 Trafficking and Cell Migration

Florian Ulrich1 and Carl-Philipp Heisenberg2,*

1 Skirball Institute of Biomolecular Medicine, New York, USA
2 Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany
*Corresponding author: Carl-Philipp Heisenberg, heisenberg@mpi-cbg.de

The migration of single cells and epithelial sheets is of great importance for gastrulation and organ formation in developing embryos and, if misregulated, can have dire consequences e.g. during cancer metastasis. A keystone of cell migration is the regulation of adhesive contacts, which are dynamically assembled and disassembled via endocytosis. Here, we discuss some of the basic concepts about the function of endocytic trafficking during cell migration: transport of integrins from the cell rear to the leading edge in fibroblasts; confinement of signalling to the front of single cells by endocytic transport of growth factors; regulation of movement coherence in multicellular sheets by cadherin turnover; and shaping of extracellular chemokine gradients. Taken together, endocytosis enables migrating cells and tissues to dynamically modulate their adhesion and signalling, allowing them to efficiently migrate through their extracellular environment.

Key words: cell migration, endocytosis, integrin, cadherin, adhesion

Received 30 November 2008, revised and accepted for publication 21 April 2009, uncorrected manuscript published online 24 April 2009, published online 20 May 2009

Cell migration

Cell migration contributes significantly to morphogenetic changes during development and disease. Cells can move as single units, e.g. during border-cell migration in Drosophila and primordial germ-cell (PGC) migration in vertebrates, or they can be connected with each others to move as a coherent sheet of cells, such as in Drosophila dorsal closure and wound healing in vertebrates (1–4). The mechanisms that regulate cell cohesion during migration are only beginning to be understood, but recent studies suggest that endocytic turnover of adhesive contacts is an important regulator of movement coherence during gastrulation (5). Misregulation of cell migration can have grave consequences. For example, during cancer progression, epithelial cells acquire motile characteristics and leave their original tissue to form metastases at distant sites of the organism (6).

Cell migration is a dynamic process that can be subdivided into four phases: polarization of the cell in response to an external stimulus, formation of a protrusion at the leading edge, adhesion to other cells or the extracellular matrix (ECM) and retraction of the trailing edge, which moves the cell forward (7).

Gradients of growth factors and chemokines are generally thought to stimulate cell migration during development. For example, a gradient of platelet-derived growth factor (PDGF) regulates gastrulation movements in zebrafish and Xenopus through activation of phospho-inositol-3-kinase and Akt/protein kinase B, leading to the assembly of actin at the leading edge of the cells (8,9). Another example is chemokine signaling in Primordial Germ Cell (PGC) migration in mice and zebrafish. The chemokine SDF-1α is secreted by somatic cells and binds to its receptor CXCR4b, which is located on PGCs, leading to the recruitment of actin–myosin contractility to the leading edge of the cell via intracellular Ca2+ signaling (2,10). Cell protrusions form contacts to either the ECM or other cells. Binding to the ECM is mediated by integrins, whereas cadherins mediate cell-to-cell adhesions (11,12). Both integrins and cadherins are linked to the actin cytoskeleton via adaptor proteins, and the initial contacts become reinforced by recruiting more integrin or cadherin molecules into the complex (‘clustering’). In parallel, the actin cytoskeleton polymerizes further, forming stress-fibers or actin bundles. Thus, the cell-to-ECM or cell-to-cell contacts mature and reinforce their link to the cytoskeleton (13–15).

In order to move over an adhesive substrate, cells must dynamically assemble and disassemble their adhesive contacts. The degradation of integrin complexes has been proposed as one possible disassembly mechanism. Integrin complex degradation is thought to be triggered by intracellular Ca2+ influx, which activates the protease calpain, cleaving components of the integrin adhesive complex that then become internalized and cleared from the plasma membrane. However, clathrin- and dynamin-mediated endocytosis have also been reported to directly regulate the disassembly of both integrin-ECM and cadherin-based cell-to-cell contacts (16,17). The following chapters will provide more insight into these mechanisms.

Integrins and cell-to-ECM adhesion

Integrins are type I transmembrane receptors consisting of a non-covalently linked α and β subunit and mediate cell-to-cell and cell-to-substrate adhesion. In mammals, there are 18 α- and 8 β-integrin subunits, and 24 different integrin combinations have been found to date (18,19). Integrins...
bind to a variety of substrates in the ECM, such as laminin, collagen, vitronectin and fibronectin (20). Integrin-based adhesion is required for single cell migration and tissue morphogenesis; important examples include leukocyte migration, gastrulation and heart development (21–23).

Upon binding to extracellular ligands, integrins change their conformation and this structural change is transmitted through the plasma membrane to their cytoplasmic tails. This, in turn, changes the binding affinity of the intracellular part of the integrin dimer and allows them to assemble a protein complex, consisting of Rho GTPases, Src family kinases (SFKs) and adaptor proteins, such as α-actinin, talin and vinculin, which bind to actin (19,24). This complex thus serves a dual purpose. On one hand, it links integrins to actin filaments, providing a connection between the ECM, membrane and cytoskeleton, which reinforces tissue integrity and cell adhesion, and stabilizes cell protrusions during migration (15). On the other hand, it also constitutes a signaling platform through which integrins can relay information to major processes, such as transcriptional control, cell death, proliferation and cell migration (18). The assembly of an intracellular protein complex and the transduction of signals to downstream components as a response to integrin-to-ECM binding is named ‘outside-in’ signaling.

Notably, the assembly of an intracellular protein complex can also increase the binding affinity of the integrin extracellular domain to ECM ligands and thus activate the integrin complex in a process called ‘inside-out’ signaling (19,24). Inside-out signaling is initiated via interactions of the integrin intracellular domains with the cytoplasmic adaptor protein talin. In its inactive state, the cytoplasmic tail of the α-integrin subunit binds to and ‘masks’ the tail of the β-subunit. Talin is thought to bind to the β-tail and thus deactivates this interaction, leading to a change in binding affinity of the integrin complex for extracellular ligands. In addition, talin provides an assembling platform for Rho GTPases, SFKs, integrin-linked kinase, focal adhesion kinase (FAK) and tensin, which have been shown to be required for integrin-mediated adhesion and signaling during organogenesis in Drosophila and mouse. Moreover, talin, FAK and SFKs have been implicated in clathrin-mediated endocytosis and disassembly of the integrin complex, suggesting that the integrin complex is dynamically regulated (25). Indeed, in tumor cells, the localization of adhesion molecules to the leading edge is mediated through endocytic trafficking, suggesting a complex and dynamic control of cell adhesion and polarity (12,26–28). Interestingly, integrin complexes have also been involved in the transduction of mechanical stimuli into biochemical signaling. The scaffolding protein p130Cas binds to the integrin complex and becomes phosphorylated upon stretching, which allows it to assemble a signaling complex that activates Rap1 signaling. Rap1 has been shown to regulate cell migration and epithelial morphogenesis in Drosophila and vertebrates (29–32).

**Cadherins and cell-to-cell adhesion**

Cadherins constitute a large family of transmembrane proteins that are involved in cell-to-cell adhesion. Cadherins are expressed in and required for the development of various tissues throughout development—gastrulation and Primordial Germ-Cell (PGC) migration, somitogenesis, morphogenesis of the neural system, and heart and vascular development (33–36).

Classic cadherins harbor several extracellular cadherin repeats that mediate homophilic binding between cadherins of the same type; this interaction requires Ca\(^{2+}\). On the cytosolic side, cadherins assemble a protein complex that mediates signaling and links them to the cytoskeleton (35). The most prominent members of this complex are α-, β- and p120-catenin. Both β- and p120-catenin directly bind to the cytoplasmic cadherin tail, and α-catenin binds to β-catenin. Because β-catenin can also bind to actin filaments, it serves as a cross-linker connecting the cadherin complex to the actin cytoskeleton (35). The cadherin–catenin complex is dynamically regulated, with α-catenin existing in an equilibrium between β-catenin- or actin-binding filaments. Using biochemical binding assays and FRAP (Fluorescent Recovery After Photobleaching) studies in epithelial cell culture, a model has been proposed in which the recruitment of cadherin molecules into nascent cell-to-cell contacts elevates local α-catenin levels. α-catenin then competes with the Arp2/3 complex for binding to actin filaments, thereby inhibiting actin branching and facilitating formation and maturation of an actin cortex (13,14). p120-catenin supports the actin cytoskeleton link provided by α- and β-catenin, because mutations in p120-catenin augment the loss-of-function phenotypes of α- and β-catenin in Drosophila and Caenorhabditis elegans, resulting in reduced actin attachment at the cell–cell junctions. Moreover, in a mutant p120 background, mammalian epithelial cells lack an intact adherens junctions (AJ) belt and thus fail to compact their cells (37–39). In Xenopus, knockdown of p120-catenin disrupts cell movements during gastrulation, suggesting that p120-catenin has a role in regulating cadherin-based cell-to-cell adhesion (40). Interestingly, the loss of p120 is accompanied by an accumulation of cadherins in intracellular vesicle stores, suggesting that p120-catenin has a role in regulating cadherin endocytosis during mesodermal cell movements. In Xenopus embryos, p120 associates with the transcriptional repressor Kaiso, and this interaction elevates the expression of Wnt11. Wnt11 in turn regulates cell movements during gastrulation by influencing E-cadherin-based cell-to-cell adhesion and endocytosis, suggesting a complex link between cell adhesion, cadherin internalization and the transcriptional regulation of gastrulation movements (5,36,41).

**Scope of the review**

Over the past 20 years, a large number of observations in cultured cells have underscored the importance of adhesion turnover during cell migration in vitro. However, how endocytosis of signaling receptors and adhesion...
molecules dynamically regulates cell migration in vivo is still a matter of intense debate. This review aims at introducing key concepts of endocytosis during cell migration in development and disease. Specifically, it will focus on the following main topics:

- Integrin trafficking and cell migration.
- Cadherin trafficking and cell migration.
- Endocytosis and sharpening of chemokine gradients.

**Integrin Trafficking and Cell Migration**

The first hint that endocytosis is linked to cell migration came from experiments in cell culture. By probing integrin receptors with radioactively labeled ligands and observing their distribution within Chinese Hamster Ovary cells, a model was proposed in which adhesive contacts became disassembled at the rear of the cell and transported to the leading edge through the endocytic pathway, where they were reassembled [Figure 1A (42,43)]. In support of the assumption that integrins are endocytosed and recycled, α5β1 integrins have been found to colocalize with clathrin-coated vesicles. Moreover, dynamin, a key regulator of endocytosis, localizes to focal contacts and is required for their disassembly in cultured migrating cells (12,16,44).

Notably, tetanus neurotoxin-mediated cleavage of cellubrevin, an early endosome-specific v-SNARE, inhibits internalization of β1 integrin at the leading edge of migrating epithelial cells and, as a consequence of this, cell motility is impaired, suggesting that endocytosis functions during cell migration by regulating the turnover of adhesion molecules at the leading edge (45). This notion is further supported by observations in ovarian cancer cells, where Rab25 (a Rab11 paralog) directly interacts with the cytoplasmic tail of β1 integrin and promotes the rapid endocytic turnover of α5β1 integrins at the tips of large pseudopods (26). Using real-time in vivo imaging of photoactivated green fluorescent protein-integrin, it was shown that the rapid internalization and recycling of integrins at the leading edge of the migrating cell restricts integrins to the front of the cell by out-competing their slow lateral diffusion towards the cell body, suggesting that endocytic turnover of adhesion molecules at the leading edge serves to accumulate them there [Figure 1B (12,15)]. This spatial restriction does not only apply to the turnover of integrin-based adhesive contacts, but also to restrict growth factor receptor signaling to the leading edge of migrating cells. During border-cell migration in Drosophila, Rab5-mediated endocytosis is required to restrict the receptor tyrosine kinases (RTKs), PDGF/VEGF and EGF receptors, to the leading edge of the cells, thus keeping the response to guidance cues spatially localized within the cell (46,47).

Recent studies have begun to shed light on the molecular mechanism underlying integrin endocytosis in cell migration. In migratory epithelial cells, knockdown of clathrin-mediated endocytosis blocks both cell migration and β1-integrin internalization, and this effect is mediated by the interaction between the endocytic adaptor protein Numb and the β1-integrin subunit (28). Numb becomes localized to focal adhesions at the leading edge of migrating fibroblasts and cytochalasin-D abolishes this localization, suggesting that the actin cytoskeleton plays an important role in this process. Interestingly, Numb forms a complex with Par-3 and atypical protein kinase C (aPKC), and the ability of Numb to bind to β1-integrins is regulated by phosphorylation through aPKC. The aPKC/Par-3 complex is localized at the leading edge of migrating cells through the activity of Cdc42 and non-canonical Wnt signaling, previously shown to regulate cell migration (48). Thus, Numb phosphorylation provides a molecular switch that links integrin endocytosis to upstream signals involved in cell migration (28). Because Numb is essential for neuronal migration in Drosophila and mice, Numb-mediated integrin turnover at the leading edge may regulate cell adhesion and cell migration in vivo (49,50).

Integrin αβ2 has also been found to bind to fibroblast growth factor (FGF)-1, and this interaction is required for epithelial cell migration. In Xenopus, fibroblast growth factor receptor (FGFR) signals through the adaptor protein RLIP, which localizes to the plasma membrane and regulates cytoskeletal dynamics by recruiting actin regulators. FGFR also binds to adaptor protein-2, which regulates clathrin-mediated endocytosis, pointing at the interesting possibility that FGFR signaling regulates cell migration by modulating the endocytic turnover of integrin adhesion molecules (51–53).
Figure 2: (A) E-cadherin endocytosis promotes EMT. E-cadherin (red bars) becomes endocytosed and transported via early endosomes into late endosomes, followed by degradation in lysosomes. As a result, the cell loses adhesion to the epithelial layer and becomes mesenchymal. (B) Endocytic turnover of E-cadherin (red bars) promotes coherent cell movements. N, nucleus; EE, early endosome; LE, late endosome; EV, endocytic vesicle; RE, recycling endosome; L, lysosome.

Cadherin Trafficking and Cell Migration

An important regulator of cell migration during development and disease are epithelial-to-mesenchymal transitions (EMT). During EMT, epithelial cells leave their surrounding tissue and become motile, often resulting in invasive growth. EMT thus contributes to a variety of processes, including morphogenesis, wound healing and neural crest development, and also cancer cell metastasis (54). To separate epithelial cells from their surrounding tissue, the cell’s AJ at the apical side have to be dissociated, and one way to achieve this is through the internalization of E-cadherin, followed by degradation in the lysosomal pathway (Figure 2A (55)).

A multitude of different signals, including Wnts, transforming growth factor beta (TGFβ), RTKs, Notch and FGFs, together with integrin signaling and ECM degradation, have been implied as upstream factors in the regulation of EMT in development and disease (56,57). A major contribution to EMT is the repression of E-cadherin transcription via the bHLH protein Snail (58). However, TGFβ- and mitogen-activated protein kinase (MAPK) signaling as well as Snail itself can also trigger the internalization of E-cadherin through clathrin-mediated endocytosis, and EGF signaling induces E-cadherin uptake through caveolae. In the case of TGFβ-, MAPK- and EGF-signaling, E-cadherin, once internalized, becomes degraded in late endosomes/lysosomes (59–61).

In addition, FGFR has been shown to be endocytosed together with E-cadherin in cultured epithelial cells, and the endocytosis of E-cadherin is required for FGFR translocation into the nucleus, where it regulates cell proliferation, growth and differentiation (62,63). Whether FGF can directly trigger E-cadherin endocytosis is yet unclear; however, RTKs can phosphorylate the E-cadherin complex, and the phosphorylated complex can be endocytosed by binding of the E3 ubiquitin ligase Hakai, thereby facilitating EMT and cell motility (64,65). Notably, FGF signaling has also been implied in the regulation of mesodermal cell migration during gastrulation in Sea Urchin, Drosophila, Xenopus, Medaka and zebrafish independent from cell fate specification, pointing at the interesting possibility that FGF-mediated endocytosis of E-cadherin is involved in this function (66–69).

In Drosophila, the gene abnormal wing disc (awd) regulates tracheal development and border-cell migration by modulating endocytosis of growth factor and cytokine receptors and thereby regulating signaling pathway activity (70,71). In cultured epithelial cells, the awd homolog Nm23-H1 becomes recruited to the AJ complex and interacts with the ADP-ribosylation factor 6. This interaction activates dynamin and suppresses the Rac-GTP exchange factor Tiam 1. As a result, Rac1-dependent actin polymerization is suppressed at the junctions, destabilizing them, whereas clathrin- and dynamin-mediated endocytosis is enhanced, facilitating the uptake of AJ components (72,73).

Several in vivo studies of Drosophila, Xenopus and zebrafish morphogenesis support the notion that endocytosis modulates cadherin-mediated cell adhesion. During gastrulation, tissues elongate not only through a specific set of movements and tissue intercalations termed ‘convergent extension’, but also through directed movements of coherent tissue (74,75). Nodal/activin signals (TGFβ homologs) have been proposed to induce convergent extension of Xenopus animal caps by modulating dynamin-mediated C-cadherin endocytosis. Nodal signals are thought to function in this process by upregulating the expression of the transmembrane protein FLRT3 and the small GTPase Rnd1, which control the internalization of C-cadherin (76–78). Similarly, Wnt11 has been suggested to regulate cell cohesion and coherent migration of mesendodermal precursors by modulating Rab5-mediated endocytosis of E-cadherin. Interestingly, Rab11-mediated endocytic trafficking of E-cadherin has also been implicated in wing disc morphogenesis and tracheal and salivary gland morphogenesis in Drosophila. It is conceivable that in all these different processes, endocytosis of cadherins plays an important role in rapid reorganization of adhesive contacts, required for cell cohesion and junctional remodeling [Figure 2B (5,79–81)].
Figure 3: Modulation of chemokine signaling in PGCs and sharpening of a gradient by endocytosis. (A, B) Endocytosis of the chemokine receptor, followed by degradation of its ligand along the endocytic pathway and recycling of the empty receptor to the plasma membrane enables PGCs to readjust their movement direction in response to a change in the chemokine gradient. EV, Endocytic vesicle; EE, early endosome; N, Nucleus. The receptor CXCR4b is depicted in blue, whereas the ligand SDF-1 is displayed as red circles. Green circles in (B) denote ligands from (A) that would not be cleared from the receptor complex without endocytic turnover. (C) SDF-1 ligands (red circles) are synthesized in and secreted by somatic tissue, yielding a gradient that determines the movement direction of PGCs [compare (C) with (A, B)]. SDF-1 ligands in the back of the PGC bind to the CXCR7 receptor (green) and become endocytosed, followed by destruction in lysosomes (L). This way, the SDF-1a gradient becomes sharpened and the cell moves into the direction of the highest SDF-1a concentration. (D) Without CXCR7 endocytosis, SDF-1a prevails, no gradient forms, and the cell loses its movement direction.

Interestingly, recent studies have suggested that endocytosis links cadherin- and integrin-based signaling and adhesion at the molecular level. The small GTPase Rap1 is required for epithelial migration in Drosophila and mesodermal cell movements during Xenopus and zebrafish gastrulation, where it acts downstream of Wnt signaling (30,31). In cultured cells, Rap1 becomes localized towards and is activated at Rab11-positive recycling endosomes that harbor internalized E-cadherin. Rap1, in turn, associates with integrin-dependent focal adhesions and is required for their formation, suggesting functional crosstalk of cadherin endocytosis and integrin assembly (29,82–85).

Endocytosis and Shaping of Chemokine Gradients

In zebrafish, PGCs migrate through the developing embryo to reach their target site, the future gonad. They
are guided by the chemokine SDF-1a, which is expressed and secreted by somatic cells along the PGC movement path. Germ cells express the GTP building protein-coupled receptor CXCR4b, and binding of SDF-1a to CXCR4b activates phospholipase C/IP$_3$ downstream signaling at the leading edge, which elevates intracellular Ca$^{2+}$ levels. Ca$^{2+}$ then activates actin–myosin contractility and protrusion formation at the leading edge of the migrating cell (10).

Germ cells exhibit two distinct phases of movement—a ‘tumbling phase’, during which they reorient their axis of polarity towards the chemokine gradient, and a ‘run phase’, in which they migrate along that gradient. Alternation of ‘tumbling’ and ‘running’ phases allows the cells to readjust to subtle changes in the chemokine concentrations and improve their movement precision. Interestingly, endocytosis of the chemokine receptor CXCR4b regulates the precision of PGC movement. Germ cells that expressed a non-internalizable version of the CXCR4b receptor displayed an extension of running phases relative to the reorientation phases, leading to a less efficient arrival at their eventual target sites. One possible model is that internalization of CXCR4b decreases its downstream signaling level and allows the cells to enter the ‘tumbling phase’, in which they reorient their movement direction. In contrast, if signaling levels are not decreased, the cells follow their initial direction of movement for too long at the expense of reorientation towards local changes in the chemokine concentrations. This suggests that although endocytosis of activated CXCR4b receptors is dispensable for cell motility and directional sensing, it is required for the ‘fine-tuning’ of the movement and the precise arrival of PGCs at their target site [Figure 3A,B (86)].

Interestingly, downregulation of a second chemokine receptor, CXCR7, in zebrafish, leads to similar defects in PGC migration as observed in sdf-1a and cxcr4b mutants. CXCR7 functions as a receptor for SDF-1a, but is expressed in somatic cells along the PGC movement path. Recent observations indicate that CXCR7 becomes endocytosed upon ligand binding and that this mechanism removes SDF-1a from the extracellular space, thus sharpening the chemokine gradient. In the absence of CXCR7 function, SDF-1a, but is expressed in somatic cells along the PGC movement path. Recent observations indicate that CXCR7 becomes endocytosed upon ligand binding and that this mechanism removes SDF-1a from the extracellular space, thus sharpening the chemokine gradient. In the absence of CXCR7 function, SDF-1a is not cleared efficiently. As a result, high chemokine levels remain in the absence of CXCR7 function, SDF-1a is not cleared efficiently. As a result, high chemokine levels remain in the space surrounding the PGCs, leaving them unable to establish polarity and exhibit directed migratory behavior [Figure 3C,D (87,88)].

**Conclusion**

Migrating cells need to rapidly turnover contacts with other cells or the ECM. Initial observations in cultured epithelial cells have suggested a model in which adhesion molecules become internalized at the trailing edge and transported to the leading edge, where they become inserted into the membrane to build new contacts. However, *in vivo* studies from invertebrate and vertebrate development suggest a more complex function of endocytic recycling in cell migration. It can also serve to regulate the spatial distribution of growth factor receptors or integrin adhesion complexes to the leading edge by countering lateral diffusion of these transmembrane receptors. Clathrin- or caveolin-mediated endocytosis plays a role in the dynamic remodeling of adhesive contacts, thus ensuring synchronous and effective cell movements during morphogenesis. Similarly, internalization of growth factor and chemokine receptors can modulate the signaling strength of the corresponding ligand or sharpen its extracellular gradient. As a consequence, cells can respond more rapidly to changes in chemokine or growth factor concentration, enabling them to move more efficiently towards their target.

**References**

Traffic 2009; 10: 811–818


