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On the move: endocytic trafficking in cell migration

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Abstract Directed cell migration is a fundamental process underlying diverse physiological and pathophysiological phenomena ranging from wound healing and induction of immune responses to cancer metastasis. Recent advances reveal that endocytic trafficking contributes to cell migration in multiple ways. (1) At the level of chemokines and chemokine receptors: internalization of chemokines by scavenger receptors is essential for shaping chemotactic gradients in tissue, whereas endocytosis of chemokine receptors and their subsequent recycling is key for maintaining a high responsiveness of migrating cells. (2) At the level of integrin trafficking and focal adhesion dynamics: endosomal pathways do not only modulate adhesion by delivering integrins to their site of action, but also by supplying factors for focal adhesion disassembly. (3) At the level of extracellular matrix reorganization: endosomal transport contributes to tumor cell migration not only by targeting integrins to invadosomes but also by delivering membrane type 1 matrix metalloprotease to the leading edge facilitating proteolysis-dependent chemotaxis. Consequently, numerous endocytic and endosomal factors have been shown to modulate cell migration. In fact key modulators of endocytic trafficking turn out to be also key regulators of cell migration. This review will highlight the recent progress in unraveling the contribution of cellular trafficking pathways to cell migration.

Keywords Endocytosis · Cellular motility · Clathrin · Chemotaxis · Signal transduction · Vesicular transport

Introduction

Diverse physiological processes ranging from embryonic development to the induction of immune responses require coordinated cell migration within the organism. In addition, migratory abilities of cells also represent a determining factor in pathological settings such as metastasis and inflammatory diseases. The term cell migration comprises a number of different migration modes with different mechanistic requirements. However, in all cases cell migration is inherently a spatially organized process that begins with the polarization of the cell, for instance triggered by the encounter of a chemotactic signal. During this polarization, cells develop a distinguishable leading edge and trailing end. For this morphological polarization and the subsequent cell migration, a plethora of proteins have to be spatially segregated and confined within the cell. The intracellular localization of proteins that are membrane associated either due to a membrane anchor or via binding of a membrane-associated protein depends predominantly on vesicular trafficking. In this review, we intend to provide a comprehensive overview of the intimate link between endocytic traffic and cell migration and highlight recent progress illustrating the emerging variety of ways in which migration is shaped by endosomal trafficking.

Directed cell migration comprises different steps: (1) generation of an environment that provides chemotactic

cues, e.g. in the form of a chemotactic gradient. (2) Sensing of this environment by the migrating cell using chemotactic and growth factor receptors that induce intracellular signaling cascades. (3) Generation of a polarized cell phenotype. (4) Cell protrusion by regulating the actin cytoskeleton, often in conjunction with the establishment of adhesive structures to generate traction forces. (5) Dynamic turnover and finally disassembly of the adhesive structures, as the cell moves on. (6) Modulation of the extracellular matrix in some cases (e.g. invasion). (7) Modulation of cell cell contacts in the case of cells migrating within or out of a cell sheet. Accordingly, there is a variety of ways in which endosomal traffic can affect cell migration: (1) by shaping the chemotactic gradient. (2) By modulating the responsiveness to chemotactic cues through regulation of chemotactic and growth factor receptors. (3) By controlling downstream signaling and actin regulatory components. (4) By regulating the dynamics of adhesive contacts. (5) By modulating the extracellular matrix organization. These aspects will be discussed in the following sections and are illustrated in Fig. 1.

Establishing and shaping chemotactic gradients

Directed cell migration is primarily governed by the cell's ability to sense an external gradient of attracting factors of which chemokines and chemotactic growth factors represent major families. Chemokines and chemotactic growth factors are both produced locally as soluble proteins, secreted into the extracellular space and are considered to be retained there by means of basic amino acid motifs that bind to extracellular matrix glycosaminoglycans, thereby establishing immobilized gradients [1]. Based on this concept, cell migration in response to a soluble attractant is commonly referred to as "chemotaxis" and is distinguished from "haptotaxis" where cells migrate along a gradient of surface-bound attractants [2]. Although this concept has been established based on in vitro assays, it is widely accepted. Two recent studies now provide direct evidence from tissues that gradients of the chemokines CXCL8 and CCL21 for attracting leukocytes are established by glycan binding [3, 4]. Interestingly, some chemokines such as CCL2 are additionally stored locally in vesicles resembling type 2 granules that are docked on actin fibers just beneath the plasma membrane of endothelial cells, and hence are not exposed at the surface and not visible [5]. However, at sites of inflammation vesicle-stored CCL2 is released within tight lymphocyte-endothelial synapses upon firm adhesion of effector (but not naïve) T cells to the inflamed endothelium. This allows the highly selective extravasation of effector T cells expressing adhesive integrins [5].

A chemotactic gradient can also be built by modifying the activity of chemokines [6]. One way to achieve this is by truncating the N-terminus of the chemokine to increase its potency as exemplified by MMP9-mediated cleavage of CXCL8 [7]. Alternatively, MMP9 proteolytically processes CXCL12 to render it inactive [6]. Furthermore, a gradient can be established by removing the chemoattractant in a regulated manner through receptor-mediated endocytosis. The first demonstration of such a chemokine sequestering mechanism was identified in zebrafish, where the somatically expressed scavenger receptor CXCR7/ACKR3 sequesters CXCL12 by internalization from the tissue, permitting germ cell migration away from the chemokine sink [8]. Subsequently, the zebrafish primordium was shown to generate a chemokine gradient across itself by sequestering CXCL12 locally in its rear via CXCR7/ACKR3 while CXCL12-producing cells at the stripe underneath the primordium provide the guidance cue to the migrating primordium [9, 10]. An analogous mechanism has been recently identified for CCR7-driven dendritic cell and lymphocyte migration in mammalian lymphoid organs [11]. Here the atypical chemokine CCRL1/ACKR4 expressed by lymphatic endothelial cells lining the ceiling of the subcapsular sinus [11], scavenges CCL21, which is produced by stromal cells in the T zone of lymph nodes [12], to form the chemokine gradient. Additional atypical chemokine receptors have been described, which in contrast to classical chemokine receptors do not transmit signals resulting in chemotaxis [13], however, their role in generating chemokine gradients and their potential regulation by endocytosis have not yet been addressed. In addition, chemokines can be scavenged by classical signal transducing chemokine receptors during cell migration as shown for the inflammatory chemokine CCL2 which is internalized and removed by monocytes during chemotaxis [14].

Keeping chemotactic receptors in place

The efficiency of cell migration is determined largely by the level of chemokine receptor expression at the plasma membrane and the receptor's responsiveness to chemokines. Chemokine receptor internalization and intracellular trafficking are, therefore, key for regulating cell migration. Chemokine binding enhances steady-state internalization and trafficking of the receptor. Internalized receptor can then be sorted either for degradation, which is considered as stop signal, or for recycling back to the plasma membrane to re-participate in chemokine sensing. The major route of chemokine receptor internalization is through clathrin-mediated endocytosis (CME) [15, 16]. In some cases, however, chemokine receptors may also be internalized through clathrin-independent pathways mainly involving caveolae or lipid rafts [15, 16]. Whether chemokine receptors or atypical scavenger chemokine

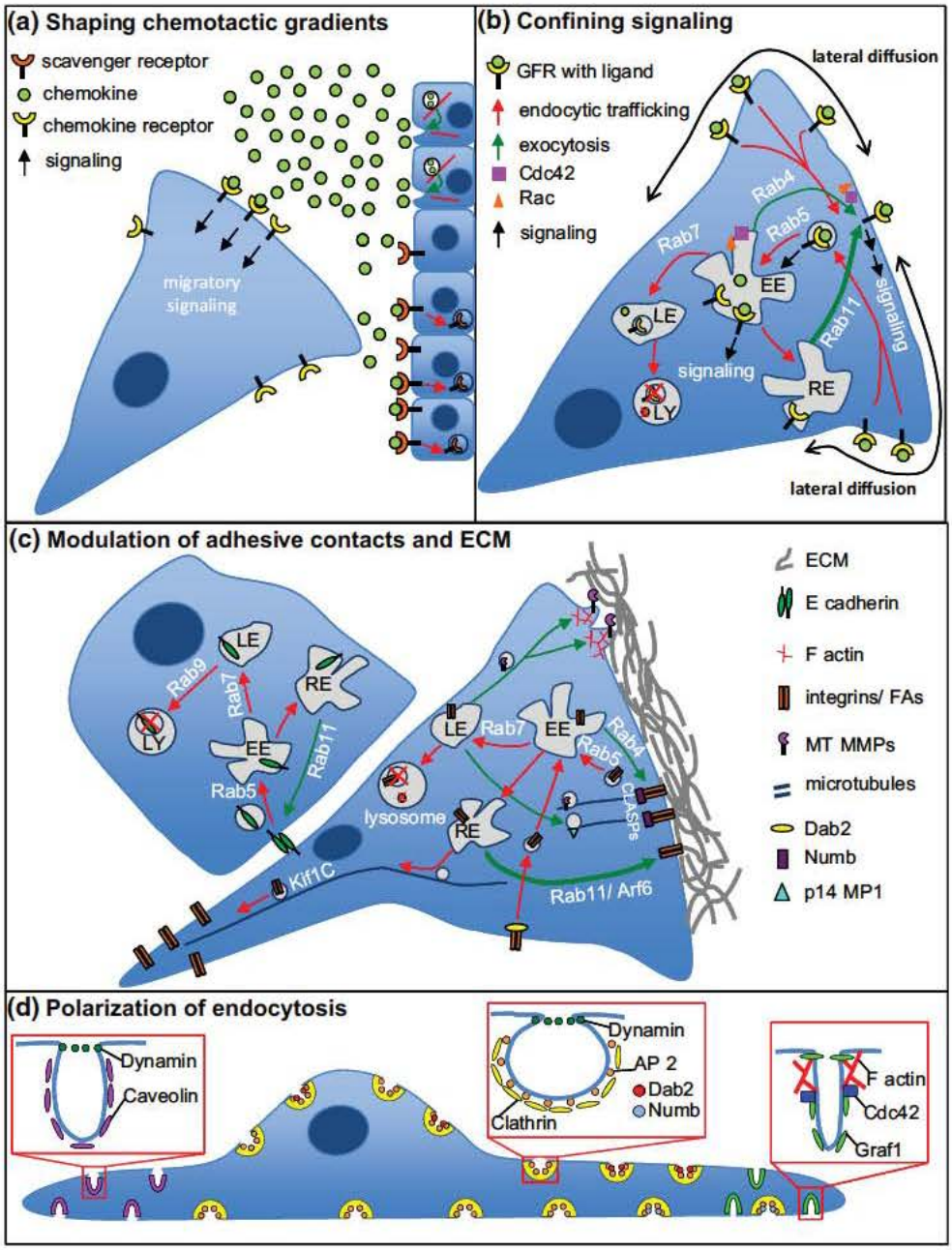

Fig. 1 Endocytic trafficking contributes at multiple steps for the regulation of the different processes required for cell migration. **a** The binding of scavenger receptors to secreted chemokines and the subsequent endocytosis of the receptor ligand complexes contributes to the shaping of chemotactic gradients. For details see paragraph 1. **b** Endocytosis and subsequent recycling of growth factor receptors and chemokine receptors to specific sites at the leading edge counteracts lateral diffusion thereby confining the pro migratory signaling to the cell front. For details see paragraphs 2 and 3. **c** Adhesive contacts between cells or between cell and extracellular matrix are regulated through the internalization of the involved adhesion molecules such as E cadherins and integrins which can be either routed to degradation or recycled to contact sites. In addition, vesicular traffic provides factors for the disassembly of focal adhesions as well as for the modulation of the extracellular matrix. For details see paragraph 5 and 6. **d** The different modes of endocytosis appear to be polarized in distinct ways in migrating cells with caveolin mediated endocytosis being enriched at the cell rear and the CLIC pathway at the leading edge, while clathrin coated pits per se do not show a strong polarization. However, certain cargo specific adaptors operating at clathrin coated pits display a strong enrichment at the leading edge. For details see "Excursion 1: polarization of the endocytic machinery during cell migration"

receptors also exploit the newly identified endophilin-dependent, clathrin-independent pathway used by certain other GPCRs [17] remains to be determined. CME is initiated upon chemokine binding to the receptor and its subsequent phosphorylation on serine and threonine residues by G-protein-coupled receptor kinases (GRKs), resulting in dissociation of the G-protein subunits from the receptor and interaction with the endocytic adaptors AP-2 and β-arrestin. The association of the chemokine receptor with the adaptors allows its concentration in nascent clathrin-coated pits (CCPs) which further invaginate and finally undergo fission from the plasma membrane giving rise to clathrin-coated vesicles (CCVs). The CCV becomes uncoated and the receptor-chemokine complex enters the early endosomal pathway. From there the chemokine receptor either proceeds to late endosomes/lysosomes to be degraded or dissociates from its ligand and enters the recycling compartment to traffic back to the plasma membrane [15]. Small GTPases of the Rab family are responsible for the regulation of the intracellular trafficking steps [18].

The chemokine receptor CCR7, for instance, follows the clathrin-dependent pathway involving dynamin, Eps15, Rab5 and early endosomal antigen-1 (EEA1) [19, 20]. After internalization, CCR7 enters the recycling compartment and traffics back to the plasma membrane to actively participate in directed cell migration, whereas its ligand CCL19 is sorted to lysosomes for degradation [19]. Interestingly, ubiquitylation of CCR7 serves as control mechanism for receptor trafficking as an ubiquitylation-deficient mutant on one hand reduces the intracellular pool of CCR7 in the steady-state and, on the other hand, shows a significant retardation in receptor recycling after ligand-mediated endocytosis, resulting in a reduced CCR7 surface pool and in inefficient cell migration [21].

Chemokine receptor recycling mainly follows the slow recycling pathway mediated by Rab11 [15]. Noteworthy, overexpressing a dominant negative mutant of Rab11 or truncation mutants of Rab11-family interacting protein 2 (Rab11-FIP2) and myosin Vb significantly impairs CXCR2 recycling and, more importantly, hampers its re-sensitization, consequently leading to a less efficient migration towards its ligand in vitro [22]. Moreover, a CXCR2 mutant lacking the C-terminal LLKIL motif responsible for binding to AP-2 partially loses its internalization capacity upon chemokine binding and shows reduced migration towards CXCL8 [23]. Similarly, AP-2 depletion diminished CXCL8-induced CXCR2 internalization, cell polarization and migration in vitro [23].

Of note, chemokine receptors are versatile in using different pathways of endocytosis and intracellular trafficking. The best example is CXCR4 which can exploit both clathrin-dependent and independent routes for endocytosis, depending on the cellular context [15]. Remarkably, the warts, hypogammaglobulinemia, infection and myelokathexis (WHIM) syndrome is an immunodeficiency disease linked to the expression of a C-terminally truncated mutant of CXCR4. Leukocytes expressing this WHIM-mutant of CXCR4 display enhanced G-protein-dependent signaling and cell migration in response to CXCL12 in vitro, but are refractory to internalization, which is G-protein coupling independent [24]. However, opposing results in terms of the role of the CXCR4 C-terminus in regulating cell migration in vitro have been published [25]. A rigorous study from the Raz group demonstrated in vivo that in zebrafish CXCR4 internalization is dispensable for cell motility and directional chemokine sensing, but essential for fine-tuning of migration allowing precise arrival of primordial germ cells [26]. Finally, CXCR4 internalization and its subsequent lysosomal degradation have also been reported [27], a mechanism that is implicated in rendering cells insensitive to chemokines and hence immobile.

Polarizing growth factor receptor signaling

Cell migration is influenced by growth factors like platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and keratinocyte growth factor (KGF) that bind to their respective growth factor receptors. A seminal study of border cell migration in *Drosophila melanogaster* revealed the importance of a spatially confined localization and signaling of these receptors for directed cell migration [28]. During *Drosophila* oogenesis, a cluster of 6 8 somatic cells which arise from the follicular epithelium migrates between the giant nurse cells towards the oocyte. These cells owe their name to the fact that they end up on the border between nurse cells and oocyte, and are used as model system for a stereotypic collective cell migration [29]. Border cells rely on the two receptor tyrosine kinases (RTKs) EGFR and PVR (PDGF and VEGF (vascular endothelial growth factor) receptor related), a homolog of the mammalian PDGF/VEGF receptor, as guidance receptors for their spatial orientation during directional migration toward the oocyte [30, 31]. Loss of the proteins Cbl, an E3 ubiquitin ligase, and Sprint, a Rab5 guanosine exchange factor (GEF), which are crucial regulators of EGFR and PVR endocytosis, led to severe migration defects [28]. These defects were shown to derive from the delocalization of RTK signaling, which was not restricted to the front any more. Thus endocytosis serves in this context to maintain RTKs and thereby guidance signaling spatially confined to the leading edge of the border cells to enable their polarization and directed migration. However, border cell migration does not only require endocytosis to counteract delocalization of guidance signaling by lateral diffusion of

guidance receptors, but likely also regulated trafficking of RTKs through the endosomal system for their recycling to a specific region of the plasma membrane. The small GTPases Rab5 and Rab11, which regulate early endosomal and slow recycling endosomal transport, were shown to be crucial for the polarization of guidance signaling during border cell migration [32]. In addition, a component of the exocyst complex, which is involved in targeting exocytosis to defined plasma membrane sites, was reported to play a role [32]. Although it could not be established yet whether RTKs themselves are the cargo of this recycling pathway or whether it serves to localize downstream factors like Rac1, the transport loop from the cell front to the recycling endosomal compartment and back to the leading edge is clearly crucial for maintaining polarized RTK signaling in border cells [32].

Also in the mammalian system cell migration was shown to depend on RTK endocytosis. For instance, in case of the PDGFRβ, an intracellular signaling pathway was unraveled by which the Rac1 GEF DOCK4 and the GTPase dynamin2 control NIH3T3 cell migration in response to PDGF [33]. Upon ligand binding, PDGFRβ becomes tyrosine phosphorylated enabling its interaction with the adaptor protein Grb2 which recruits DOCK4 and dynamin2 resulting in receptor endocytosis and Rac activation. Like in the case of border cells [28], blocking RTK endocytosis in NIH3T3 fibroblasts causes impaired migration [33]. Of note, internalized PDGFR recycled via Rab11 or Rab4 positive endosomes to the surface of NHI3T3 cells [33], whereas in human fibroblasts the same receptor is predominantly degraded in lysosomes [34]. Consistent with the importance of balanced PDGFR trafficking for cell migration and other processes, it is subject to regulation by kinases and phosphatases. Activation of PKCα was identified as critical determinant for sorting PDGFRβ into a Rab4a-dependent recycling route [35]. Loss of PKCα impaired the routing of activated PDGFR into the short recycling pathway thereby promoting its degradation, while loss of the phosphatase TC-PTP, which restricts PKCα activation, promoted Rab4a-dependent recycling of PDGFR in mouse embryonic fibroblasts (MEFs). Increased recycling of PDGFR promoted an increased chemotaxis of MEFs towards PDGF-BB [35].

Endocytosis can not only serve to spatially restrict receptors and terminate their signaling, but it can also provide, in the form of endosomes, a platform for the intracellular signaling of receptors. This renders a tight regulation of the endocytic trafficking of active receptors even more crucial for controlling their signaling. Impaired PDGFR endocytosis was in fact shown to decrease its mitogenic signaling [34]. Moreover, alternative receptor internalization and trafficking routes might bring the receptor into contact with distinct downstream signaling components and thus allow for selective activation of different signaling cascades thereby promoting either migration or proliferation [34].

In addition to PDGFR, the keratinocyte growth factor receptor (KGFR) was shown to be endocytosed in a polarized manner in migrating cells. The KGFR is an epithelial cell-specific splicing variant of fibroblast growth factor receptor 2 (FGFR2) and promotes keratinocyte migration upon binding to KGF/FGF7 or FGF10 (see references in [36]). In migrating keratinocytes the polarized localization of this receptor at the leading edge upon ligand stimulation depended on the kinase Src and on cortactin which contribute to KGFR endocytosis and thus polarization. In fact Src inhibition as well as cortactin depletion resulted in impaired KGFR internalization [36].

The EGFR was likewise reported to be internalized in a polarized manner in migrating mammalian cells. When the endocytosis of EGFR was studied in the context of chemotactic cancer cell invasion, CME was found to be polarized towards the front of the migrating breast cancer cells and to be indispensable for EGF-dependent invasion of MDA-MB-231 cells [37]. Again, EGFR trafficking is subject to regulation, and dysregulation of EGFR is in fact linked to disease progression and metastasis in many types of cancer. Ligand-stimulated EGFR internalization commonly leads to degradation of the receptor to limit its signaling. The protein Bif-1, a tumor suppressor, modulates EGFR degradation and thereby chemotactic migration of breast cancer cells [38]. Loss of Bif-1 impairs the recruitment of Rab7 to EGF-positive vesicles and its activation, which in turn leads to the sequestration of internalized EGF in Rab5 positive endosomes and delayed EGFR transport to lysosomes for degradation. This results in a sustained Erk1/2 activation in response to EGF stimulation and to increased chemotactic cell migration. Thus, Bif-1 is able to suppress breast cancer cell migration by promoting EGFR degradation through the regulation of endosome maturation [38]. The protein tyrosine phosphatase PTPN3, which was reported to inhibit border cell migration [39], likewise promotes EGFR degradation thereby inhibiting lung cancer cell migration. By dephosphorylating the endocytic factor Eps15, PTPN3 promotes lipid raft-mediated endocytosis of EGFR leading to its lysosomal degradation [39]. In addition, a screen for new regulators of EGFR endocytosis in human HBL100 breast cancer cells recently identified annexin A2, an adapter protein with several interaction partners. Depletion of annexin A2 in MDA-MB-231 cells inhibited EGFR transport beyond the early endosome, increased downstream signaling and promoted EGF-induced cell migration as well as lung metastasis in mice [40]. The effect of annexin A2 on EGFR endocytosis is linked to activation of the actin-severing protein cofilin establishing annexin A2 as a mediator of EGFR endocytic trafficking

and signaling in breast cancer cells via regulation of cofilin activation [40]. Furthermore, the Rab11 effector Rab-coupling protein (RCP) has pro-invasive effects by forming a complex with the integrin α5β1 and EGFR1 and by coordinating their recycling [41]. EGFR-dependent signaling likely occurs partly from the plasma membrane, leading to the activation of PKB/Akt via phosphoinositide-3-kinase (PI3K), and partly from late endosomal compartments, resulting in the activation of the Ras-MEK-ERK axis (see references in [41]). RCP-dependent recycling might keep EGFR from efficiently reaching late endosomes thereby promoting pro-invasive PKB/Akt signaling [41]. These examples illustrate vividly the crucial role of endocytic pathways for correctly localizing growth factor receptors to enable directed migration as well as for limiting or enhancing depending on cellular context potentially oncogenic growth factor receptor signaling.

Regulating downstream signaling and actin regulatory components

Cell surface receptors relay the guidance information they gather by ligand binding into the cell via diverse signaling cascades. In the context of cell migration, these cascades converge often on actin regulatory components, as the dynamic reorganization of the actin cytoskeleton forms the basis for cell migration. In the past years it has become clear that not only the guidance receptors themselves are regulated by endocytic trafficking, but also a number of downstream effectors. A pivotal example is the small GTPase Rac1 which is activated by GEFs downstream of growth factor receptors. Rac stimulates nucleation promoting factors (NPFs), that activate actin nucleators like the ARP2/3 complex, and is therefore crucial for the rearrangement of the actin cytoskeleton. Also here *Drosophila* border cell migration served as a valuable experimental system to unravel in how far endocytic trafficking of Rac is needed for the spatial restriction of signaling [42]. CME and the early endosomal Rab5 GTPase were crucial for activation of Rac by growth factors in line with the finding that Rac activation in fact occurs on early endosomes due to the recruitment of the Rac GEF Tiam 1 to this location. Subsequently, Rac recycles in an Arf6-dependent and spatially controlled manner to the leading edge which ensures the localized induction of actin-based migratory protrusions [42]. Activating Rac within the endosomal system couples this crucial step to the targeted delivery of the protein to specific plasma membrane sites and thereby limits Rac-dependent actin remodeling to the leading edge. This mechanism reinforces the theme that recycling serves as a tool to achieve the spatial confinement of signaling molecules.

Border cell migration requires an additional layer of spatial regulation, as the cells do not migrate independently but collectively. Here Rac signaling does not only need to be restricted to the leading edge of an individual cell, but should be specifically enriched in the leading cell. The expression of dominant negative Rab5 in border cells abolishes Rac activation which reinforces the crucial role of endocytic trafficking for the regulation of Rac activity [43]. Interestingly, the expression of a dominant negative Rab11 variant caused a divergent phenotype: Instead of being confined to the leading cell the signal peak for activated Rac alternated between different cells or was present in multiple cells at once [43]. Thus, Rab11 seems to regulate the polarization and persistence of Rac activity in the leading cell of a collectively migrating cell cluster and to control the spatial pattern of Rac activity across the cluster [43]. How Rab11 as an endosomal trafficking protein achieves this intercellular coordination of Rac activity remains a mystery for the moment.

Another small GTPase whose polarized localization during cell migration was shown to depend on endocytic trafficking is Cdc42. Cdc42 is known to control cell polarization and the direction of cell migration in chemotaxis and wound-induced migration. Like for Rac, its activation needs to be spatially restricted to the leading edge to promote directed migration. In a wound-induced astrocyte migration assay dynamic Cdc42 positive vesicles were observed to move in a directed manner towards the leading edge [44]. Again Arf6 served as decisive factor for vesicular transport of Cdc42 and its GEF βPIX to the leading edge [44] reinforcing the notion that Arf6 controls crucial membrane transport steps in directed cell migration.

Excursion 1: polarization of the endocytic machinery during cell migration

Originally the rate of endocytosis had been proposed to be higher at the cell's trailing end to achieve polarized bulk recycling of membrane and/or adhesion molecules to the leading edge during migration [45, 46]. However, as discussed above, nowadays it is rather endocytosis at the leading edge which is regarded to be crucial for cell migration by spatially restricting localization of signaling receptors. Does this mean that endocytosis itself is polarized to the leading edge, taking place more frequently there than at the cell rear? Endocytosis is of course an umbrella term for different modes of internalization [47]. CME is the best-understood pathway to date. However, there are also clathrin-independent internalization routes like caveolin-mediated endocytosis or the clathrin-independent carrier (CLIC) pathway [47]. The first attempts to study the localization and dynamics of CME during cell migration

were made more than a decade ago by combining overexpression of fluorescently tagged endocytic proteins with dual-color total internal reflection fluorescence (TIRF) microscopy of MDCK cells in a scratched monolayer [48]. When dividing migrating cells into a lagging, middle and leading region, Rappoport et al. observed an enrichment of clathrin, dynamin2 and transferrin in the middle and front region versus the rear of the cell. However, there was no difference between middle and front region arguing for a rather weak polarization of the endocytic machinery in the direction of cell migration [48]. The finding that CCPs per se are not specifically enriched at the leading edge is in line with the broad distribution of AP-2 observed in migrating ECV304 cells [49] and the non-polarized uptake of the bona fide CME cargo transferrin in migrating fibroblasts [50]. When looking more specifically at CCP dynamics in different cell regions, the number of disappearing CCPs per area was reported to be increased in the front region [48]. However, as this number was not normalized to the total, this difference might originate from the likewise increased total number of clathrin puncta. When CCP dynamics were studied recently in 3D cell migration, clathrin appeared more dynamic in the front region of migrating cells, even though no significant increase in the likelihood for leading edge CCPs to undergo endocytosis was found [37]. Clearly more experiments are needed to conclusively resolve the question whether CCP dynamics at the leading edge are different. However, the most striking difference between leading edge CCPs and other CCPs is in fact their composition in terms of certain cargo-specific endocytic adaptor proteins like Numb [49] that are specifically enriched in leading edge CCPs, likely to mediate the internalization of cell migration-related cargo proteins such as integrins.

In the case of caveolin-mediated endocytosis the main component, Cav1, has been shown to localize predominantly to the cell rear during 2D migration [51, 52]. This was confirmed by ultrastructural analyses finding the typical flask-like invaginated caveolae structures mainly localized at the trailing end [50]. Conversely, a strong leading edge polarization was observed for the CLIC pathway in fibroblasts migrating into a scratch wound [50]. CLICs are the vesicles involved in uptake via the Cdc42 regulated clathrin-independent endocytosis mechanism [53]. This pathway involves the uptake of extracellular fluid and GPI-anchored proteins and requires neither clathrin nor dynamin [53]. As the CLIC pathway depends on Cdc42 [54] and as Cdc42 itself is polarized to the leading edge, this might explain the strong polarization of the CLIC pathway to this site. Using proteomic approaches, Howes et al. [50] identified additional cargo proteins for the CLIC pathway such as the proteins Thy-1 and CD44 which have functions at focal adhesions and thus imply a role for CLICs in adhesion turnover. Inhibition of the CLIC pathway in fact impaired fibroblast migration into a wound. Recently, an additional clathrin-independent mode of endocytosis has been described [17]. This fast endophilin-mediated endocytosis (FEME) was shown to mediate the ligand-triggered uptake of GPCRs and RTKs, such as EGFR and PDGFR, at the leading edge of cells consistent with a role in cell migration. In fact, the migration of cells depleted for all three endophilin-A proteins was strongly decreased [17].

Thus, CME, caveolin-mediated endocytosis, the CLIC pathway and FEME all seem to contribute to cell migration. A recent study quantified that at least 95 % of the earliest detectable endocytic vesicles arise from CCPs [55] leaving not much room for additional contributions of other endocytic pathways to endocytic flux. However, uptake was analyzed in non-migrating cells. The possibility that pro-migratory signaling might change the relative contribution of the different endocytic pathways to overall endocytic flux has not been investigated so far. CLICs have been claimed to account for the major uptake of fluid and bulk membrane in fibroblasts and thus could constitute a pathway that provides rapid membrane turnover at the leading edge of migrating cells [50]. A study in *Dictyostelium* quantified that the cellular surface area changes so substantially during cell movement that endo-/exocytosis is required to adjust the cell surface area to shape changes [56]. Maybe the CLIC pathway contributes to the required dynamic membrane remodeling.

Excursion 2: unexpected additional roles for trafficking proteins in cell migration

Recently, a number of well-characterized endocytic and endosomal proteins have been reported to promote cell migration in ways that are not directly linked to their primary function in vesicular transport (Fig. 2). The first example pertains to the just discussed regulation of Rac1. Unexpectedly, the endocytic fission GTPase dynamin2 does not only regulate Rac1 activation indirectly due to its role in endocytosis, but also via direct binding to the Rac1 GEF Vav1 [57]. Complex formation between dynamin2 and Vav1 has proven crucial for preventing Hsc-70-mediated lysosomal degradation of Vav1. In this manner, dynamin2 contributes to Rac1 activation independent of its function in fission, at least in cell types where Vav1 is expressed such as pancreatic tumor cells whose invasive migration is potentiated by dynamin2-dependent stabilization of Vav1 [57]. Another key player of CME, clathrin itself, was reported to act independently of the endocytic machinery as a recruiting factor that localizes the ARP2/3-activating WAVE complex to lamellipodial membranes

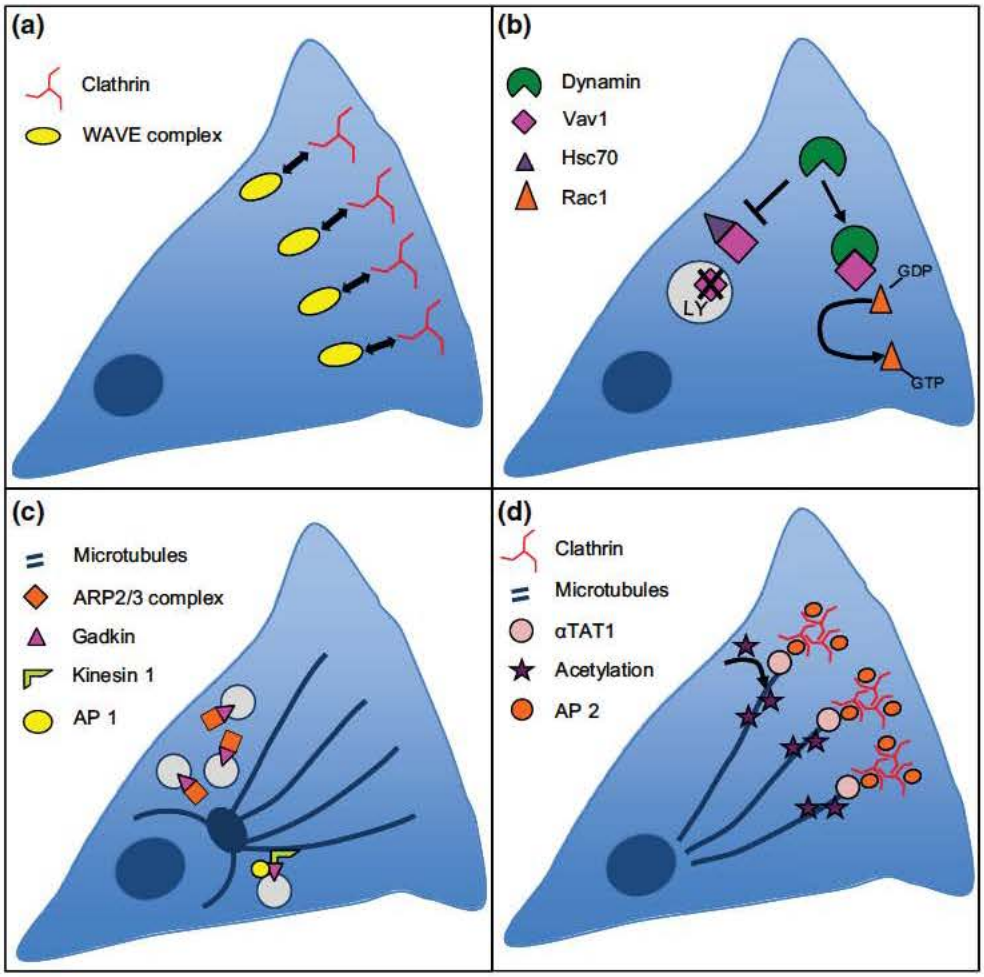

Fig. 2 Unexpected additional roles for trafficking proteins in cell migration. **a** Additional non endocytic involvement of clathrin in cell migration as recruiter of the WAVE complex. **b** Additional non endocytic role of dynamin2 in cell migration by activation of Rac1 via binding of its GEF Vav1. **c** Additional role for Gadkin as inhibitor of the actin nucleating ARP2/3 complex. **d** Additional non endocytic role of AP 2 in cell migration as anchor point for the microtubule acetylating enzyme αTAT1. For details please refer to "Excursion 2: unexpected additional roles for trafficking proteins in cell migration" in the main text

[58], thereby promoting lamellipodium formation. However, as clathrin does not bind to membranes, it remains enigmatic how it is recruited in the first place without the involvement of the classical endocytic recruitment factors. Finally, the main endocytic adaptor protein complex AP-2 was demonstrated to serve a dual function by participating on one hand in endocytosis and serving on the other hand as an anchor point for acetylated microtubules [59]. Acetylation is a means of stabilizing microtubules, which are then especially found in cell protrusions and contribute to cell polarization, migration and invasion (see references in [59]). Acetylation is conferred by the α-tubulin acetyltransferase αTAT1 which was shown to directly interact with AP-2. As the sites of endocytosis, the CCPs, where AP-2 is present, are supposed to be somewhat enriched at the leading edge, linking αTAT to AP-2 might ensure the polarized acetylation of microtubules towards the leading edge supporting their role in directional cell migration and invasion. Accordingly, the loss of AP-2 results in decreased microtubule acetylation as well as reduced directionality of 2D and invasive migration [59]. Another adaptor, the AP-1-associated protein Gadkin [60], that regulates recycling endosomal traffic by linking AP-1 positive vesicles to the microtubule-dependent motor protein kinesin1 [61], was likewise found to have a complementary role in cell migration. Gadkin binds additionally to the actin nucleator ARP2/3, which is crucial for lamellipodia formation. In the absence of activated NPFs Gadkin sequesters, the ARP2/3 complex on endosomal vesicles thereby inhibiting cell migration [62]. Thus, it becomes more and more common for proteins of the endocytic trafficking machinery to serve additional roles in cell migration.

Regulating adhesive contacts

Integrin trafficking

Cells form adhesive contacts to the substratum and potentially also to neighboring cells. These contact sites need to be dynamically turned over to allow for cell migration. This again involves endocytic trafficking. Adhesive contacts to the substratum are generally established via focal

adhesions. The first step in focal adhesion assembly is the binding of transmembrane integrin heterodimers to extracellular matrix ligands such as fibronectin, vitronectin, laminin or collagen. Upon ligand binding, a conformational change occurs that renders the cytosolic tail of integrins accessible for intracellular interactors leading to the recruitment of diverse proteins such as talin, vinculin and α-actinin that link integrins to the actin cytoskeleton which in turn allows the generation of traction forces through actomyosin contraction. Adhesions are and have to be highly dynamic structures to enable cell migration. They generally start as small, short-lived so-called nascent adhesions which either turn over rapidly or connect to the actin cytoskeleton and mature into larger focal complexes. These can keep growing into the still larger, often elongated focal adhesions in response to actomyosin contractile forces (for more details see [63]). Mature focal adhesions couple the extracellular matrix to the intracellular actin cytoskeleton and thereby provide the necessary anchorage for cell migration. They are estimated to comprise a network of about 180 protein protein interactions [64] illustrating the complexity of these structures. Even mature focal adhesions are still very dynamic, sliding sometimes along while the cell moves and exchanging proteins over the course of their lifetime. Finally, focal adhesions have to disassemble to allow the cell to move on.

The function of integrins crucially depends on their position in the cell, respectively, on their availability at the plasma membrane, which accordingly is subject to intricate regulation. It is well-established that many integrins continually and rapidly cycle between the plasma membrane and endosomal compartments. In fact, integrins are estimated to cycle about once every 30 min [65]. In mammals there are 24 types of integrin heterodimers composed of 18 α- and 8 β-subunits. These different integrin heterodimers can have quite divergent effects on different aspects of cell migration. Accordingly, their trafficking is a highly selective process distinguishing between active and inactive integrin heterodimers [66] which lets certain heterodimers cycle rapidly while others remain for longer times at the plasma membrane. As integrins are crucial for cell adhesion and migration, there is a wealth of literature already on their trafficking. The basic principles of their transport are well understood. However, there are still many details left to resolve, as the precise trafficking itinerary of individual integrins depends on several factors such as their exact heterodimer composition, their activation status, the trafficking of other integrins and growth factor receptors, extracellular stimuli as well as the cell type. Here, we will summarize the basic principles of integrin trafficking and highlight some recent progress illustrating the close connection between integrin functionality, endocytic transport and cell migration. For more details, we refer the reader to a number of reviews specifically dedicated to integrin trafficking [65, 67 70]. Integrins are internalized by many different pathways including CME, caveolin-dependent endocytosis and clathrin-independent pathways like macropinocytosis at circular dorsal ruffles (see also table in [68]). CME of different types of integrins is often facilitated by cargo-specific adaptors like Dab2 [71], ARH [72] and Numb [49]. These endocytic adaptor proteins have been shown to interact with the NPxY motif in β-integrin cytoplasmic tails via their phosphotyrosine-binding domains. While Dab2 was reported to colocalize with integrin β1 in CCPs that are dispersed over the cell surface, suggesting that it regulates bulk endocytosis of inactive integrins [71], Numb localizes specifically to CCPs at the substratum-facing surface of the leading edge and facilitates there the uptake of integrin β1- and β3-containing heterodimers [49]. Thus, different functional pools of the same integrins might employ different endocytic adaptors depending on their cellular location. At the same time the phosphorylation-dependent regulation of Numb turns this adaptor protein into a molecular switch that links integrin endocytosis to upstream signals involved in cell migration [73]. Endocytosed integrins are transported to early endosomes where sorting into degradative or recycling pathways occurs [70]. While integrins can be routed to degradation, the majority normally recycles rapidly to the cell surface. The decision between degradation and recycling is influenced by proteins like sorting nexin 17 which was shown to prevent the lysosomal degradation of β1 integrins by binding within early endosomes to their cytoplasmic tail [74]. For recycling, integrins can employ either the short Rab4-dependent recycling pathway or the long Rab11- and Arf6-dependent recycling loop which transits through the perinuclear recycling compartment [70]. By modulating the internalization and the transport itinerary of integrins, the endocytic trafficking machinery can influence the composition of focal adhesions which in turn determines cell migration parameters like directionality. This is due to the fact that different integrins affect focal adhesion dynamics in distinct ways thus having diverging effects on directionality. While α5β1 integrins promote focal adhesion turnover and thereby random cell motility, the heterodimer αvβ3 rather suppresses focal adhesion dynamics thereby promoting directionally persistent migration [75]. Thus, the balance between the different integrin heterodimers has to be tightly controlled to achieve the degree of directionality adapted to the acute cellular need. The Rab4-dependent recycling of αvβ3 in fibroblasts and the activity of this integrin for instance suppresses the recycling of α5β1 thereby coordinating their activities and promoting directionally persistent migration [70]. Blocking αvβ3 on the other hand promotes the association of RCP with α5β1 and its RCP-dependent

recycling [41] as well as random movement. As mentioned earlier, RCP also coordinates trafficking of α5β1 integrin and EGFR1 which leads to increased activation of the pro-invasive PKB/Akt kinase downstream of EGFR1. Recently RacGAP1 was identified as Akt substrate in this pathway [76], which locally suppresses Rac activity while enhancing RhoA activity thereby promoting invasive migration into fibronectin-containing matrices [76]. The regulation of Akt signaling via the endosomal adaptor protein APPL1 illustrates the emerging importance of subcellular compartments as platforms for the coordination of migration-relevant signaling cascades. APPL1 decreases Src-mediated activation of Akt1 at the leading edge thereby impairing migration by hindering the turnover of adhesions [77]. Thus, APPL1 likely acts as scaffold for bringing together signaling molecules on endosomes which allows for their regulated targeting to specific regions within the cell [77]. A further regulatory layer controlling αvβ3 versus α5β1 integrin recycling is exerted by syndecan-4 [78]. This transmembrane heparan sulfate proteoglycan acts as a switch in the recycling of those integrins. Syndecan-4 was shown to be phosphorylated by Src which promotes its binding to syntenin, which in turn suppresses Arf6 activity. Indeed, suppression of Arf6 activity via syndecan-4 or by depletion of Arf6 itself resulted in increased recycling of αvβ3 to the plasma membrane at the expense of α5β1 which promoted focal adhesion stabilization and directionality [78]. Directional persistence is also promoted by kinesin Kif1C-mediated transport of integrins [79], which is needed to stabilize trailing adhesions. Kif1C distributes integrin α5β1 heterodimers within the cell and transports them particularly from the perinuclear recycling compartment to the cell tail to ensure a sufficient supply for the reinforcement of rear adhesions thereby stabilizing the extended cell tail to sustainably resist traction forces. Loss of Kif1C causes the rapid and frequent retraction of the cell tail which is accompanied by changes in migration direction [79]. Another microtubule-dependent motor protein which was recently identified in a screen for regulators of α2 integrin endocytosis is the kinesin KIF15 [80]. Depletion of KIF15 inhibited the intracellular accumulation of α2 integrin, which is likely due to the fact that it also induced a loss of plasma membrane-associated Dab2, as Dab2 depletion mimicked KIF15 depletion [80].

Not only factors for the uptake and decision between recycling and degradation of integrins have been identified, but also proteins involved in the transport between vesicular compartments and in the delivery back to the plasma membrane. For trafficking along the endosomal pathway and back to the cell surface vesicular membranes have to fuse with target membranes such as other vesicles or the plasma membrane. These fusion events are mediated by the zippering up of SNARE proteins present on both membranes thereby bringing the membranes in close enough contact for fusion [81]. Accordingly, SNARE proteins have been found to modulate cell migration. The SNARE SNAP29 is for instance implicated in the endocytic recycling of β1-integrin. Loss of SNAP29 caused a longer retention of β1 integrin in intracellular compartments and impaired wound healing [82]. Furthermore, the SNAREs VAMP3 and syntaxin6 were revealed as crucial factors for the surface delivery of integrins in HeLa cells [83]. Loss of syntaxin6, which is located at the trans-Golgi network, or VAMP3, which is present on recycling endosomes, led to the accumulation of α3β1 in perinuclear recycling endosomes and thereby impaired chemotactic cell migration. Syntaxin6 und VAMP3 presumably form a SNARE complex that catalyzes vesicle fusion which is necessary for the transport of integrins from VAMP3 positive recycling endosomes to the syntaxin6-containing trans-Golgi network, before they are delivered to the plasma membrane, thus delineating a new transport route [83].

Integrins also play an important role for lymphocyte migration, and part of the regulatory mechanism controlling their surface distribution in lymphocytes was recently elucidated. Here, the surface delivery of the lymphocyte integrin LFA-1 was shown to depend on Rab13 which needs to be activated by its GEF DENND1C which in turn is stimulated by phosphorylation through the kinase Mst1 [84]. Active Rab13 associates with Mst1 to deliver LFA-1 to the leading edge, and inhibition of Rab13 reduced lymphocyte migration on the LFA-1 ligand ICAM-1 [84]. Finally, as an additional layer of complexity integrins are not only regulated by endocytic trafficking themselves but also regulate in turn the endocytic transport of other migration-relevant cargos like the above mentioned EGFR (for details see [68 70]).

Focal adhesion disassembly

Apart from undergoing continuous exchange of protein components focal adhesions finally also have to disassemble. As integrins are a major component of focal adhesions, their removal is an integral part of focal adhesion disassembly. While the protease calpain contributes to this by cleaving focal adhesion proteins like integrins and talin [85], dynamin and CME of active β1 integrins were also shown to be instrumental for this process [72, 86]. Proteins of the endocytic machinery like clathrin, AP-2, Dab2 and dynamin2 localize to adhesion sites prior to adhesion disassembly. The importance of CME for focal adhesion disassembly is highlighted by the fact that depletion of dynamin2 blocks β1 integrin internalization leading to impaired focal adhesion disassembly and consequently to defects in cell migration [86]. CME depends

on phosphatidylinositol(4,5)bisphosphate [PI(4,5)P_2] which is generated by the enzyme PIPK1β and contributes to the recruitment of endocytic proteins like AP-2. Consistently, depletion of PIPK1β likewise decreased internalization of active β1 integrin, focal adhesion turnover and migration due to the impaired recruitment of the endocytic machinery to focal adhesion sites [87].

In addition, focal adhesion disassembly has long been known to be induced by microtubules contacting focal adhesions [88]. In fact, a common focal adhesion disassembly assay is based on the finding that microtubule regrowth after nocodazole washout induces massive disassembly of focal adhesions [72, 88]. However, the mechanism behind this observation has only recently become apparent. The microtubule-associated proteins CLASP1 and CLASP2 were identified as the tethering factors that actually link microtubules to focal adhesions thereby establishing a focal adhesion directed transport pathway, e.g. for the delivery and localized fusion of exocytic vesicles secreting proteases involved in extracellular matrix degradation [89]. Thus, microtubules may serve here to establish a local secretion pathway that facilitates focal adhesion turnover by severing cell matrix connections. At the same time Schiefermeier et al. [90] identified another vesicle type using the microtubule tracks leading up to focal adhesions. They demonstrated that a pool of Rab7 positive late endosomes, which carry the p14-MP1 (LAMTOR2/3) complex, moves to the cell periphery along microtubules in a kinesin1 and Arl8b-dependent manner. There they specifically target focal adhesions to induce the dissociation of the protein IQGAP1, which has been suggested to regulate cell migration. Loss of functional p14-MP1 complex causes several phenotypes: a decrease in focal adhesion turnover, an accumulation of peripheral elongated focal adhesions containing elevated IQGAP1 levels, and a reduction in migration speed. Depletion of IQGAP1 rescued the migration defect of p14 deficient cells suggesting that removal of excess IQGAP1 constitutes an essential step for focal adhesion disassembly [90]. However, which role IQGAP1 actually plays at focal adhesions still needs to be determined. Moreover, it remains a challenge to unravel the spatiotemporal coordination of the diverse processes (calpain-mediated proteolysis, CME of integrins, endosomally mediated dissociation of IQGAP1, targeted delivery of proteases for secretion) contributing to focal adhesion formation and disassembly.

Trafficking of other cell surface adhesions proteins

While integrins most often take center stage, a number of additional proteins exists that mediate contacts to the extracellular matrix such as syndecans and other proteoglycans like NG2/CSPG4 which act as receptors for extracellular matrix molecules and as co-receptors for growth factors and cytokines [91, 92]. Syndecans are removed from the plasma membrane by endocytosis. However, the endocytic mechanism underlying their uptake is only incompletely understood. Internalized syndecan-2 enters like many integrins an endosomal recycling compartment [93]. For the exit of syndecan from perinuclear Arf6-positive recycling endosomes, an interaction with the PIP_2 binding protein syntenin is required [94]. NG2, a chondroitin sulfate proteoglycan acting as co-receptor for PDGFR, which has been shown to promote migration and invasion [91], is very likely also regulated by endocytic trafficking.

In addition, there are those adhesion proteins that mediate cell cell contacts. The remodeling of these contacts is pivotal during collective cell migration and relies on endocytic trafficking of the involved cell cell adhesion proteins. A well-studied example is the cadherin protein family, which plays a role in different types of cell cell contact sites such as adherens junctions and desmosomes [95]. Classic cadherins connect cells at adherens junctions via Ca^{2+}-dependent homophilic interactions between their extracellular domains. Like integrins they are linked on the intracellular side via intermediate proteins to the actin cytoskeleton. Desmosomes are built up by the non-classical cadherins desmoglein and desmocollin which are indirectly connected to intermediate filaments. As a prototypical cadherin, we will discuss E-cadherin trafficking as an example. Epithelial cells are connected by E-cadherin-based adherens junctions which have to be dissolved as part of a process called epithelial-to-mesenchymal transition (EMT) to allow migration. EMT is on one hand a physiological process involved in many developmental processes as well as wound healing, but on the other hand it is also intimately linked to metastasis initiation [96]. As E-cadherin is the main component of epithelial adherens junctions, its removal is crucial for the dissolution of epithelial cell cell contacts. While E-cadherin is also heavily regulated at the transcriptional level, its internalization and lysosomal degradation represent a very rapid means to disassemble these contacts. Cadherins are like integrins internalized via different endocytic pathways like CME, caveolin-mediated endocytosis and macropinocytosis (see table in [97]) depending on cell type and cellular context. CME of E-cadherin depends on its dileucine motif [98], a sequence recognized by the endocytic adaptor AP-2. Access to this motif is regulated by competitive binding of p120 catenin. Depletion of p120 catenin unmasks the endocytosis motif of E-cadherin resulting in its endocytosis and accumulation in internal vesicles and concomitant dissolution of adherens junctions. The p120 catenin-dependent endocytosis block can be overridden by binding of the endocytic

adaptor Numb to p120 catenin and its recruitment of AP-2 [99] or by direct interaction of Numb with E-cadherin which probably prevents the binding of p120 catenin [100]. Alternatively, the interaction between p120 catenin and E-Cadherin can be abolished by Src-mediated phosphorylation of E-Cadherin. In addition, this phosphorylation promotes the binding of Hakai, an E3 ubiquitin ligase, which promotes E-cadherin endocytosis and degradation via ubiquitylation [101]. Also phosphorylation of E-cadherin takes place in the context of EMT [102]. In addition, E-cadherin binds the endocytic adaptor Dab2 [103]. Growth factor signaling promotes E-cadherin endocytosis additionally by activation of RIN2, which activates Rab5 [104], and of Arf6, which recruits Nm23-H1 to facilitate dynamin-mediated endocytosis [105, 106]. Studies in *Drosophila* have also shown a requirement for Cdc42, the Par6/aPKC polarity pathway and ARP2/3 to maintain normal rates of E-cadherin internalization [107]. An interaction between E-cadherin and the type Iγ phosphatidylinositol phosphate kinase isoform 2 splice variant (PIPK1γi2), which links E-cadherin to the clathrin adaptor protein complex AP-1B, seems to play a role in E-cadherin endocytosis and recycling [108] as well. Internalized E-cadherin can principally be targeted for lysosomal degradation or enter the recycling pathway for redelivery to the plasma membrane. The sorting decision must be tightly controlled to prevent unwanted EMT and metastasis. Mutations in the E-cadherin binding site for PIPK1γ in gastric carcinogenesis [109] highlight the importance of regulated E-cadherin trafficking to keep cell motility in check. While the underlying regulatory layers are only incompletely understood, a number of components involved in E-cadherin degradation and recycling have been identified. E-cadherin destined for lysosomal degradation is transported through Rab5 and Rab7 positive endosomes. Expression of inactive mutants of Rab5 or Rab7 delays E-cadherin degradation [102]. Further, it was demonstrated that the ubiquitylation of E-cadherin is crucial for its sorting into the intraluminal vesicles of multivesicular bodies (MVBs) [101, 102]. The ubiquitylation is recognized by the ESCRT-0 complex protein Hrs which recruits ESCRT machinery to catalyze the intraluminal sorting of the ubiquitylated cargo [110]. This degradative pathway was recently shown to be promoted by association of isoform 5 splice variant of PIPKIγ (PIPKIγi5) with E-cadherin [111] in a phosphorylation-dependent manner. SNX5 and SNX6 were reported to associate with PIPKIγi5 and to inhibit PIPKIγi5-mediated E-cadherin degradation. Src-induced phosphorylation of PIPKIγi5 downstream of HGF signaling impaired SNX5 binding thereby relieving the inhibition of PIPKIγi5-driven E-cadherin degradation [111]. The exact mechanism behind this regulation remains to be determined. Thus, both PIPKIγi2 and PIPKIγi5 interact with E-cadherin and synthesize PIP_2, but have opposite effects on E-cadherin trafficking.

Furthermore, Rab11 and the sorting nexin SNX1 have been implicated in E-cadherin recycling to the plasma membrane. Recently, efficient E-cadherin recycling was shown to depend on the lipid raft protein reggie/flotillin-1. Reggie/flotillin1 interacts with Rab11a and SNX4, and overexpression of constitutively active variants of these proteins rescued the E-cadherin recycling defect in reggie/flotillin-1 depleted cells arguing that reggie/flotillin exerts its influence on E-cadherin recycling via these two proteins [112]. Thus, E-cadherin trafficking is another example for how endocytic transport provides the organism with tools to rapidly match cellular adhesion and cellular motility exactly to its acute needs, for growing a new structure during development or preventing tumor metastasis. However, it is becoming increasingly clear that the trafficking of the different types of adhesion molecules cannot be viewed in isolation, but is often coordinately regulated. The surface levels of cadherins and integrins for instance tend to be inversely modulated during cell migration. This is achieved via the small GTPase Rab35 which promotes the surface localization of cadherins while at the same time inhibiting Arf6 and thereby down-regulating the Arf6-dependent recycling pathway for β1-integrins and EGFRs [113].

The influence of endocytic trafficking on extracellular matrix reorganization

The extracellular matrix provides on one hand the substrate for cell adhesion and traction, but on the other hand acts as physical barrier for invading cells [114]. Contact-dependent remodeling of the extracellular matrix hence determines rate and speed of cell migration along and through the extracellular matrix [115]. To achieve extracellular matrix remodeling, mesenchymal cells, such as solid tumor cells and fibroblasts, have developed a strategy to pericellularly remodel and proteolytically degrade the extracellular matrix using matrix metalloproteinases (MMPs) [114]. Cancer cells were shown to coordinate mechanotransduction and extracellular matrix remodeling by segregating the anterior force-generating leading edge containing integrins, F-actin and the membrane type 1 (MT1)-MMP from the posterior proteolytic zone where extracellular matrix fibers are cleaved [116]. Of note, the delivery of MT1-MMP to the leading invasive pseudopods of a migrating cell is coordinated by the NPF Neural Wiskott Aldrich syndrome protein (N-WASP) [117]. N-WASP is upregulated in invasive cancer cells and promotes the trafficking of MT1-MMP from late endosomes to the plasma membrane where it stabilizes MMT1-MMP via direct tethering of its cytoplasmic tail to F-actin [117].

Delivery of MT1-MMP to the invadosome further requires the master regulators of endocytosis Rab5 and Rab4 [118], as well as a vSNARE and VAMP7 for fusing the MT1-MMP-containing vesicles with the plasma membrane and presumably Rab8 for exocytosis [119]. Notably, overexpression of Rab5 in cancer cells was found to be necessary and sufficient for cell invasion and tumor dissemination by enhancing MT1-MMP driven extracellular matrix degradation and increasing intratumoral cell motility [118]. Finally, in vitro data provide evidence that extracellular matrix proteins, such as fibronectin, collagen and fragments thereof, themselves are able to influence integrin recycling and regulate gene expression of MMPs (e.g. MMP9) [67] thereby providing additional feed-forward signals influencing cell migration in vivo.

Conclusion

Cell migration involves multi-step cascades bringing about the coordinated establishment of extracellular guidance cues, the recognition and transmission of these cues by receptors, the induction of cell polarity, cell adhesion and partial detachment, and the remodeling of the extracellular matrix. These complex processes need to be tightly coordinated in space and time to finally enable a cell to move forward. Endocytic trafficking forms not only the basis for the essential spatial segregation and confinement of many molecules that contribute to cell migration, but emerges more and more as a key player in the coordination of the diverse processed by providing master regulators as well as transport routes and platforms where components of the different migration-related signaling pathways intersect.

Cells possess a remarkable migratory plasticity; they are able to adapt to numerous intra- and extracellular conditions and to integrate many factors to achieve the right mode of migration for a particular context. Endocytic trafficking takes central stage in this dynamic adaptation process. We are only at the beginning of understanding the molecular complexity underlying and connecting the processes summarized in this review. For the future, it will be crucial not only to describe the role of different trafficking proteins in cell migration, but also to unravel how decisions for different sorting steps are taken along a particular trafficking route, which molecules intersect during vesicular trafficking and potentially modulate each other and to integrate this information into a bigger context to understand how a cell will adapt its vesicular transport routes to modulate its migration mode. With the advancement of the new super resolution microscopy techniques and improved in vivo imaging of vesicles and their cargo, we will hopefully soon be able to tackle these questions and to decipher the trafficking-based coordination of cell migration in health and disease.

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