immediately after a spinal cord injury in an animal model, introduction of peptide amphiphile molecules that self-assemble into nanofibres reduces glial scarring and promotes motor and sensory neuron outgrowth through the wounded region. While more investigation is required to understand how topographic physical cues are involved in directional migration *in vivo*, it is clear that association of cells with ECM with a defined structure, whether a 2D surface, a 3D matrix, or a 1D line/nanofibre, can strongly affect cell polarity, cell morphology and cell migration.

Topography of ECM fibrils and cancer invasion

Cell migration can now be studied in native *in vivo* environments using new imaging approaches. For example, studies of *in vivo* explants to analyse breast cancer metastasis reveal metastatic tumour cells and macrophages migrate rapidly along collagen fibres [42]. Highly metastatic tumour cells migrate preferentially along fibres. The reticular orientation of the collagen matrix surrounding mammary glands may anchor and/or restrain cells [43]. However, the dense fibrous collagen characteristic of breast cancer stroma forms radial patterns extending away from tumours (FIG. 2C). *In vitro* experiments show that parallel collagen fibres radiating outward from tumour explants can promote tumour epithelial cell invasion, while non-linear matrix reduces invasive behaviour [44]. Tumour cells remodel the matrix into these parallel fibres in order to migrate. These data suggest that oriented ECMs play a role *in vivo* in directional migration and invasion. Understanding these mechanisms may provide better models for cancer metastasis and developmental processes.

Connecting topography to directional migration

It will be important to determine how ECM topography links to intracellular signalling to promote directional cell migration. Integrin receptors and the physical arrangement of adhesions could trigger orientation of the cytoskeleton that favours directional cell migration. Alternatively, specific matrix topography could influence cell polarity or integrin trafficking (see below). Although matrix orientation can stabilize leading-edge protrusions to promote directionally persistent migration, the specific signalling pathways remain to be determined.

Polarity and directional migration

Cells contain polarity signalling machinery that can influence directional cell motility. This polarization influences the formation of the leading and trailing cell edges. The Par (partitioning defective) complex, consisting of Par3, Par6 and atypical protein kinase C (aPKC), connects Rho GTPase signalling, centrosome reorientation, microtubule stabilization and membrane trafficking to the regulation of directional persistence during intrinsic cell migration (FIG. 3). Par activation polarizes a broad spectrum of cellular processes, including the formation of the front–rear axis in moving cells, as well as asymmetric cell division and basal–apical polarity in epithelial cells [45]. The stability of the front–rear axis correlates with the extent of persistent directional cell movement [46].

The Rho GTPase family member Cdc42 is a master regulator of cell polarization that influences directional migration [47]. Integrin engagement by components of the ECM can locally activate Cdc42 at subregions of the plasma membrane [48]. Active Cdc42 then recruits the Par complex to the plasma membrane where aPKC is activated [48]. Cdc42 activity can also be regulated at the leading edge of migrating cells via the phosphoprotein Nudel [49]. Nudel is phosphorylated by the Ser/Thre kinase ERK (extracellular signal regulated kinase) at the leading edge to locally sequester the Cdc42 GTPase-activating protein Cdc42GAP. This sequestration can prevent Cdc42GAP from downregulating local Cdc42 activity and may contribute to Cdc42-dependent activation of the Par complex to trigger polarized protrusions and directionally persistent cell migration [49].

Cdc42 can promote directional cell motility in fibroblast scratch-wound healing assays *in vitro* as cells migrate into a region denuded of cells [50, 51]. A caveat, however, is that such non-epithelial monolayers are seldom seen *in vivo*. Cdc42 activates p21 protein-activated kinase 1 (Pak1), which recruits the Rac guanine nucleotide-exchange factor (GEF) βPIX to the leading edge where it can locally activate Rac to initiate protrusions and directional migration [52]. Simultaneously, Par6 and aPKC act downstream of Cdc42 to stabilize microtubules at the leading edge while the dynein motor acts to keep the centrosomes in position, resulting in the

final arrangement of the nucleus, centrosome and leading edge along the front–rear axis [50] in 2D cell culture. Microtubule-binding proteins stabilize microtubules at the leading edge [53] to promote local protrusion and directional migration by regulating adhesion formation, and by facilitating the anterograde transport of material from the Golgi to the active leading edge to replenish material removed by a combination of protrusion and retrograde actin flow [54, 55]. During this process, Cdc42 triggers actomyosin retrograde flow via its downstream effector, myotonic dystrophy kinase-related Cdc42 binding kinase (MRCK), in a complex with myosin 18A and the adaptor protein LRAP35a [50, 56]. Cdc42-regulated actomyosin retrograde flow repositions the nucleus to the rear of the cell.

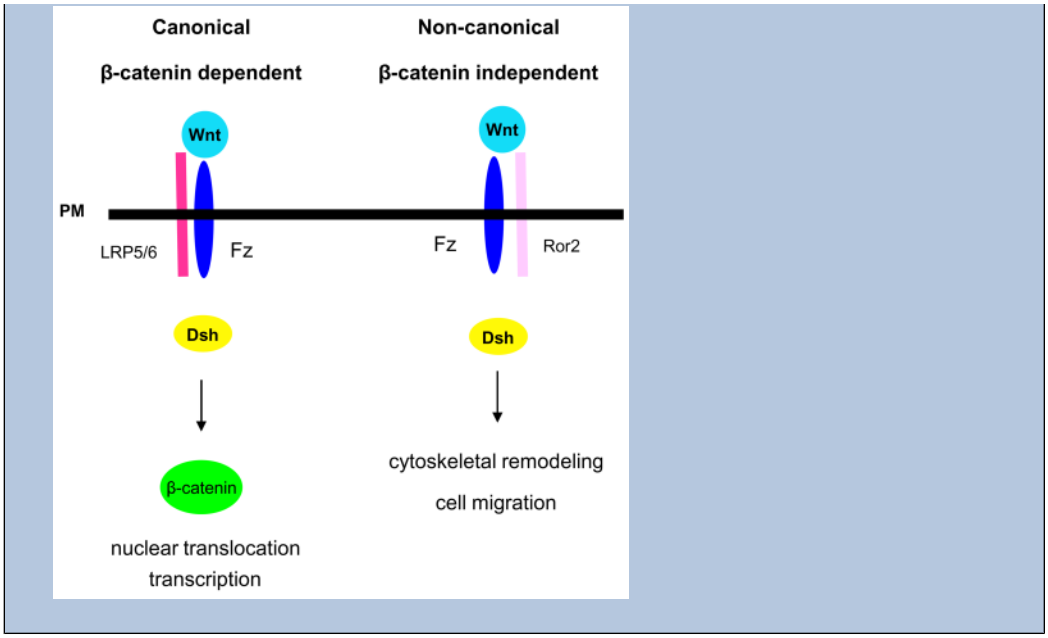
Wnt signalling and directional migration

Additional pathways can cooperate with Cdc42 and the Par complex to promote directional migration. Wnts are a family of secreted proteins that regulate cell fate and tissue patterning. Wnt signalling classically contributes to polarization of tissues within developing embryos and, more recently, has been shown to contribute to cell polarity and directional motility. Wnts regulate gene expression via a canonical pathway or cytoskeletal dynamics and cell polarization via a non-canonical pathway (BOX 3). Wnt5a triggers non-canonical Wnt signalling and cell motility by binding to the receptor Frizzled and the alternative or co-receptor Ror2, a Tyr kinase receptor [57]. In scratch-wounded monolayers of fibroblasts, Wnt5a binding to these receptors causes the cytosolic mediator Disheveled to trigger Golgi and centrosome reorientation via the tumour-suppressor protein APC (adenomatous polyposis coli) and stabilizes microtubules toward the newly formed leading edge in cooperation with the Cdc42, Par–aPKC pathway [58]. Importantly, engagement of Ror2 by Wnt5a is required for directional migration of fibroblasts during scratch-wound healing in the presence of Wnt5a and chemotaxis towards a source of Wnt5a [57, 59].

Box 3

Canonical and non-canonical Wnt signalling

In higher vertebrates, the 19 members of the Wnt family of proteins induce intracellular signalling that is important for developmental processes such as cell migration, proliferation or differentiation [138]. Historically, this complex signalling system has been categorized into canonical (part a) and non-canonical Wnt signalling pathways (part b). While these pathways share common elements, such as the receptor Frizzled and the cytoplasmic protein Disheveled (Dsh), they diverge in their biological effects. Canonical Wnt signalling can induce dorsal–ventral embryonic patterning [139], whereas non-canonical Wnt signalling can trigger convergent extension of tissues — a simultaneous narrowing and lengthening of a tissue that occurs during gastrulation and other formative processes [140]. The type of receptor engaged by a particular Wnt determines the output of the signalling pathway. During canonical signalling (see the figure part a), Wnt (1, 3a, or 8) binding to Frizzled and its co-receptor LRP5/6 (low-density lipoprotein receptor-related protein 5/6) increases levels of the transcriptional co-activator β-catenin in the cytoplasm by inhibiting its phosphorylation-dependent ubiquitylation and degradation. This permits β-catenin to enter the nucleus and trigger transcription. Thus, an alternative name for canonical Wnt signalling is β-catenin-dependent Wnt signalling [140]. Non-canonical Wnt signalling (see the figure part b) is triggered by Wnt (5a or 11) binding to Frizzled and the co-receptor Ror2 [57]. This complex activates JNK (c-Jun N-terminal kinase) and leads to Rho family GTPase activation, cell polarity, cytoskeletal remodelling and cell migration. An alternative name for this pathway is β-catenin-independent Wnt signalling [140].



Regulation of Rho GTPases by the Par complex

In addition to forming the front–rear axis that is important for directional cell migration, the Par complex is a focal point of crosstalk between the small GTPases Cdc42, Rac and RhoA. Rac and Cdc42 can promote RhoA activity at the back of the cell to aid in the formation of the leading and trailing edges that are required for efficient cell migration [60, 61]. This crosstalk may also occur at the front of the cell to coordinate adhesion, protrusion and retraction of the leading edge [62]. Thus, Par-mediated crosstalk between the Rho family of GTPases may be a crucial factor regulating cell morphology and migration.

In addition to recruiting βPIX, Cdc42 may activate Rac at the leading edge via the polarity complex. In neuroblastoma cells, active Cdc42 binds the Par complex and helps recruit the GEF Tiam1 to the leading edge, locally activating Rac [63]. Similarly, Tiam1 is targeted to the leading edge by direct binding to Par3 in epithelial cells [64]. Depletion of either Tiam1 or Par3 decreases front–rear polarization, increases random cell migration and reduces sensitivity of cells to a chemotactic cue [64, 65]. Active Rho kinase (ROCK) downstream of GTP-bound RhoA can antagonize Rac activation at the leading edge by phosphorylating Par3 and disrupting the complex to prevent Rac activation by Tiam1 [65]. This phosphorylation also occurs at the leading edge, and these signalling circuits are required for cell polarization and directed cell migration [65]. By targeting Tiam1 to the cell front, Par3 promotes microtubule stabilization and lamellipodium formation to generate directionally persistent migration for both intrinsic and directed cell motility. Although the precise link between Tiam1 and localized microtubule stabilization is not yet known, the microtubule plus-end binding protein CLASP2 mediates the stable association of microtubules with the cell cortex at the leading edge [66]. As with Par3 and Tiam1, depletion of CLASP2 reduces the number of stable microtubules and increases random motility. While reduced Rac activity in fibroblasts leads to directionally persistent migration [14], loss of the Rac GEF Tiam1 in keratinocytes leads to a decrease in total Rac activity that increases random migration [64]. Consequently, local restriction of active Rac to the leading edge by the combined action of Par3 and Tiam1 may be a key factor promoting directionally persistent motility. In fibroblasts, however, Tiam1-mediated activation of Rac is associated with an increase in cell–cell interactions and loss of cell motility. These discrepancies indicate that the function of specific GEFs may be context dependent [61].

Caveolin-1, the principal component of caveolae, may act in parallel with the Par complex and contribute to polarity and directional migration [67]. Directional migration in both wound healing and chemotaxis assays requires phosphorylation of caveolin-1. Deficiency of caveolin-1 decreases Rho activity while increasing the levels of active Rac and Cdc42 [68]. These increased activity levels are associated with faster turnover of nascent adhesions and enhanced random protrusions in mouse embryonic fibroblasts [68]. These cells show impaired directional cell migration during scratch-wound healing consistent with a global increase in Rac activity. Src activation of p190RhoGAP in these cells may contribute to inhibition of Rho activity and decreased directional migration. Alternatively, removal of caveolin-1 might promote Rac activity and increase random migration by reducing internalization of Rac binding sites from the plasma membrane [69]. Together, these findings are consistent with the notion that the mutual antagonism between Rac and RhoA activity coordinated by the Par complex and caveolin-1 can be important for directionally persistent cell migration.

Protrusion and directional migration

The main factors that determine the orientation of cell migration are the frequency and direction of local lamellipodial protrusions extending laterally from the main longitudinal axis of the cell [5, 15]. Intracellular signalling pathways at the leading edge [2] that regulate actin cytoskeleton remodelling or adhesion formation to create or stabilize local protrusions therefore likely contribute to directional migration [6].

Calcium regulation of the leading edge

Local changes in the concentration of intracellular calcium regulate directional cell migration. Transient, spatially restricted increases of intracellular calcium guide growth cone migration during haptotaxis [70] and chemotaxis [71]. Local fluxes of intracellular calcium can activate Rac and Cdc42 and inactivate RhoA, regulating growth cone motility [72]. In migrating fibroblasts undergoing chemokinesis, TRPM7 (transient receptor potential) calcium channels intermittently open and trigger intense local bursts of intracellular calcium at the leading edge [73]. Symmetric application of PDGF increases random fibroblast migration along with the number and amplitude of the local calcium bursts, whereas inhibition of TRPM7 channels prevents fibroblast chemotaxis towards PDGF. Whether calcium is an upstream mediator of Rho family GTPase function or regulates additional signalling pathways during directional fibroblast migration [74] is currently unresolved.

PI3K and Rac signalling at the leading edge

The non-overlapping distribution and combined action of PI3K and the lipid phosphatase PTEN (PI3K phosphatase and tensin homolog) produces $PtdInsP_3$ at the leading edge during intrinsic and directed migration [75]. In *D. discoideum*, PI3K controls the rate of pseudopod generation during chemotaxis [15], where it may cooperate with other pathways such as PLA_2 to trigger efficient chemotaxis of these cells [12, 76]. In fibroblasts, $PtdInsP_3$ is localized to the leading edge of fibroblasts during intrinsic and directed cell migration [77, 78]. During chemotaxis, local PIP3 generation within lamellipodia may trigger actin polymerization and protrusion of the lamellipodia towards the source of guidance cue [5].

Rac may be a key target of PI3K signalling at the leading edge during cell migration [79]. Inhibiting PI3K during intrinsic fibroblast motility partially reduces Rac activity and random migration compared with directly reducing active Rac by siRNA [14], indicating that other pathways may cooperate to regulate Rac at the leading edge during cell migration. Phospholipase D (PLD) hydrolyses the phospholipid phosphatidylcholine (PC) to generate phosphatidic acid (PA) in response to growth factor or integrin engagement [80]. PA binds directly to the membrane targeting motif of active Rac to recruit it to the plasma membrane to

promote fibroblast migration [81, 82]. Interestingly, PLD cooperates with PI3K signalling to mediate Rac activation during neutrophil chemotaxis [83]; a similar interplay may occur in other cell types during intrinsic cell motility.

The level and localization of Rac activity plays a central role in determining the choice between random and directionally persistent motility, though this relationship may not be universal [84]. Rac is highly active at the leading edge during intrinsic migration [85] and the level of Rac activity determines whether the intrinsic migration of a cell is random or directional [14, 86]. Relatively high levels of Rac activity induce formation of multiple lamellae, leading to more non-directional, random cell migration. Moderate levels of Rac activity support fewer lateral lamellae, thereby promoting directional cell migration and chemotaxis. Compared with cells migrating on 2D surfaces, cells migrating in complex 3D environments have lower levels of Rac activity with an elongated morphology, fewer lateral lamellae, and more rapid and directional migration [14, 35, 87]. Increased levels of Rac activity likely increase the targeting of active Rac to the plasma membrane and formation of lateral lamellipodia to promote random intrinsic migration [14, 88].

Mechanistically, Rac activation at the leading edge may promote directional cell migration by triggering local actin polymerization or adhesion formation. Rac is known to trigger formation of adhesive structures in the lamellipodium [89] and this contributes to epidermal growth factor (EGF)-triggered motility in carcinoma cells [90]. The WAVE (WASP family verprolin-homologous protein) family (WAVE1, 2, and 3) and Pak1 link Rac signalling to membrane ruffling and lamellipodia formation [91, 92]. However, fibroblasts deficient in WAVE2 migrate randomly during wound healing and chemotax less effectively [93], contrary to the affects of diminished Rac signalling [14]. By contrast, expression of kinase-inactive Pak1 in fibroblasts increases random migration [94]. Independent of its kinase activity, Pak1 can recruit the protein kinase Akt to the plasma membrane where it is activated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) [95], an important pathway for endothelial cell migration [96]. Consistent with the scaffolding function of Pak1 acting downstream of Rac, Akt activity can compensate for changes in the directionality of migration caused by diminished Rac activation [14].

The cofilin pathway and directional migration

Localized activity of the actin-severing protein cofilin is important in directional migration. Cofilin functions at the leading edge by severing F-actin filaments at the minus end to provide more free-barbed ends for actin polymerization [97] When cofilin activity is decreased in fibroblasts either via siRNA treatment [98] or $\alpha_5\beta_1$ integrin-triggered phosphorylation of cofilin on Ser14 by ROCK (Rho-associated kinase), cells undergo increased random migration [99, 100]. However, in metastatic cancer cells, depletion of cofilin leads to more directionally persistent intrinsic migration in response to EGF [101]. This increase in directional migration is associated with stable and persistent lamellipodial protrusions and a decrease in sensitivity to a point source of the chemoattractant EGF at the posterior of the cell [101]. Thus, lamellipodial dynamics controlled by cofilin and Arp2/3 are a critical factor in dictating the directional migration of these cells.

Cofilin activity at the leading edge is also sensitive to local changes in intracellular pH (pH_i). The ubiquitously expressed NHE1 Na-H exchanger is targeted to lamellipodia, and locally modulates intracellular pH_i to promote directional migration of fibroblasts in a scratch-wound assay [102]. NHE1-mediated deprotonation of His133 of cofilin prevents cofilin binding to its negative regulator phosphatidylinositol-4,5-bisphosphate (PIP2) [103]. This process may activate cofilin at the leading edge to promote directional migration [104].

ECM receptors, trafficking and motility

Integrin trafficking and co-receptors contribute to integrin function and adhesion formation during cell migration [105]. Integrin trafficking may contribute to directional migration by facilitating the formation of new adhesions at the leading edge [105]. Recent work shows that integrin trafficking and the co-receptor syndecan-4 can contribute to directional migration, in part, by modulating Rho GTPase signalling to control protrusion formation.

Integrin trafficking and persistent migration

The Par complex can contribute to polarized integrin trafficking and adhesion formation at the leading edge of migrating cells [106] (FIG. 4). Par3 cooperates with the endocytic machinery by regulating Numb, an adaptor that couples specific cargo to clathrin-coated pits [107]. Numb directs internalization of integrin β_1 or β_3 subunits behind the leading edge. Par3 binds directly to Numb and promotes its Ser phosphorylation by aPKC [106] (FIG. 4A). Phosphorylation of Numb prevents its interaction with the integrin β subunits and inhibits their internalization. Inhibiting either phosphorylation or dephosphorylation of Numb blocks the directed migration that occurs during wound healing of fibroblast monolayers in the presence of serum. This mechanism links the trafficking of integrins on the cell surface to the polarization machinery at the leading edge.

Recycling of specific integrins is another process that contributes to directional migration in both 2D and 3D contexts (FIG. 4B and C). Integrin $\alpha_V\beta_3$ expression in wounded epithelial cell monolayers promotes stable centrosome re-orientation and directional migration compared to cells expressing integrin $\alpha_5\beta_1$ 99. Integrin $\alpha_V\beta_3$ suppresses RhoA–ROCK-mediated phosphorylation and inhibition of the actin-severing protein cofilin, thereby triggering broad lamellipodia, stable adhesions and increased directional migration. Cofilin functions at the leading edge by severing F-actin filaments at the minus end to provide more free-barbed ends for actin polymerization [97]. In fibroblasts, PKD1 and Rab4 (a small GTPase of the Rab family) drive the rapid recycling of integrin $\alpha_V\beta_3$ from early endosomes to the cell surface [108], whereas Rab11 controls integrin $\alpha_5\beta_1$ recycling via a longer pathway from a perinuclear endosomal compartment [109]. Perturbation of the rapid Rab4-dependent recycling of integrin $\alpha_V\beta_3$ increases the rate of integrin $\alpha_5\beta_1$ recycling and promotes random fibroblast migration [100]. Consistent with epithelial cells, integrin $\alpha_5\beta_1$-mediated random migration in fibroblasts is triggered by the ROCK-dependent phosphorylation and inactivation of cofilin [99, 100].

3D matrix and integrin trafficking

Matrix dimensionality influences Rab11-dependent recycling of integrin $\alpha_5\beta_1$ and directional cell migration. Inhibition of integrin $\alpha_V\beta_3$ in an epithelial cancer cell line increases integrin $\alpha_5\beta_1$ recycling by stimulating the Rab11-mediated return of integrin $\alpha_5\beta_1$ to the plasma membrane [110]. This switch of integrin trafficking correlates with an increase in random migration of fibroblasts on a 2D surface, but it promotes directional cell migration in 3D fibronectin-containing Matrigel and cell-derived matrices. Integrin $\alpha_5\beta_1$ recycling in epithelial cells driven by the epithelial-specific Rab11 family member Rab25 promotes the directional migration of these cells within 3D environments without affecting their mode of migration on 2D surfaces [111]. Cell-derived matrix increases the association of Rab25 with β_1 integrin and restricts its recycling to the tips of leading-edge protrusions in cell-derived matrix to promote directionally persistent migration [111] (FIG. 4D).

It is not yet known how matrix dimensionality regulates Rab25 activity or its association with integrin β_1, but the Rab-coupling protein (RCP) mediates the formation of a tripartite complex between the EGF receptor 1 (EGFR1) and integrin $\alpha_5\beta_1$ in recycling endosomes to increase integrin $\alpha_5\beta_1$ and EGFR1 recycling to the cell surface [110]. Thus, the trafficking of integrin

$\alpha_5\beta_1$ and an EGFR1 co-receptor can initiate intracellular signalling leading to cytoskeletal rearrangements that promote directional cell migration. How these integrin trafficking pathways guide cell migration within tissues is uncertain, but different components of the extracellular matrix can modulate recycling of specific integrins to promote cell motility *in vitro* 112.

Syndecan-4 and directional cell migration

The transmembrane proteoglycan syndecan-4 may sense ECM topography to control directional migration in 3D environments. Syndecan-4 cooperates with integrin $\alpha_5\beta_1$ to bind fibronectin, form focal adhesions and support cell migration [113, 114] by activating Rac downstream of protein kinase C (PKC) [88]. Syndecan-4, via PKC, restricts Rac activity to the leading edge of fibroblasts migrating on cell-derived matrices. Correspondingly, deletion of syndecan-4 leads to an increase in active Rac around the cell periphery and more random migration on cell-derived 3D matrix [88]. Thus, syndecan-4 restricts Rac activation to generate a dominant lamella to drive directionally persistent fibroblast migration in response to linear fibrils in the extracellular matrix. Unlike Par3 targeting of the Rac GEF Tiam1 to the leading edge to locally activate Rac [64], syndecan-4 suppresses Rac activation except at the leading edge of the cell to promote directionally persistent cell migration. The mechanism by which syndecan-4 limits Rac activation resembles a mechanism used by the integrin α_4–paxillin–Arf GAP (GTPase-activating protein) complex to inhibit Rac activation around the periphery of migrating epithelial-like CHO cells [115].

Syndecan-4 also cooperates with non-canonical Wnt signalling to control directional migration of neural crest cells in 3D environments during development [116, 117]. Migration of neural crest cells to specific locations is important for their differentiation [118]. Ablation of syndecan-4 expression by morpholino injection blocks neural crest differentiation by diminishing neural crest migration from the dorsal neural tube. *In vitro* cultures of neural crest cells lacking syndecan-4 undergo increased random migration resulting from larger numbers of random membrane protrusions. As in mouse embryonic fibroblasts migrating on cell-derived matrix [88], syndecan-4 via PKC restricts Rac activation to the leading edge for directionally persistent neural crest cell migration *in vitro* and *in vivo* 117. Perturbing Disheveled function decreases RhoA activity and prevents neural crest cell emigration from the dorsal neural tube. This pathway mediates contact inhibition [119] that is partially responsible for the directional migration of neural crest cells [120]. In contact-inhibited cells, Wnt11 and Disheveled cooperate to trigger RhoA-dependent collapse of protrusions that contact neighbouring neural crest cells. Thus, mutually exclusive zones of Rac and RhoA activity, controlled by syndecan-4 contacting extracellular matrix and the non-canonical Wnt receptor, respectively, drive directional cell migration in response to contact inhibition [117, 120].

Steering from the back

The trailing edge of a migrating cell contributes to the maintenance of directional migration by generating contraction forces to pull the cell rear forward and limiting the formation of protrusions to maintain the orientation of migration.

RhoA activates ROCK, which phosphorylates myosin phosphatase and the regulatory light chain on myosin II to increase actin–myosin contractility[121] and trigger tail retraction and disassemble focal adhesions [122]. Inhibition of RhoA by active Rac contributes to the formation of myosin-mediated contractility at the rear of migrating neutrophils during chemotaxis [60], in part by limiting the formation of protrusions at the rear of the cell [123]. An analogous pathway may be mediated by PTEN in *D. discoideum*. PTEN localized to the cell rear promotes intrinsic and directed migration by suppressing pseudopod formation [124]. Fibroblasts commonly express two isoforms of myosin II, A and B [125]. Myosin IIA (MIIA) deficiency leads to

formation of broad lamellipodia, increased Rac activation and random migration, and a defect in tail retraction [125, 126]. By contrast, myosin IIB (MIIB) depletion causes unstable protrusions, increases random intrinsic migration, and inhibits haptotaxis [127]. MIIB promotes directional migration by forming contractile actomyosin bundles at the cell rear, which prevent protrusion formation and thereby promote directional migration [128]. Similarly, during migration of EC tip cells in 3D collagen matrix, MIIB activity prevents protrusion initiation away from the leading edge to maintain directional migration [21].

Intracellular membrane trafficking in response to Wnt5a may be a novel mechanism to direct MIIB-mediated retraction at the cell rear. Increased Wnt5a expression in metastatic melanoma cells is associated with increased migration and invasiveness [129]. Cultured melanoma cells require Wnt5a plus a chemokine gradient in order to polarize and migrate effectively [130]. Under these conditions, Wnt5a polarizes the cell by promoting the recycling of specific membrane components, such as the melanoma cell adhesion molecule (MCAM), to the rear of the cell. In these cells, the coupling of adhesion with MIIB-mediated retraction may establish the polarity of cell migration. Whether Wnt5a is required more generally for directed cell migration remains to be determined.

Conclusions

Specific molecular mechanisms operate at each step of cell motility to control directional cell migration. These mechanisms are used by the cell to integrate information provided the topography of the ECM, constituents of the matrix, distribution of soluble or substrate-bound guidance cues and/or other factors. The cell distils this array of guidance information to select a direction of migration. While not all steps of this process are known, it is clear that Rho GTPase signalling and control of directional protrusions are critical for directional cell migration. A morphological view of directional cell migration highlights the frequency and direction of local protrusions extending laterally away from the front–rear axis of migration as being important in determining directionality [5, 15]. In other words, if the protrusions and subsequent new adhesions formed by a polarized cell are themselves directionally persistent, the cell will move in a directionally persistent manner. Processes occurring at each step of the cell motility cycle can act to regulate Rho GTPase signalling in order to promote stable and directionally persistent protrusions, which in turn promote directional migration.

The diverse array of mechanisms that contribute to directional migration may be a reflection of the complex environments cells must navigate. For example, axonal growth cones integrate guidance information provided by matrix components, soluble and matrix-bound guidance molecules, and cell–cell contacts, in order to arrive at the correct position in the body [131]. Similar complexity is illustrated in fibroblast-mediated wound-healing [132] and in the immune system, where cells must often prioritize between competing guidance cues [133]. In addition, the conceptual model of a distinct steering mechanism coupled to the cell motility machinery may be oversimplified in some cases. For example, roles of $\alpha_5\beta_1$ and $\alpha_V\beta_3$ in directional migration may suggest that the steering mechanism is embedded within the underlying motile apparatus to respond to environmental cues and trigger directional migration.

Future efforts to understand the processes driving cell migration will need to use models that recapitulate the competing guidance cues and the physically and biochemically complex environments found *in vivo*. Doing so should clarify whether the many molecular mechanisms controlling directional migration operate within a hierarchical framework or whether they are functionally redundant or even synergistic.

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Glossary

Motogenic signal

A signal, such as a growth factor, which activates the cell motility machinery without providing directional information to trigger intrinsic cell migration

Extracellular matrix

A network of proteins and polysaccharides secreted by cells; provides structural support for cells within tissues

Matrigel

Commercially available basement membrane matri composed primarily of laminin and collagen, which can be used as a 3D tissue culture model for studying cell migration and differentiation

Focal adhesion

A large protein complex that mediates the attachment of the extracellular matrix to the actin cytoskeleton through an integrin heterodimer

Morpholino

A synthetic molecule that binds to specific mRNAs and blocks their translation; used to assay protein function

Gastrulation

The process during embryogenesis when the embryo is transformed from a hollow sphere of cells to a structure with three germ layers: ectoderm, mesoderm, and endoderm

Metastasis

The spreading of cancer cells from a site of origin to distant parts of the body that often involves cell motility

Contact guidance

he process by which cells are guided by topographical structures often associated with the extracellular matrix

Glu-tubulin

A posttranslational modification of tubulin associated with MT stabilization. Also known as detyrosinated tubulin

Lamellipodium

A flattened, actin-rich protrusion found at the leading edge of a migrating cell

Scratch-wound healing assay

An in vitro cell motility assay. When an area of cells is cleared (scratched) in a monolayer of cells, cells will directionally migrate into and close the wound

Anterograde transport

Transport of material from the Golgi to the cell surface through the secretory pathway

Retrograde flow

Net movement of filamentous actin away from the cell edge

Cell cortex

An actin-rich layer near the inner surface of the plasma membrane

Caveolae

Flask-shaped invaginations of the plasma membrane that contribute to cell polarity and directional cell migration

GEF

Guanine nucleotide exchange factor; activates small G proteins by catalyzing the exchange of GDP for GTP

GAP

GTPase activating protein; accelerates the intrinsic GTPase activity of small G proteins to inactivate them

Integrins

A large family of transmembrane proteins that exists in the plasma membrane as heterodimers of α and β subunits; they frequently mediate the interaction of cells with the extracellular matrix

Lamellae

Flattened region immediately behind the lamellipodium

Clathrin coated pits

Invaginations in the plasma membrane coated by lattices made up of the protein clathrin which are precursors to endocytic vesicles

Disheveled

A cytoplasmic protein which participates in Wnt signalling immediately downstream of frizzled receptors

Neural crest

A group of cells that migrates to various parts of the embryo and form, in part, the bones of the skull, teeth, and portions of the peripheral nervous system

Proteoglycan

A protein core linked to one or more long, linear, and highly charged polysaccharide chains

Contact inhibition

Response when a migrating cell contacts another and changes direction to move away from the point of contact

Rab proteins

Large family of small GTPases found on organelles and the plasma membrane that confer specificity on vesicle docking and membrane trafficking

Arp2/3

A protein complex that nucleates actin filament growth from the sides of pre-existing actin filaments to form branched actin networks

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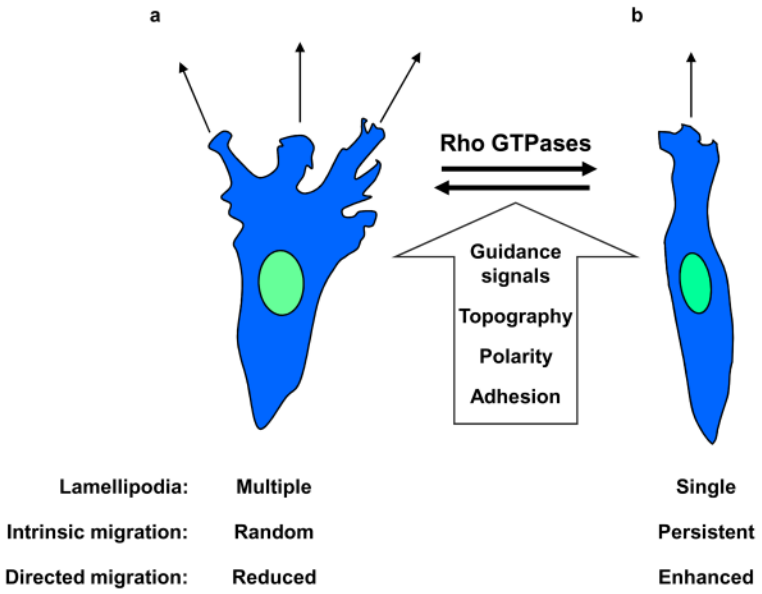

Figure 1. Control of lamellipodial protrusions promotes directional migration

Directional migration is a result of regulated formation of lamellipodia during both intrinsic and directed cell motility. A variety of signals including external guidance cues, topography of the extracellular matrix, the intracellular polarity machinery and adhesion receptors can converge on the Rho GTPases to direct the adhesion and cytoskeleton remodelling necessary for lamellipodium formation. Increased lateral lamellipodia can result in random intrinsic migration and a reduced capacity to respond to external cues during directed cell migration (see the figure part a). Restricting lateral lamellipodia formation results in a single dominant leading edge, directionally persistent intrinsic cell migration, and enhanced directed migration during chemotaxis (see figure part b).

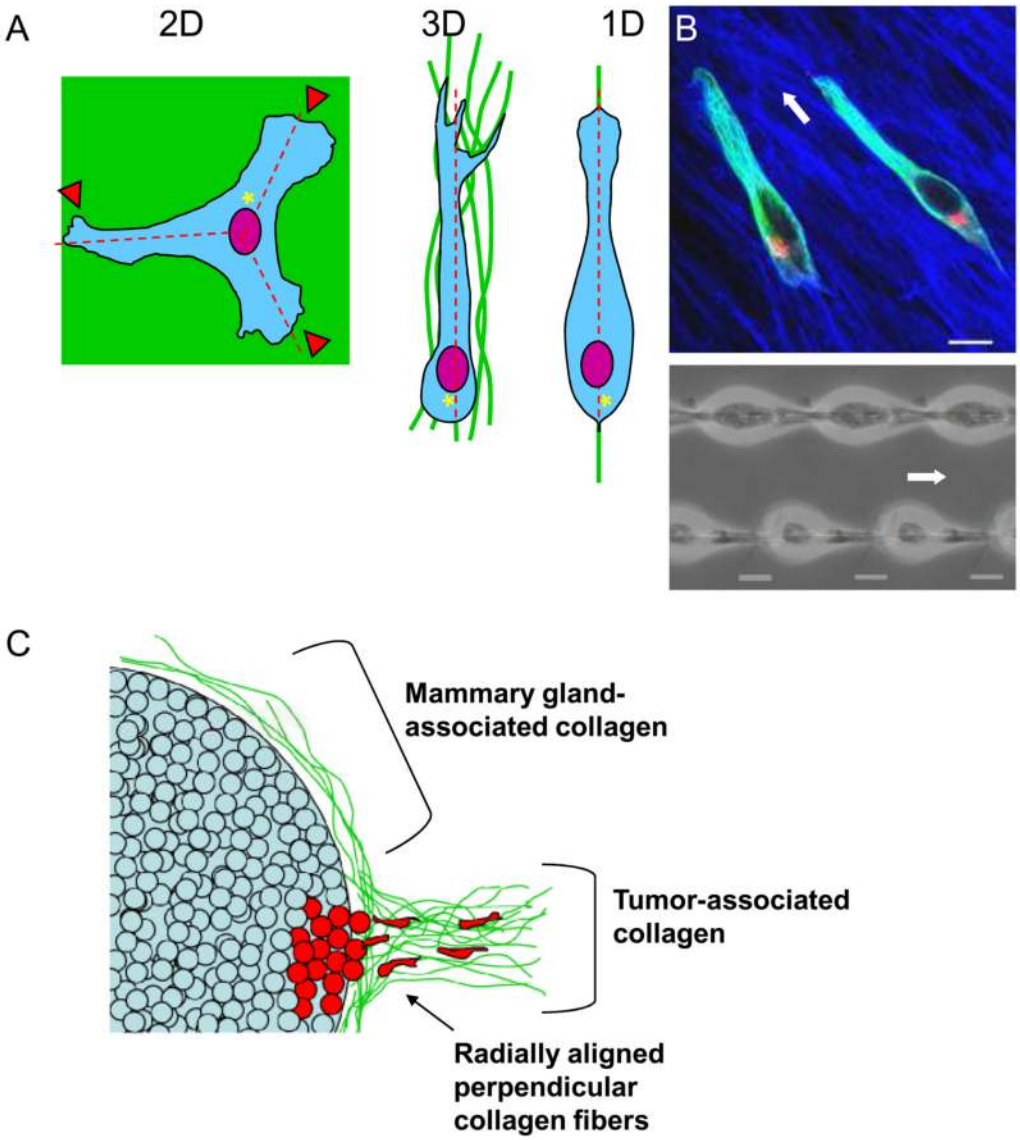

Figure 2. Topographical control of directional migration

A. Representation of how different topographical cues (2D, 3D and 1D) result in different cell morphologies and migration. When plated on 2D surfaces, cells demonstrate multiple lamellae (arrowheads) compared to the single lamella and uni-axial or spindle morphology associated with cells in an oriented 3D matrix or on 1D lines. Centrosome and Golgi (asterisks) are oriented towards the posterior of the cell in 3D and 1D but towards the anterior of the cell in 2D. Cells in both 3D and 1D demonstrate a single directional axis of travel (dashed lines), whereas the 2D surface promotes multiple axes and reduced directional migration. B. 1D mimics 3D. The upper panel shows a confocal image of NIH/3T3 fibroblasts migrating through 3D cell-derived matrix (fibronectin is shown in blue) demonstrating a uni-axial phenotype and a posterior-oriented Golgi complex (red, with microtubules in green). Fibroblasts migrating on 1D lines have similar morphology (lower panel). White arrows indicate the direction of migration. Scale bar, 10 μm. C. Schematic of differences in stroma associated with normal mammary gland (top) and malignant mammary tumours (bottom). Collagen (pink) associated with mammary tissue often tightly surrounds the epithelial cells and is oriented along the axis of the gland. By

contrast, invasive tumour cells (blue) reorient the collagen fibres perpendicular to the gland and may use these structures as highways for migration to initiate metastasis.

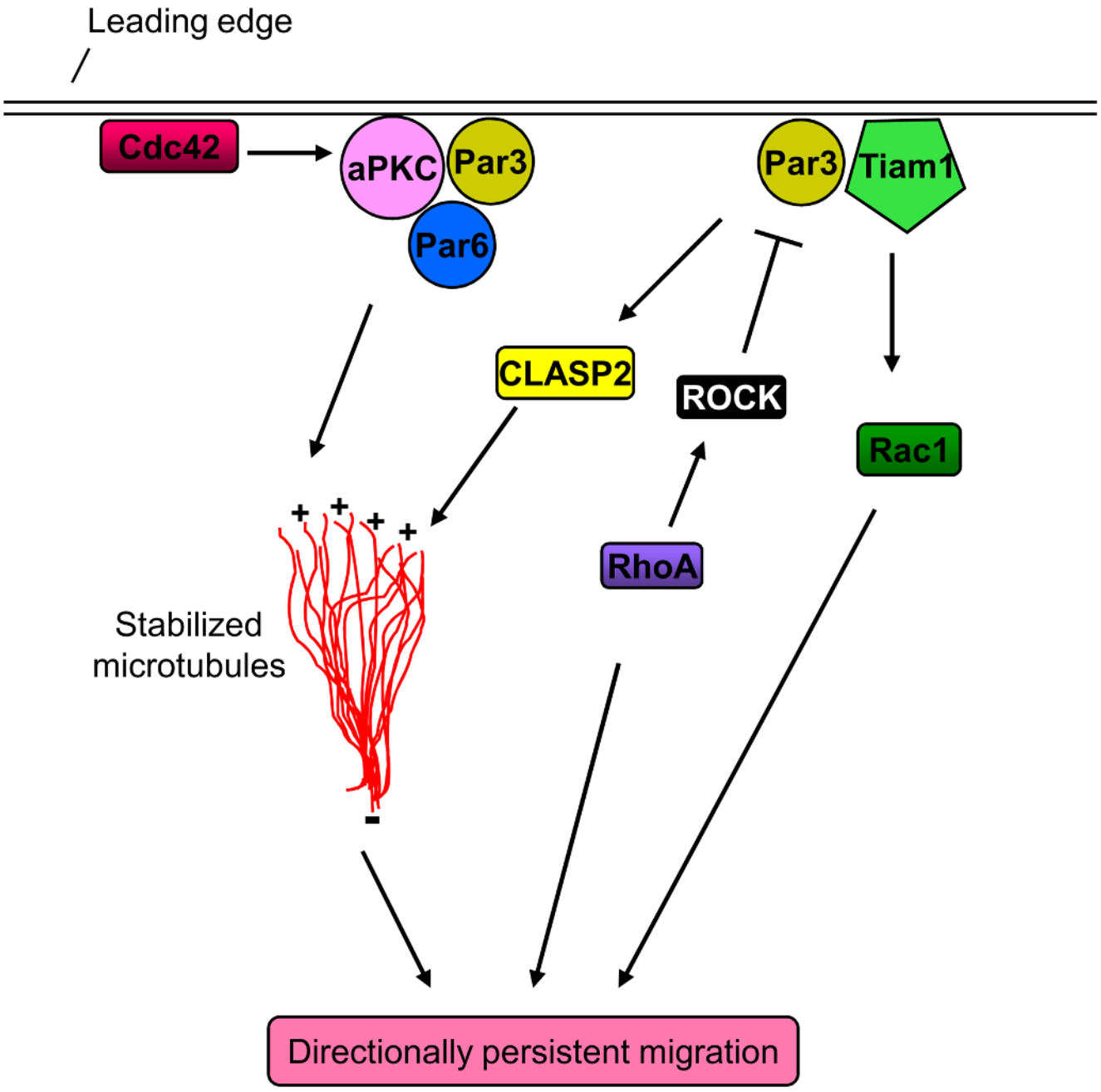

Figure 3. The Par polarity complex and directional migration

Cdc42 targets and activates aPKC and Par6 at the leading edge to stabilize microtubules and promote directional migration. Par3 and the guanine nucleotide-exchange factor Tiam1 activate Rac1 and stabilize microtubules, possibly via the action of CLASP2, at the leading edge to promote front–rear polarity and directionally persistent migration. RhoA-activated ROCK phosphorylates Par3 and disrupts the formation of the Par3–Tiam1 complex, thereby preventing Rac activation. This pathway may coordinate mutual antagonism of Rac1 and RhoA locally within the cell to dictate directional migration.

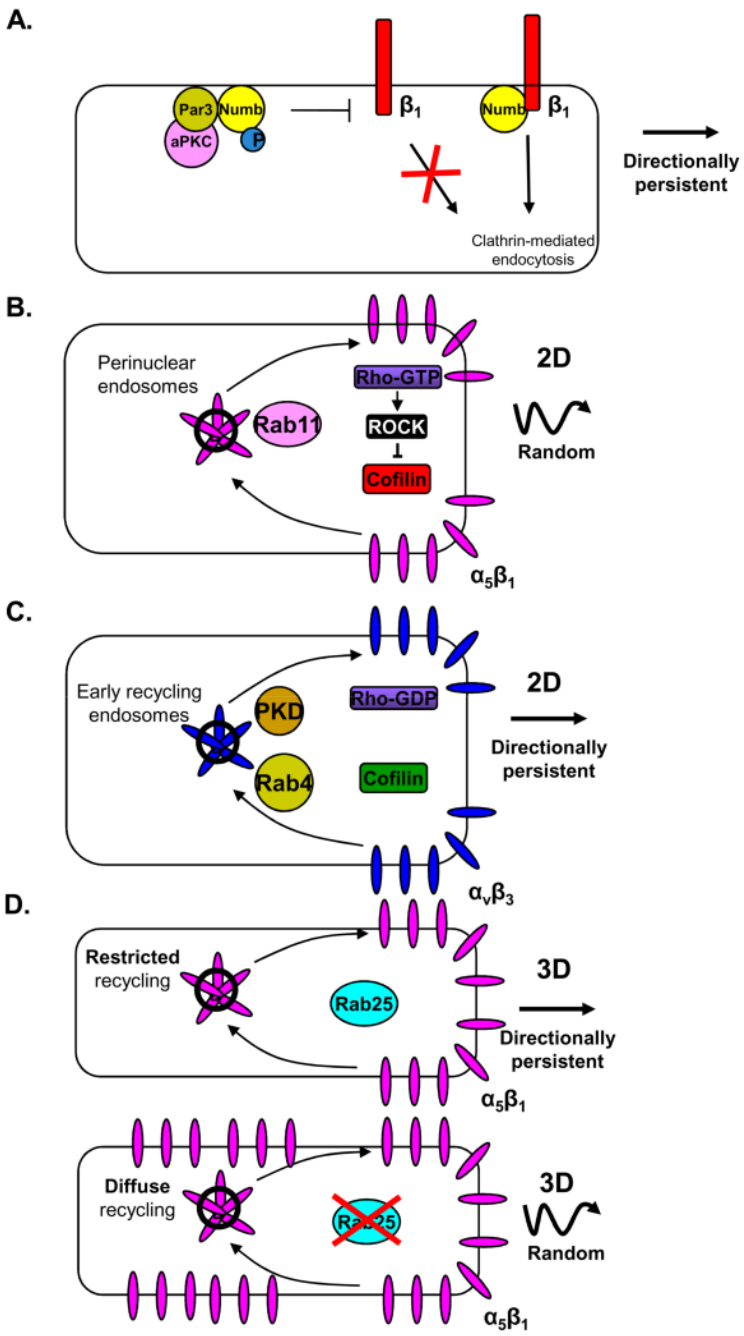

Figure 4. Integrin trafficking and directional migration

Trafficking of specific integrin heterodimers contributes to the directional persistence of cell migration. A. The endocytic cargo adaptor protein Numb binds to the cytoplasmic tail of integrin β_1 to trigger its clathrin-mediated endocytosis. Numb binds to Par3 and is phosphorylated by aPKC. Phosphorylation of Numb prevents its binding and initiating the internalization of integrin β_1 at the leading edge. This spatial regulation of integrin β_1 endocytosis leads to directional migration. B. Integrin $\alpha_5\beta_1$ is trafficked from the cell surface to a perinuclear endosomal compartment and is recycled back to the plasma membrane via a Rab11-dependent trafficking pathway. Trafficking of integrin $\alpha_5\beta_1$ via this pathway increases Rho activity, which triggers ROCK phosphorylation and inactivation of cofilin, which

stimulates random cell migration. C. Integrin $\alpha_V\beta_3$ traffics from the leading edge to an early endosome compartment and is recycled to the cell surface via a pathway that depends on the activity of PKD and Rab4. This pathway leads to cofilin activation and directionally persistent migration. D. During epithelial cell migration in 3D environments, Rab25 trafficking restricts integrin $\alpha_5\beta_1$ recycling to protrusions at the leading edge of the cell, which in turn results in fewer lateral protrusions and more directionally persistent migration. Upon inhibition of this recycling pathway, trafficking of $\alpha_5\beta_1$ is no longer restricted to the leading-edge pseudopodia, thereby increasing random protrusions and decreasing directional migration.