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 the topography of the extracellular matrix, the cellular polarity machinery, receptor signalling, integrin trafficking, integrin co-receptors and actomyosin contraction converge on regulation of the Rho family of GTPases and the control of lamellipodial protrusions to promote directional migration.

Motogenic signal
A signal, such as a growth factor, that activates the cell motility machinery without providing directional information and thereby triggers intrinsic cell migration.

Extracellular matrix
A network of proteins and polysaccharides secreted by cells that provides structural support for cells in tissues.

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

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Cell migration is important for embryogenesis, immune surveillance and wound healing. The basic mechanisms of cell motility are fairly well understood. To migrate efficiently, cells must have 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, and 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 various factors. It is not clear how this basic motility machinery is coupled to a steering mechanism that integrates environmental cues with polarized signalling, adhesion and cytoskeletal remodelling to promote directionally persistent migration.

Conceptually, directional cell migration has two sources: intrinsic cell directionality 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 has 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 in the extracellular matrix (ECM)[7], electrotaxis in response to electric fields[8] and durotaxis in response to mechanical signals in the environment[9].

Both intrinsic and externally directed migration can be characterized quantitatively by their velocity and directional persistence[10]. Factors can change the velocity of migration by perturbing the basic mechanism of cell motility. For example, inhibition of the enabled/vasodilator-stimulated phosphoprotein (ENA/VASP) family of actin-binding proteins slows the dynamics of lamellipodia 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 phosphoinositide 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 is 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 seem to converge on a fundamental mechanism that underlies 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 review here, multiple factors can influence this process, including the topography of the ECM, cell polarity and cell adhesion.

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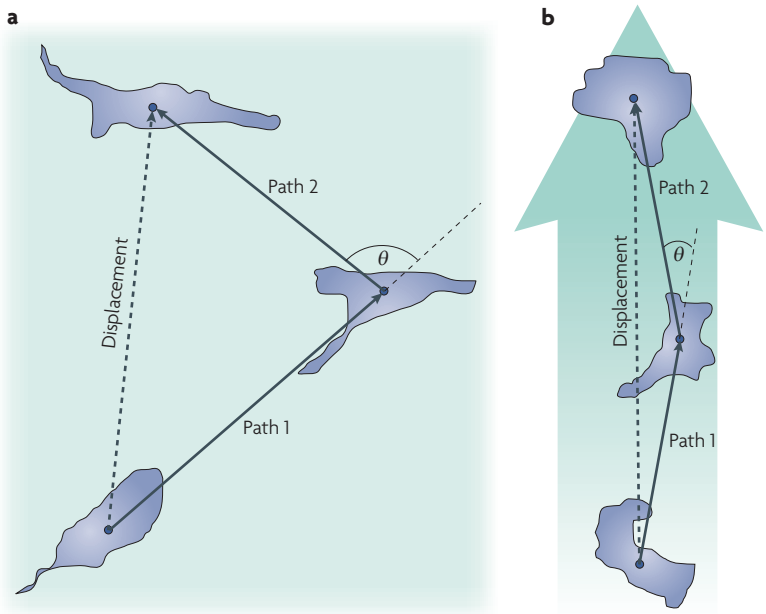
Box 1 | **Chemokinesis, chemotaxis and directional migration**



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. Part **a** of the figure depicts a cell at three time points as it migrates in a uniform concentration. Part **b** depicts a cell at three time points as it migrates in a gradient of increasing concentration 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. Directionality 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 factors that decrease directionality can inhibit chemotaxis[14,64,68]. More studies will be required to determine whether this relationship is universal.

Integrin
A member of a large family of transmembrane proteins, which exist in the plasma membrane as heterodimers of α and β subunits and frequently mediate the interaction of cells with the ECM.

Chemokinesis
Non-directional cell migration triggered by an extracellular cue.

Contact guidance
The process by which cells are guided by topographical structures that are often associated with the ECM.

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.

This Review first discusses 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, contraction and detachment — can regulate directionally persistent migration. A recurring theme is that these processes achieve this by regulating the number and orientation of lamellipodia (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]. The process that restricts 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 the role of this migration method in random or directional motility is not yet clear. Because of space restrictions, this Review focuses 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 in 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 in the central nervous system consist 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 their 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 regulate cell motility through physical cues that geometrically constrain adhesion sites to guide directional migration (FIG. 2). During durotaxis, in which the pliability of the underlying ECM affects rates of migration, fibroblasts migrate towards a rigid surface or a local region of higher tension in an elastic polyacrylamide gel[9]. Consequently, when cells probe their physical surroundings, they acquire mechanical information or signals that help to determine the direction of migration — for example, in cell migration towards 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 reorient 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 to 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 (for example,

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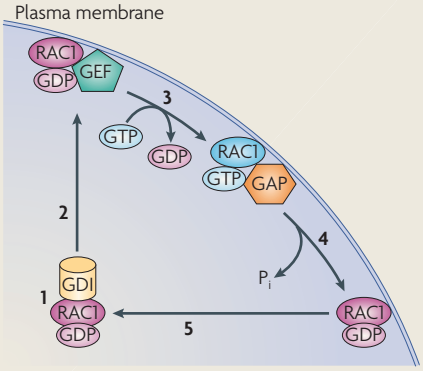
Box 2 | **Regulatory proteins and the Rho GTPase cycle of activation**



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 cytoskeletal remodelling and adhesion formation[135]. The Rho family of GTPases cycle between GTP-bound (active) and GDP-bound (inactive) states. 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 using unique combinations of GEFs and GAPs, specific plasma membrane receptors can generate unique activation profiles of the Rho GTPase family, with different functional outputs. An inactive GDP-bound GTPase, such as RAC1 (see the figure), forms a complex with a GDI in the cytosol (step 1). The GDI regulates the interaction of the GTPase with membranes and blocks the GTPase from binding to downstream effectors. Dissociation of the GDI and delivery to the appropriate membrane allows binding by a GEF (step 2). The GEF catalyses the release of the GDP, which is replaced by GTP because of the higher concentration of GTP in the cytosol (step 3). Once active, the GTPase binds and activates downstream effectors as a result of the conformational shift induced by GTP binding. GAPs bind the active GTPase and accelerate its intrinsic activity to convert GTP to GDP, releasing inorganic phosphate (P_i) and inactivating the protein (step 4). The inactive GTPase is bound by the GDI, removed from the membrane and sequestered in the cytoplasm (step 5). Members of the Rho family that play a prominent part in regulating directed cell migration include RAC1, CDC42 and RHOA.

growth factors bound to the ECM). Consequently, several 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, elongate, polarize and migrate along the grooved longitudinal axis[30]. This polarizing effect of topographic patterns has been observed for a wide range 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). These studies suggest that cell interactions with physical structures can induce responses and signalling independently 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 surfaces. However, three-dimensionality can substantially affect fibroblast cell morphology, signalling and migration[35]. Single fibroblasts migrating in a 3D cell-derived matrix often display a spindle-shaped or uni-axial morphology[24,25] (FIG. 2b). Mechanical flattening of 3D cell-derived matrices or coating of 2D surfaces with solubilized 3D matrix molecules mimics simple 2D substrata with respect to cell morphology, adhesion and random migration. Interestingly, the spindle-shaped uni-axial morphology of cells in 3D matrices 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 to regulate the shape and mode of migration of fibroblasts. It should be noted that the cellular response to fibrillar 3D structures might be cell-type-specific and dependent on the mode of cell migration (that is, single cells versus sheets). Further, amoeboid cells undergoing integrin-independent migration might 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], whereas other matrices show little orientation. Cell migration along a highly oriented matrix is 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 μm lines generated by micro-photoablation and coated with a matrix. These essentially 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 on a 2D surface), 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, which are not shared by 2D models, include the distinctive localization of key adhesion components (α5 integrin and activated β1 integrin), the presence of stabilized Glu-tubulin in an axon-like pattern, the rearward-oriented Golgi apparatus and centrosome (both of which point towards the leading edge in 2D wound-healing models (see below)) and the sensitivity of cell migration to the inhibition of cellular contractility and the disruption of microtubules. Consequently, cell association with fibrillar structures seems to provide important physical cues to initiate cell polarization by regulating cell shape and orientation of cellular organelles, which results 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 a spinal cord injury, the failure of axons to regenerate results in paralysis. This clinical problem is partly due to the inability of axons to traverse scar tissue, which is generated locally by glial cell infiltration into the wound and physically blocks axon regeneration[41]. Immediately after a spinal cord injury in an animal model, the introduction of peptide amphiphile molecules that self-assemble into nanofibres reduces glial scarring and promotes motor and sensory neuron

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

Lamella
The flattened region immediately behind the lamellipodium.

Glu-tubulin
(Also known as detyrosinated tubulin). A post-translational modification of tubulin that is associated with microtubule stabilization.

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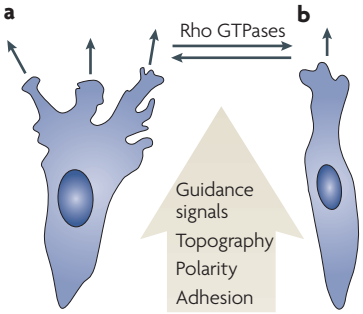
outgrowth through the wounded region. Although more investigation is required to understand how topographic physical cues are involved in directional migration *in vivo*, it is clear that the association of cells with ECM that has a defined structure, whether a 2D surface, a 3D matrix or a 1D line, can strongly affect cell polarity, cell morphology and cell migration.

Topography of ECM fibrils and cancer invasion. Cell migration can now be studied *in vivo* using new imaging approaches. For example, studies of *in vivo* explants to analyse breast cancer metastasis reveal that 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 that surrounds the mammary glands may anchor and/or restrain cells[43]. However, the dense fibrous collagen that is characteristic of breast cancer stroma forms radial patterns that extend away from tumours (FIG. 2c). *In vitro* experiments show that parallel collagen fibres that radiate outwards from tumour explants can promote tumour epithelial cell invasion, whereas a 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 part in directional migration and invasion *in vivo*. Understanding these mechanisms might 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 an orientation of the cytoskeleton that favours directional cell migration. Alternatively, specific matrix topographies 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.



Lamellipodia:	Multiple	Single
Intrinsic migration:	Random	Persistent
Directed migration:	Reduced	Enhanced

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. Various signals, including external guidance cues, the topography of the extracellular matrix, the intracellular polarity machinery and adhesion receptors, can converge on the Rho GTPases to direct the adhesion and cytoskeletal remodelling that is necessary for lamellipodium formation. **a** | Increased numbers of lateral lamellipodia can result in random intrinsic migration and a reduced capacity to respond to external cues during directed cell migration. **b** | Restricting lateral lamellipodium formation results in a single dominant leading edge, directionally persistent intrinsic cell migration and enhanced directed migration during chemotaxis.

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 range of cellular processes, including the formation of the front–rear axis in migrating 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 by the phosphoprotein NUDEL (also known as NDEL1)[49]. NUDEL is phosphorylated by the Ser/Thr kinase extracellular signal-regulated kinase (ERK) at the leading edge to locally sequester CDC42 GTPase-activating protein (CDC42GAP; also known as ARHGAP1). This sequestration can prevent CDC42GAP from downregulating local CDC42 activity and might contribute to CDC42-dependent activation of the Par complex to trigger the formation of 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-activated kinase 1 (PAK1), which recruits the RAC1 guanine nucleotide exchange factor (GEF) βPIX (also known as ARHGEF7) to the leading edge, where it can locally activate RAC1 to initiate protrusions and directional migration[52]. PAR6 and aPKC act downstream of CDC42 to stabilize microtubules at the leading edge while the dynein motor simultaneously 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]

Metastasis
The spreading of cancer cells from a site of origin to distant parts of the body, which often involves cell motility.

GTPase-activating protein
(GAP). A protein that accelerates the intrinsic GTPase activity of small G proteins to inactivate them.

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

Guanine nucleotide exchange factor
A protein that activates small G proteins by catalysing the exchange of GDP for GTP.

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in 2D cell culture. Microtubule-binding proteins stabilize microtubules at the leading edge[53], thereby promoting 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 retrograde actomyosin flow through its downstream effector, myotonic dystrophy kinase-related CDC42-binding kinase (MRCK; also known as CDC42BPA), in a complex with myosin 18A and the leucine repeat adaptor protein LRAP35A[50,56]. CDC42-regulated retrograde actomyosin flow repositions the nucleus to the rear of the cell.

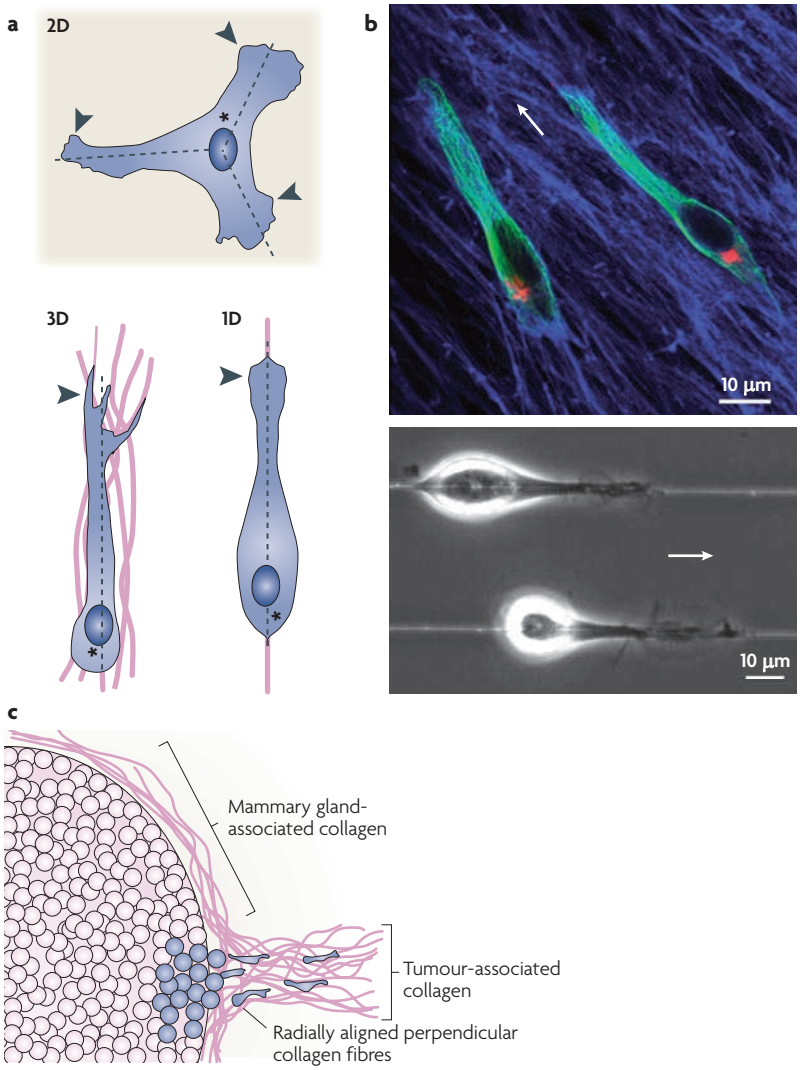

Figure 2 | **Topographical control of directional migration. a** | Representation of how different topographical cues (one-, two- and three-dimensional) result in different cell morphologies and migration. When plated on 2D surfaces, cells produce multiple lamellae (indicated by arrowheads), whereas cells in an oriented 3D matrix or on 1D lines produce a single lamella and have a uni-axial or spindle morphology. The centrosome and Golgi complex (asterisks) are oriented towards the posterior of the cell in 3D and 1D topographies but towards the anterior of the cell on 2D substrates. Cells either in a 3D matrix or on a 1D line have a single directional axis of travel (dashed lines), whereas the 2D surface promotes multiple axes and reduces directional migration. **b** | Migration on a 1D substrate is similar to that in a 3D matrix. The upper panel shows a confocal image of NIH 3T3 fibroblasts migrating through a 3D cell-derived matrix (fibronectin is shown in blue). The cells have a uni-axial phenotype and a posterior-oriented Golgi complex (red). Microtubules are shown in green. A similar morphology is seen in fibroblasts migrating on 1D lines (lower panel). White arrows indicate the direction of migration. **c** | Schematic of differences between stroma that is associated with normal mammary gland and stroma that is associated with malignant mammary tumours. 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 to be perpendicular to the gland and can then use these structures as highways for migration to initiate metastasis.

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 in developing embryos and, more recently, has been shown to contribute to cell polarity and directional motility. Wnt proteins regulate gene expression through a canonical pathway or regulate cytoskeletal dynamics and cell polarization through a non-canonical pathway (BOX 3). WNT5A triggers non-canonical Wnt signalling and cell motility by binding to the receptor Frizzled and the alternative receptor or co-receptor ROR2, a receptor Tyr kinase[57]. In scratch wounded monolayers of fibroblasts, the binding of WNT5A to the Frizzled receptors causes the cytosolic mediator Dishevelled to trigger Golgi and centrosome reorientation through the tumour-suppressor protein adenomatous polyposis coli (APC) and, in cooperation with the CDC42–Par–aPKC pathway[58], to stabilize microtubules towards the newly formed leading edge. Importantly, the engagement of ROR2 by WNT5A is required for directional migration of fibroblasts during scratch wound healing in the presence of WNT5A and for chemotaxis towards a source of WNT5A[57,59].

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 for crosstalk between the small GTPases CDC42, RAC1 and RHOA. RAC1 and CDC42 can promote RHOA activity at the rear 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 can 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 might be a crucial factor that regulates cell morphology and migration.

In addition to recruiting βPIX, CDC42 can activate RAC1 at the leading edge through the Par complex. In neuroblastoma cells, active CDC42 binds to the Par complex and helps to recruit the GEF T-lymphoma invasion and metastasis-inducing protein 1 (TIAM1) to the leading edge, where it locally activates RAC1 (REF. 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 the sensitivity of cells to a chemotactic cue[64,65]. Active Rho-associated protein kinase 1 (ROCK1), which is downstream of GTP-bound

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RHOA, can antagonize RAC1 activation at the leading edge by phosphorylating PAR3 and disrupting the complex, such that RAC1 activation by TIAM1 is prevented[65]. This phosphorylation of PAR3 at the leading edge is required for cell polarization and directed cell migration[65]. By targeting TIAM1 to the front of the cell, PAR3 promotes microtubule stabilization and lamellipodium formation to generate directionally persistent migration for both intrinsic and externally directed cell motility. Although the precise link between TIAM1 and localized microtubule stabilization is not yet known, the microtubule plus-end-binding protein cytoplasmic linker-associated protein 2 (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. Although reduced RAC1 activity in fibroblasts leads to directionally persistent migration[14], loss of TIAM1 in keratinocytes leads to a decrease in total RAC1 activity, which increases random migration[64]. Consequently, local restriction of active RAC1 to the leading edge by the combined actions of PAR3 and TIAM1 might be a key factor for the promotion of directionally persistent motility. In fibroblasts, however, TIAM1-mediated activation of RAC1 is associated with an increase in cell–cell interactions and a loss of cell motility. These discrepancies indicate that the function of specific GEFs might be context dependent[61].

Caveolin 1, the principal component of caveolae, might 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 the phosphorylation of caveolin 1. Deficiency of caveolin 1 decreases RHOA activity and increases the levels of active RAC1 and CDC42 (REF. 68). These increased activity levels in mouse embryonic fibroblasts are associated with a faster turnover of nascent adhesions and an increase in random protrusions[68]. Mouse embryonic fibroblasts show impaired directional cell migration during scratch wound healing, which is consistent with a global increase in RAC1 activity. SRC kinase-mediated activation of p190RhoGAP (also known as GRLF1) in these cells might contribute to the inhibition of RHOA activity and decreased directional migration. Alternatively, removal of caveolin 1 might promote RAC1 activity and increase random migration by reducing the internalization of RAC1-binding sites from the plasma membrane[69]. Together, these findings are consistent with the notion that the mutual antagonism between RAC1 and RHOA activity that is 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 that extend laterally from the main longitudinal axis of the cell[5,15]. The intracellular signalling pathways at the leading edge[2] that regulate actin cytoskeleton remodelling or adhesion formation to create or stabilize local protrusions are therefore likely to contribute to directional migration[6].

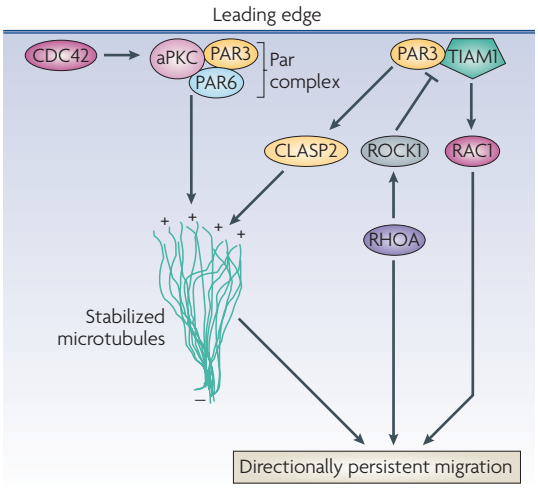

Figure 3 | **The Par polarity complex and directional migration.** The Rho GTPase family member CDC42 targets and activates atypical protein kinase C (aPKC), PAR3 and PAR6 (which constitute the partitioning defective (Par) complex) at the leading edge to stabilize microtubules and promote directional migration. PAR3 and the guanine nucleotide exchange factor TIAM1 (T-lymphoma invasion and metastasis-inducing protein 1) activate the GTPase RAC1 and stabilize microtubules, possibly through the action of CLASP2 (cytoplasmic linker-associated protein 2), at the leading edge to promote front–rear polarity and directionally persistent migration. RHOA-activated Rho-associated protein kinase 1 (ROCK1) phosphorylates PAR3 and disrupts the formation of the PAR3–TIAM1 complex, thereby preventing RAC1 activation. This pathway may coordinate mutual antagonism of RAC1 and RHOA locally in the cell to dictate directional migration.

Ca^{2+} regulation of the leading edge. Local changes in the concentration of intracellular Ca^{2+} regulate directional cell migration. Transient, spatially restricted increases of intracellular Ca^{2+} guide growth cone migration during haptotaxis[70] and chemotaxis[71]. Local fluxes of intracellular Ca^{2+} can activate RAC1 and CDC42 and inactivate RHOA to regulate growth cone motility[72]. In migrating fibroblasts that are undergoing chemokinesis, TRPM7 Ca^{2+} channels intermittently open and trigger intense local bursts of intracellular Ca^{2+} at the leading edge[73]. Symmetric application of PDGF increases random fibroblast migration along with the number and amplitude of the local Ca^{2+} bursts, whereas inhibition of TRPM7 channels prevents fibroblast chemotaxis towards PDGF. Whether Ca^{2+} is an upstream mediator of Rho-family GTPase function or a regulator of additional signalling pathways during directional fibroblast migration[74] is currently unresolved.

PI3K and RAC1 signalling at the leading edge. The non-overlapping distribution and combined action of PI3K and the lipid phosphatase PTEN (phosphatase and tensin homologue) produces phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) at the leading edge during intrinsic and directed migration[75]. In *D. discoideum*,

Anterograde transport
The transport of material from the Golgi to the cell surface through the secretory pathway.

Retrograde actin flow
The net movement of filamentous actin away from the cell edge.

Dishevelled
A family of cytoplasmic proteins that participate in Wnt signalling immediately downstream of the Frizzled receptors.

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

Caveola
A flask-shaped invagination of the plasma membrane that contributes to cell polarity and directional cell migration.

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PI3K controls the rate of pseudopod generation during chemotaxis[15] and might also cooperate with other pathways, such as PLA_2 signalling, to trigger efficient chemotaxis of the cells[12,76]. In fibroblasts, PtdIns(3,4,5)P_3 is localized at the leading edge during intrinsic and externally directed cell migration[77,78]. During chemotaxis, local PtdIns(3,4,5)P_3 generation in lamellipodia might trigger actin polymerization and protrusion of the lamellipodia towards the source of the guidance cue[5].

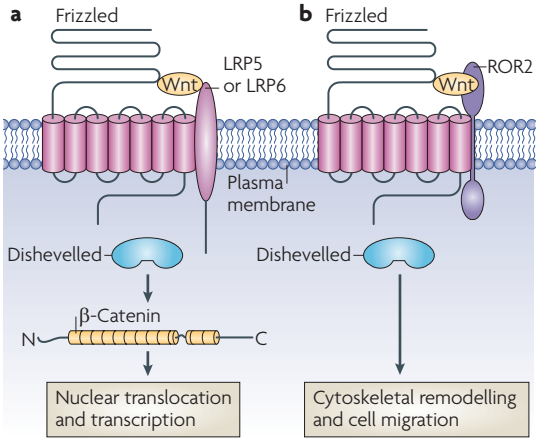
RAC1 might be a key target of PI3K signalling at the leading edge during cell migration[79]. Inhibiting PI3K during intrinsic fibroblast motility partially reduces RAC1 activity and random migration compared with directly reducing active RAC1 with small interfering RNA (siRNA)[14], indicating that other pathways might cooperate to regulate RAC1 at the leading edge during cell migration. Phospholipase D hydrolyses the phospholipid phosphatidylcholine to generate phosphatidic acid in response to growth factor or integrin engagement[80]. Phosphatidic acid binds directly to the membrane-targeting motif of active RAC1 to recruit it to the plasma membrane and thereby promote fibroblast migration[81,82]. Interestingly, phospholipase D cooperates with PI3K signalling to mediate RAC1 activation during neutrophil chemotaxis[83]; a similar interplay may occur in other cell types during intrinsic cell motility.

The level and localization of RAC1 activity plays a central part in making the choice between random and directionally persistent motility, although this relationship might not be universal[84]. RAC1 is highly active at the leading edge during intrinsic migration[85] and its level of activity determines whether the intrinsic migration of a cell is random or directional[14,86]. High levels of RAC1 activity induce the formation of multiple lamellae, which leads to more non-directional, random cell migration. Moderate levels of RAC1 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 RAC1 activity, an elongated morphology, fewer lateral lamellae and more rapid and directional migration[14,35,87]. Increased levels of RAC1 activity probably increase the targeting of active RAC1 to the plasma membrane and the formation of lateral lamellipodia to promote random intrinsic migration[14,88].

Mechanistically, RAC1 activation at the leading edge might promote directional cell migration by triggering local actin polymerization or adhesion formation. RAC1 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 Wiskott–Aldrich syndrome protein (WASP) family, comprising WAVE1 (verprolin homology domain-containing protein 1; also known as WASF1), WAVE2, WAVE3 and PAK1, link RAC1 signalling to membrane ruffling and lamellipodium formation[91,92]. However, fibroblasts deficient in WAVE2 migrate randomly during wound healing and chemotax less effectively[93], contrary to the effects of diminished RAC1 signalling[14]. Expression of a 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 (PDPK1)[95] in a pathway that is important for endothelial cell migration[96]. Consistent with the scaffolding function of PAK1 downstream of RAC1, Akt activity can compensate for changes in the directionality of migration caused by diminished RAC1 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 filamentous actin at the minus end to provide more free barbed ends for actin polymerization[97]. When cofilin activity is decreased in fibroblasts, either by siRNA treatment[98] or by α5β1 integrin-triggered phosphorylation of cofilin on Ser14 by ROCK1, 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

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 (see the figure, part **a**) and non-canonical (see the figure, part **b**) Wnt signalling pathways. Although these pathways share common elements, such as the Frizzled receptors and the Dishevelled cytoplasmic proteins, 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 tissues 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, the binding of WNT1, WNT3A or WNT8 to Frizzled and its co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6 increases the levels of the transcriptional co-activator β-catenin in the cytoplasm by inhibiting its phosphorylation-dependent ubiquitylation and degradation. This allows 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 is triggered by the binding of WNT5A or WNT11 to Frizzled and the co-receptor ROR2 (REF. 57). This complex activates c-Jun N-terminal kinase (JNK) and leads to Rho-family GTPase activation, cell polarization, cytoskeletal remodelling and cell migration. An alternative name for this pathway is β-catenin-independent Wnt signalling[140].

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point source of the chemoattractant EGF at the posterior of the cell[101]. Thus, lamellipodial dynamics controlled by cofilin and the Arp2/3 complex are crucial factors 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 Na^+/H^+ exchanger 1 (NHE1) 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 PtdIns-4,5-bisphosphate[103]. This process might 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 might 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 the internalization of β1 integrin or β3 integrin subunits behind the leading edge. PAR3 binds directly to NUMB and promotes its phosphorylation on Ser residues by aPKC[106] (FIG. 4a). Phosphorylation of NUMB prevents its interaction with the β integrin 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, c). The expression of αvβ3 integrin in wounded epithelial cell monolayers promotes stable centrosome reorientation and directional migration, whereas expression of α5β1 integrin does not[99]. Suppression of RHOA–ROCK1-mediated phosphorylation and inhibition of cofilin by αvβ3 integrin triggers the formation of broad lamellipodia, stable adhesions and increased directional migration. In fibroblasts, protein kinase D1 (PRKD1) and RAB4 (a small GTPase of the Rab family) drive the rapid recycling of αvβ3 integrin from early endosomes to the cell surface[108], whereas RAB11 controls α5β1 integrin recycling through a longer pathway from a perinuclear endosomal compartment[109]. Perturbation of the rapid RAB4-dependent recycling of αvβ3 integrin increases the rate of α5β1 integrin recycling and promotes random fibroblast migration[100]. Consistent with epithelial cells, α5β1 integrin-mediated random migration in fibroblasts is triggered by the ROCK1-dependent phosphorylation and inactivation of cofilin[99,100].

3D matrix and integrin trafficking. Matrix dimensionality influences RAB11-dependent recycling of α5β1 integrin and directional cell migration. Inhibition of αvβ3 integrin in an epithelial cancer cell line increases α5β1 integrin recycling by stimulating the RAB11-mediated return of α5β1 integrin to the plasma membrane[110]. This switch in integrin trafficking correlates with an increase in random migration of fibroblasts on a 2D surface and with the promotion of directional cell migration in 3D fibronectin-containing Matrigel and cell-derived matrices. Recycling of α5β1 integrin in epithelial cells, driven by the epithelia-specific RAB11-subfamily member RAB25, promotes the directional migration of these cells in 3D environments without affecting their mode of migration on 2D surfaces[111]. A cell-derived matrix increases the association of RAB25 with β1 integrin and restricts the recycling of RAB25 to the tips of leading-edge protrusions in the cell-derived matrix to promote directionally persistent migration[111] (FIG. 4d,e).

It is not yet known how matrix dimensionality regulates RAB25 activity or its association with β1 integrin, but Rab-coupling protein (RCP; also known as RAB11FIP1) mediates the formation of a tripartite complex between itself, EGF receptor (EGFR) and α5β1 integrin in recycling endosomes to increase α5β1 integrin and EGFR recycling to the cell surface[110]. Thus, the trafficking of α5β1 integrin and an EGFR co-receptor can initiate intracellular signalling, which leads to cytoskeletal rearrangements that promote directional cell migration. How these integrin trafficking pathways guide cell migration in tissues is uncertain, but different components of the ECM can modulate recycling of specific integrins to promote cell motility *in vitro*[112].

Syndecan 4 and directional cell migration. The transmembrane proteoglycan syndecan 4 can sense ECM topography to control directional migration in 3D environments. Syndecan 4 cooperates with α5β1 integrin to bind fibronectin, form focal adhesions and support cell migration[113,114] by activating RAC1 downstream of PKC[88]. Syndecan 4, by signalling through PKC, restricts RAC1 activity to the leading edge of fibroblasts migrating on cell-derived matrices. Correspondingly, deletion of syndecan 4 leads to an increase in active RAC1 around the cell periphery and more random migration on a cell-derived 3D matrix[88]. Thus, syndecan 4 restricts RAC1 activation to generate a dominant lamella and thereby drive directionally persistent fibroblast migration in response to linear fibrils in the ECM. Unlike PAR3 targeting of TIAM1 to the leading edge to locally activate RAC1 (REF. 64), syndecan 4 suppresses RAC1 activation, except at the leading edge of the cell, to promote directionally persistent cell migration. The mechanism by which

Arp2/3
A protein complex that nucleates actin filament growth from the sides of pre-existing actin filaments to form branched actin networks.

Clathrin-coated pit
An invagination in the plasma membrane that is coated by lattices made up of the protein clathrin and is the precursor to an endocytic vesicle.

Rab family
A large family of small GTPases, found on organelles and the plasma membrane, that confer specificity on vesicle docking and membrane trafficking.

Matrigel
A commercially available basement membrane matrix, composed primarily of laminin and collagen, which can be used as a 3D tissue culture model for studying cell migration and differentiation.

Proteoglycan
A protein core that is linked to at least one long, linear and highly charged polysaccharide chain.

Focal adhesion
A large protein complex that mediates the attachment of the ECM to the actin cytoskeleton through an integrin heterodimer.

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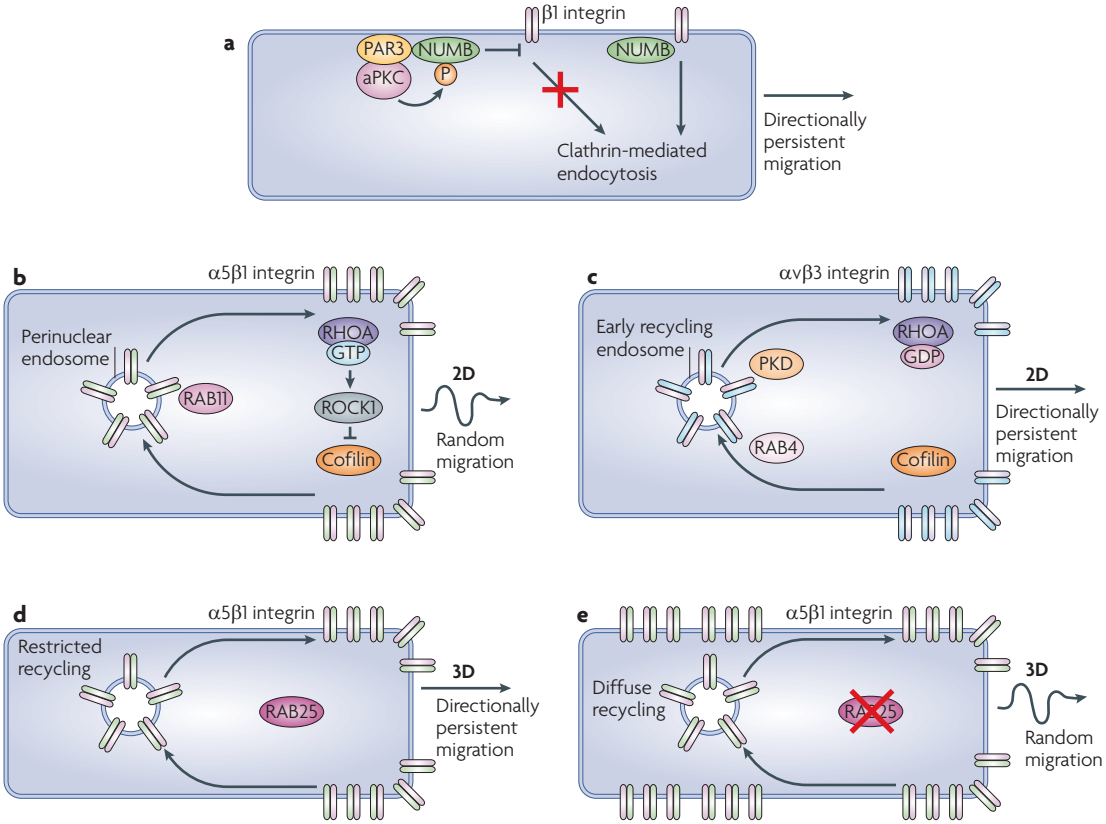

Figure 4 | **Integrin trafficking and directional migration.** Trafficking of specific integrin heterodimers contributes to the directional persistence of cell migration. **a** | The endocytic cargo adaptor protein NUMB binds to the cytoplasmic tail of β1 integrin to trigger its clathrin-mediated endocytosis. NUMB binds to partitioning defective protein 3 (PAR3) and is phosphorylated by atypical protein kinase C (aPKC). Phosphorylation of NUMB prevents it from binding and initiating the internalization of β1 integrin at the leading edge. This spatial regulation of β1 integrin endocytosis leads to directional migration. **b** | On a two-dimensional surface, α5β1 integrin is trafficked from the cell surface to a perinuclear endosomal compartment and is recycled back to the plasma membrane by a RAB11-dependent trafficking pathway. Trafficking of α5β1 integrin by this pathway increases RHOA activity, which triggers Rho-associated protein kinase 1 (ROCK1)-mediated phosphorylation and inactivation of cofilin, thereby stimulating random cell migration. **c** | Trafficking of αvβ3 integrin from the leading edge to an early endosomal compartment and recycling to the cell surface occurs by a pathway that depends on the activity of protein kinase D (PRKD1) and RAB4. This pathway does not activate RHOA, thereby allowing cofilin to remain active and leading to directionally persistent migration. **d** | During epithelial cell migration in 3D environments, RAB25 trafficking restricts α5β1 integrin recycling to protrusions at the leading edge of the cell, which in turn results in fewer lateral protrusions and more directionally persistent migration. **e** | On inhibition of this recycling pathway, trafficking of α5β1 integrin is no longer restricted to the leading edge pseudopodia, thereby increasing random protrusions and decreasing directional migration.

Neural crest
A group of cells that migrate to various regions of the embryo and form, in part, the bones of the skull and teeth and portions of the peripheral nervous system.

Morpholino
A synthetic molecule that binds to specific mRNAs to block their translation and is thereby used to assay protein function.

Contact inhibition
The response that occurs when a migrating cell contacts another migrating cell and changes direction to move away from the point of contact.

syndecan 4 limits RAC1 activation resembles a mechanism used by the integrin α4–paxillin–ArfGAP complex to inhibit RAC1 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 that lack syndecan 4 undergo increased random migration resulting from larger numbers of random membrane protrusions. As with mouse embryonic fibroblasts migrating on a cell-derived matrix[88], syndecan 4 and PKC signalling restricts RAC1 activation to the leading edge for directionally persistent neural crest cell migration *in vitro* and *in vivo*[117]. Perturbing Dishevelled function decreases RHOA activity and prevents neural crest cell migration from the dorsal neural tube. This pathway mediates contact inhibition[119], which is partially responsible for the directional migration of neural crest cells[120]. In contact-inhibited cells, WNT11 and Dishevelled cooperate to trigger RHOA-dependent collapse of protrusions that contact neighbouring neural crest cells. Thus, mutually exclusive zones of RAC1 and RHOA activity, which contact the ECM and the non-canonical Wnt receptor, respectively, are controlled

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by syndecan 4 and 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 that pull the rear of the cell forward and limiting the formation of protrusions to maintain the orientation of migration. RHOA activates ROCK1, which phosphorylates myosin phosphatase and the regulatory light chain on myosin II to increase actin–myosin contractility[121] and trigger tail retraction and the disassembly of focal adhesions[122]. Inhibition of RHOA by active RAC1 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 is mediated by PTEN in *D. discoideum*. PTEN localized to the rear of the cell promotes intrinsic and directed migration by suppressing pseudopod formation[124]. Fibroblasts commonly express two isoforms of myosin II[125]. Myosin IIA deficiency leads to the formation of broad lamellipodia, increased RAC1 activation and random migration, and a defect in tail retraction[125,126]. By contrast, myosin IIB depletion causes unstable protrusions, increases random intrinsic migration and inhibits haptotaxis[127]. Myosin IIB promotes directional migration by forming contractile actomyosin bundles at the rear of the cell, which prevent protrusion formation and thereby promote directional migration[128]. Similarly, during migration of endothelial tip cells in a 3D collagen matrix, myosin IIB activity prevents protrusion initiation away from the leading edge to maintain directional migration[21].

Intracellular membrane trafficking in response to WNT5A might be a novel mechanism to direct myosin IIB-mediated retraction at the rear of the cell. Increased WNT5A expression in metastatic melanoma cells is associated with increased migration and invasiveness[129]. Cultured melanoma cells require WNT5A plus a chemokine gradient to polarize and migrate effectively[130]. Under these conditions, WNT5A polarizes the cell by promoting the recycling of specific membrane components, such as melanoma cell adhesion molecule, to the rear of the cell. In these cells, the coupling of adhesion with myosin IIB-mediated retraction might 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 the information provided by the topography of the ECM, the constituents of the matrix, the distribution of soluble or substrate-bound guidance cues and/or other factors. The cell integrates this array of guidance information to select a direction of migration. Although not all steps of this process are known, it is clear that Rho GTPase signalling and control of directional protrusions are crucial 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 that occur at each step of the cell motility cycle can act to regulate Rho GTPase signalling to promote stable and directionally persistent protrusions, which in turn promote directional migration.

The diverse array of mechanisms that contribute to directional migration might be a reflection of the complex environments that 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 to arrive at the correct position in the body[131]. Similar complexity is seen in fibroblast-mediated wound healing[132] and in the immune system, in which cells must often prioritize competing guidance cues[133]. In addition, the conceptual model of a distinct steering mechanism coupled to the cell motility machinery might be oversimplified in some cases. For example, the roles of α5β1 integrin and αvβ3 integrin in directional migration suggest that the steering mechanism is embedded in the underlying motile apparatus to respond to environmental cues and trigger directional migration.

Future efforts to understand the processes that drive 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 that control directional migration operate in a hierarchical framework or whether they are functionally redundant, or even synergistic.

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DATABASES

UniProtKB: http://www.uniprot.org
βPIX | CDC42 | CDC42GAP | NHE1 | NUDEL | NUMB | PAK1 | PAR3 | PAR6 | PTEN | RAB4 | RAB11 | RAB25 | RAC1 | ROR2 | TIAM1 | WNT5A | WNT11

FURTHER INFORMATION

Kenneth M. Yamada's laboratory homepage:
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