Mechanisms of force generation and force transmission during interstitial leukocyte migration

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For innate and adaptive immune responses it is essential that inflammatory cells use quick and flexible locomotion strategies. Accordingly, most leukocytes can efficiently infiltrate and traverse almost every physiological or artificial environment. Here, we review how leukocytes might achieve this task mechanistically, and summarize recent findings on the principles of cytoskeletal force generation and transduction at the leading edge of leukocytes. We propose a model in which the cells switch between adhesion-receptor-mediated force transmission and locomotion modes that are based on cellular deformations, but independent of adhesion receptors. This plasticity in migration strategies allows leukocytes to adapt to the geometry and molecular composition of their environment.

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In line with the morphological definition of ‘amoeboid migration’, leukocytes constantly change shape while migrating. These shape changes predominantly occur independently of the molecular composition of the tissue (Lammermann & Sixt, 2009). By contrast, mesenchymal cells—such as fibroblasts or smooth muscle cells—are highly adhesive; their cytoskeleton couples to the extracellular matrix (ECM) or to neighbouring cells through transmembrane receptors. This adhesiveness largely determines cell shape, such that mesenchymal cells align along ECM fibres and the structure of the actin cytoskeleton usually looks like an intracellular extension of the extracellular substrate, with thick actin cables crossing the cytoplasm (Cukierman *et al*, 2001; Wolf *et al*, 2007).

The adhesive potential of a cell is defined by its repertoire of integrin family transmembrane adhesion receptors. Even when migrating, mesenchymal cells remain strictly bound to their ECM—a principle termed *haptokinesis*, meaning migration holding on to a substrate (Fig 1A). Locating and binding to a substrate is essential for the survival of adherent cells. Removal of the signal that allows this behaviour—transmitted by ligand-engaged integrins—leads to a form of programmed cell death termed *anoikis*, the Greek word for homelessness (Chiarugi & Giannoni, 2008). Haptokinetic movement is strictly controlled and completely deterministic; cells

exclusively locate to the tissue compartment that they can bind to with their adhesion receptors.

Haematopoietic cells are different from the relatively sessile mesenchymal cells, as they constantly shuttle between different tissue compartments. They are born in the bone marrow and, once differentiated, exit into the blood-stream. They then eventually enter tissues, before returning to the blood circulation through lymphatic vessels (Luster *et al*, 2005; Massberg *et al*, 2007). How is the locomotion strategy of leukocytes suited to this life cycle?

One obvious factor is that leukocytes migrate extremely quickly, up to 100 times faster than mesenchymal cells in migration. Neutrophil granulocytes and lymphocytes, for example, reach velocities of up to 20 µm/min, whereas fibroblasts usually migrate at around 0.5 µm/min (Friedl *et al*, 2001; Friedl & Weigelin, 2008). A second factor is that leukocytes are largely autonomous from their environment. Similarly to some metastatic tumour cells that have gained autonomy by uncoupling survival signalling from adhesion receptors, leukocytes are not anchorage-dependent and can survive without being adherent. Hence, leukocytes subvert a central metazoan paradigm and instead behave like single-celled organisms. Leukocytes not only survive in the absence of adhesion receptors, but also migrate. Although they require integrins when extravasating from the blood-stream and seem to frequently migrate along guidance structures within tissues (Bajenoff *et al*, 2006; Boissonnas *et al*, 2007; Wilson *et al*, 2009), their locomotion in the interstitial space does not depend on adhesion receptors (see below). This autonomous behaviour is well suited to situations in which cells migrate along soluble guidance cues that distribute irrespective of the molecular composition of the tissue (Fig 1B).

In this review, we discuss which morphodynamic features determine the difference between adhesion-dependent mesenchymal cells and adhesion-independent leukocytes. We also consider how leukocytes generate traction forces in the absence of transmembrane force coupling.

Mechanics of the leading edge I

Locomotion driven by retrograde actin flow. The mesenchymal mode of movement is driven by the force that is generated and transmitted at the leading edge of the cell. Here, the contractile and protrusive forces of the actin cytoskeleton are transformed into traction forces. The prototypic cellular protrusion is the lamellipodium, and numerous studies and reviews have addressed its mechanical details (Vicente-Manzanares *et al*, 2009). To illustrate the differences

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between this and the leukocyte leading edge, we do not cover the controversial topic of lamellipodial force generation (Danuser, 2009; Vallotton & Small, 2009). Instead, we focus on a simplified mechanical principle: the leading edge pulls the cell forward by coupling the retrograde force of the actin cortex—through transmembrane receptors—to the extracellular environment (Fig 2A). The retrograde force of the actin cytoskeleton is driven by two engines. First, actin grows at the leading plasma membrane and filaments get pushed backward into the cell body where the actin network is disassembled in a process termed ‘treadmilling’ (Lai *et al*, 2008; Mogilner & Oster, 2003). Second, myosin II—located behind the leading edge—pulls the cortex backward, supporting the polymerization-driven retrograde movement (Fournier *et al*, 2010; Henson *et al*, 1999; Lin *et al*, 1997; Medeiros *et al*, 2006; Renkawitz *et al*, 2009). Although this paradigm is most established for the flat, sheet-like lamellipodium, it is likely that the same principle applies to other actin-based protrusions—such as filopodia—that also contain a treadmilling array of actin filaments and can help in pulling the leading edge forward (Galbraith *et al*, 2007; Nemethova *et al*, 2008). Retrograde flow-driven movement is totally dependent on transmembrane force-coupling receptors, which generate a frictional interface between rearward flowing actin and the extracellular environment. Without this friction, the cell will ‘run on the spot’, causing the treadmilling actin cortex to slide backward in relation to the substrate.

The coupling between actin and adhesion receptor has been described as a ‘clutch’ (Hu *et al*, 2007; Mitchison & Kirschner, 1988). It has been demonstrated in several cell types that this clutch is rarely a 1:1 transmission, meaning that polymerization is not completely turned into migration. Usually, there is slippage between actin and the substrate, which can occur at different molecular levels (Hu *et al*, 2007). Therefore, cells with the same actin polymerization speed can either slip (low transmission) and migrate slowly or grip (high transmission) and migrate quickly (Cramer, 1997; Jurado *et al*, 2005; Lin & Forscher, 1995). This simplified picture of retrograde flow-driven force transmission illustrates one important point: this mode can only cause migration when the actin cortex mechanically communicates with the substrate.

Adhesion versus traction

Cells that form lamellipodia are usually adhesive, and the lamellipodium is where adhesions are initiated and assembled. This does not, however, mean that it is a mechanical necessity for cells driven by retrograde actin flow to adhere to surfaces. Adhesion means that the cell is anchored to the substrate. Adhesion receptors resist forces that pull away from the membrane, and thereby ‘stick’ the cell to a surface. However, this ‘stickiness’ is not necessary for locomotion, as forward locomotion is a consequence of traction forces that act in parallel to the membrane. In principle, force-coupling receptors could generate traction without anchoring the cell, as long as the cell was in contact with the substrate. In large systems, this contact is usually achieved by gravity but, at the cellular level, gravity is insufficient for stable contact between the cell and a two-dimensional (2D) surface—as Brownian motion dominates and prevents any prolonged interaction with the substrate. This has led to the assumption that surface anchorage is necessary for the transmission of the traction forces required for migration. However, every confined environment in which the cell has to squeeze between rigid objects—for example within three-dimensional (3D) scaffolds—forces contact with the substrate. Three-dimensional environments therefore allow

Fig 1 | Migratory patterns of mesenchymal cells compared with leukocytes. Both cell types migrate through a three-dimensional meshwork of interstitial matrix containing different molecular components. Cells follow a gradient of a soluble guidance cue. (A) Mesenchymal cells are strictly confined to the matrix track to which they can bind due to their adhesion receptor equipment, whereas (B) leukocytes prioritize the guidance cue and move directly along the gradient.

the transmission of traction forces, although the cell does not ‘stick’ to the substrate. Adhesion is, therefore, unnecessary for the generation of traction forces, and this is relevant in the discussion of leukocyte force transduction.

The traction forces required to move a non-adherent cell are very low; many orders of magnitude lower than the traction forces exerted by mesenchymal cells (Oliver *et al*, 1994). A non-adherent cell only has to move its mass and overcome the viscous drag of the surrounding fluid; high traction forces are only required when tightly coupled receptors at the back of the cell need to be detached (Chen, 1981; Munevar *et al*, 2001; Gupton & Waterman-Storer, 2006; Lammernann & Sixt, 2009). Mesenchymal cells seem to ‘waste’ enormous amounts of energy by adhering to and contracting substrates, whereas non-adherent cells can efficiently move with minimal traction forces.

Mechanics of the leading edge II

Locomotion by blebbing. The model of retrograde flow-based force transmission described above is not the only principle that can drive cell motility. Alternative models have been proposed that are also dependent on the actin cytoskeleton, but independent of leading edge extension driven by actin polymerization. These are variants of ‘blebbing motility’, in which the cell generates hydrostatic pressure by actomyosin contraction. This pressure is dependent on myosin II motor activity that contracts the cortical actin network. Increased pressure and the resulting increased cortical tension are eventually released, either by rupture of the actin cortex (Paluch *et al*, 2005, 2006) or by detachment of the cellular membrane from the underlying cortex (Charras *et al*, 2005, 2006). When cytoplasm is squeezed beyond the cortical defect and the bleb becomes inflated, the resulting protrusions grow faster than those of polymerization-driven extensions (Charras *et al*, 2006; Paluch *et al*, 2005). Growing blebs are free of cortical actin but, once they are older, the inner surface becomes coated with newly polymerized actin that contracts due to myosin II recruitment, leading to shrinkage of the bleb (Charras *et al*, 2006). A bleb can generate a transient membrane protrusion, and early studies by Trinkhaus clearly documented membrane blebbing

Fig 2 | Modes of force generation and force transmission in two and three dimensions. (A) The lamellipodial principle: cells migrate on a two-dimensional (2D) substrate and use the combined force of actin polymerization and myosin II-mediated contractility to pull substrate-ligated integrin receptors backward and the cell body forward. (B,C) Bleb driven migration is initiated by polarized rupture of the actin cortex or detachment of the plasma membrane and subsequent inflation of a membrane bleb. The bleb is then contracted, before the next bleb forms 'on top of' the former bleb. Traction force to move the cell body is either generated by transmembrane receptors that couple the retracting actin cortex to a 2D substrate (adhesion-based, shown in B) or by mechanical immobilization within a confined three-dimensional (3D) space (deformation-based, shown in C). (D) Polymerizing actin causes the leading edge to protrude and thereby deforms the cell body, which subsequently becomes mechanically immobilized at the cell front. As in (C), local friction is generated by 'pushing against the walls' of a 3D confined environment. (E) The cell migrates within a 3D confined environment that is partly adhesive and partly non-adhesive. It simultaneously uses the lamellipodial principle (A) and deformation-based movement. The actin cortex that is in contact with the non-adhesive surface undergoes retrograde slippage.

at the leading edge of migrating *Fundulus* deep cells (Fink & Trinkhaus, 1988).

However, an extending protrusion pushes the cell backwards once it binds to or collides with an obstacle, so how can a bleb possibly translocate the cell body? There are two suggestions of how a bleb can generate traction forces that result in locomotion. First, the retracting actin cortex of the 'ageing' bleb is coupled with the substrate by transmembrane adhesion receptors. As the bleb retracts, the cell is thereby pulled forward. If the next blebbing cycle occurs in the same direction as the previous, the cell can move forward (Fig 2B). This has been demonstrated for zebrafish primordial germ cells (Kardash *et al*, 2010). Second, the bleb protrudes into the open space—which could be a pore within the interstitial matrix—and inflates, which entangles it within the meshwork of the ECM or jams it into a confined space. This polar expansion creates local friction, leading to an asymmetry in friction that pulls the trailing edge forward as it is contracting and shrinking. A cell might bleb forward by pushing itself from matrix compartment to matrix compartment or, when confined between two surfaces, it might periodically 'chimney' through the confined space (Fig 2C). This concept is mechanically appealing (Charras & Paluch, 2008), but so far there is no experimental evidence that it is used by cells for locomotion. The first of the proposed mechanisms depends on transmembrane coupling of retrograde actin forces by adhesion receptors, and is therefore conceptually similar to migration driven by retrograde flow. It can also account for movement along 2D surfaces, as well as 3D scaffolds. The second mechanism functions independently of adhesion receptors and is therefore dependent on the sustained surface contact, guaranteed in 3D environments. As this relies on shape changes of the cell we call it 'deformation-based migration'.

Mechanisms used by leukocytes

Do leukocytes use principles based on retrograde flow? In certain situations, they do. Lymphoblasts (activated lymphocytes) or neutrophil granulocytes that are activated with chemoattractants adhere to 2D surfaces. They form a sheet-like protrusion that, although it might not have all the features of a lamellipodium, is flat, spread out and rich in actin. As in mesenchymal and epithelial cells, this mode of movement is strictly dependent on force coupling by integrins, and interference with integrins abrogates 2D migration (Jacobelli *et al*, 2009; Lammermann *et al*, 2008; Malawista & de Boisleury Chevance, 1997; Malawista *et al*, 2000; Smith *et al*, 2005).

The situation changes when leukocytes are embedded in 3D environments. Here, in contrast to mesenchymal cells, leukocytes do not adapt their shape to the ECM fibres in order to move. They change shape 'from inside', exemplified by the fact that they can deform the cell body even in suspension (Haston *et al*, 1982), whereas adherent cells most often round up once detached from their substratum. Accordingly, leukocytes in 3D environments do not form leading edge protrusions that align with the ECM fibres, but rather form rounded or cylindrical extensions (lobopodia) that protrude between the fibres (Friedl *et al*, 1995, 2001). Such observations suggest that leukocytes do not need to adhere to their substrates in order to migrate and leave the possibility that leukocytes can use their adhesion receptors to exclusively transmit traction forces without anchoring the cells. However, *in vitro* and *in vivo* studies have shown that integrins—the largest and most important family of adhesion receptors—are unnecessary for 3D leukocyte motility (see below). This raises the question of how the frictional

interface between the cytoskeleton and the actin cortex is established. One possibility is that alternative force-coupling receptors are used: for example, transmembrane glycoproteins such as the sydecans and CD44 have been implicated in force transduction, as well as non-integrin collagen receptors such as the discoidin domain receptors (DDR) family of receptor tyrosine kinases (Schmidt & Friedl, 2010). Although alternative force transmission systems might contribute to motility, *in vitro* studies with inert 3D substrates indicate that without any transmembrane force coupling, leukocytes can still move rather efficiently (Renkawitz *et al*, 2009). How can this work?

Blebbing motility is one alternative mechanism that does not rely on transmembrane receptors. Blebbing has been demonstrated in several migrating tumour cells (Keller *et al*, 2002; Sahai & Marshall, 2003; Sanz-Moreno *et al*, 2008; Wicki & Niggli, 2001), but whether blebs are epiphenomena or critical for traction-generation remains to be demonstrated. Functional evidence that blebs are the driving motile force comes exclusively from zebrafish primordial germ cells (Blaser *et al*, 2006). By contrast, many dynamic and static imaging studies have shown that the protrusions of leukocytes are actin-rich throughout the cell front, with no evidence for membrane blebs (Haston, 1987; Lammermann & Sixt, 2009). Furthermore, it has been shown that dendritic cells, B cells, granulocytes and *Drosophila* haemocytes can migrate in the absence of myosin II motor activity, which largely excludes bleb formation (Bardi *et al*, 2003; Lammermann *et al*, 2008; Redd *et al*, 2006; Xu *et al*, 2005). Myosin II is required to squeeze the rigid nucleus through narrow pores, but in environments where the pore size of the interstitium is large enough, leukocytes can reach normal peak velocities without myosin II (Bardi *et al*, 2003; Jacobelli *et al*, 2009; Lammermann *et al*, 2008). This contraction-independent mode of migration was also found to be independent of adhesion receptors (Lammermann *et al*, 2008), suggesting that in confined environments leukocytes can adopt a migration mode that is non-blebbing and independent of transmembrane force coupling. Although these results do not exclude the possibility that leukocytes can use blebbing migration, they indicate that blebbing is not the general principle explaining independence from adhesion.

Mechanics of the leading edge III

Locomotion by polymerization-driven deformation. If neither retrograde flow nor bleb driven migration applies to leukocytes, how do they transduce their force instead? A mode of non-blebbing migration that does not depend on transmembrane force coupling was proposed more than 20 years ago (Haston *et al*, 1982) and is often mentioned synonymously with amoeboid migration. Put simply, it is reasonable to think that leukocytes move entirely by deformation of the cell body and that these deformations are generated by actin polymerization. In contrast to migration driven by retrograde flow, it is not the retrograde force of the actin that moves the cell, but rather the lobopodia that intercalate in the 3D environment and push or pull the cell forward. In contrast to non-adhesive blebbing, the leading protrusions are not inflated by hydrostatic pressure but by actin polymerization, which pushes out the membrane and deforms the cell. This mode of movement is similar to both blebbing and retrograde-flow-driven migration. The leading protrusions increase by the force of polymerization and, like an inflating bleb, they get entangled within a matrix compartment. Similarly to non-adhesive blebbing, the swelling protrusion might jam the cell front

between two surfaces; both jamming or entangling generates more friction at the front than at the back and this asymmetry causes translocation when the rear end of the cell contracts (Fig 2D).

This mode of movement seems plausible, and theoretical modelling has shown that a deforming actin network can move forward within a microchannel by pushing against the wall of the channel and extending at the front (Hawkins *et al*, 2009). However, as with non-adhesive blebbing locomotion, it remains to be demonstrated with biophysical methods that leukocytes indeed migrate in such a deformation-based, polymerization-driven manner.

Plasticity of migratory modes

One question that arises from studies demonstrating adhesion-independent leukocyte movement is whether leukocytes rely entirely on principles independent of transmembrane force coupling (Sidebar A). If so, adhesion receptors do not have any role in leukocyte force transduction. One argument against this view is that in some model systems of interstitial migration, integrins seem to have a role in locomotion, albeit a minor one. Neutrophil granulocytes migrating through the interstitium of mouse peritoneum or rat cremaster muscle have been shown to decelerate by 30% in the presence of antibodies against $\beta 1$ integrin (Lindbom & Werr, 2002; Werr *et al*, 1998; 2000). T lymphocytes migrating within lymph nodes slowed down by 10% when $\beta 1$ and $\beta 2$ integrins—which are likely to be the only integrin classes expressed by the cells—were deleted or blocked (Woolf *et al*, 2007). Similarly, *in vitro* studies measuring lymphoblast chemotaxis in 3D collagen gels showed slight reductions in migration velocities on the blockade of integrins (Franitza *et al*, 1999; Friedl *et al*, 1995). By contrast, randomly migrating T-cell blasts in collagen gels and neutrophil granulocytes moving between two adjacent glass surfaces were unaffected by integrin blockade (Friedl *et al*, 1998). The same was demonstrated for granulocytes, B-cell blasts and mature dendritic cells migrating along chemotactic gradients through collagen gels or under agarose, as well as for dendritic cells migrating *in vivo* from the skin to the T-cell area of the draining lymph node (Lammermann *et al*, 2008; Renkawitz *et al*, 2009). These data demonstrate that integrins are dispensable for migration, but also suggest that they might make some contribution.

Further evidence comes from studies of dendritic cells, T-cell blasts and granulocytes migrating on 2D surfaces; traction generation during this mode of movement was entirely dependent on integrins (Lammermann *et al*, 2008; Malawista & de Boisleury Chevance, 1997; Malawista *et al*, 2000; Smith *et al*, 2005). This suggests that leukocytes can use integrins to generate traction and that either the integrin-mediated mode of movement is switched off once cells are embedded in 3D environments, or integrins are used in 3D but become dispensable when cells can revert to an alternative mode of movement. A recent study has suggested that the latter might be the case; by using dendritic cells as a model system it was shown that integrin-mediated force transduction is used in confined environments, but that once integrins are deleted, uncoupled from the cytoskeleton or—physiologically more realistic—when no integrin ligands are available, cells can generate traction without them (Renkawitz *et al*, 2009). The switch from integrin-mediated to integrin-independent movement was not accompanied by changes in migration velocity or directional persistence. A difference could only be detected at the level of the actin cytoskeleton. On adhesive substrates the cells migrated with maximal efficiency, indicating

Sidebar A | In need of answers

- (i) How does deformation of the cell body translate into locomotion? Which forces act on the environment?
- (ii) Which mode of movement dominates in a physiological interstitium?
- (iii) How is actin polymerization at the leading edge of leukocytes regulated?
- (iv) Are there leukocyte-type specific differences in locomotion strategies, and do some metastatic tumour cells use similar principles?

that the growth of the actin network that occurred below the leading membrane was entirely transformed into forward movement, resembling the lamellipodial principle with almost 1:1 force transmission. On non-adhesive substrates or on uncoupling or deletion of integrins, the actin network slipped backward in relation to the substrate. Such slippage is in perfect accordance with the clutch hypothesis; once the clutch is removed, actin filaments growing below the leading membrane do not experience any retrograde resistance and slide towards the centre of the cell. If locomotion driven by retrograde flow was the only mechanism occurring here, then the cell would treadmill but not move, as explained above. Interestingly, however, the ‘slipping cells’ protruded and migrated with the same speed and shape as the ‘gripping cells’. This was possible because slippage was compensated for by higher actin polymerization rates. These data indicate that higher polymerization rates accompanied the switch between the retrograde-flow-driven and the deformation-driven principle, and suggest that dendritic cells can switch between the two modes of movement, without changing shape.

The switch between adhesion-dependent and deformation-based modes of movement was also shown to occur instantaneously: the cells maintained the round, protrusive shape of their leading edge when crossing from adhesive to non-adhesive areas. This implies that the two motility modes represent an inherent plasticity of the cytoskeletal mechanics, rather than a remodelling of the cellular proteome. More strikingly, the cells were able to spatially adapt to the substrate. Similarly to the gears of a car, they ‘slipped’ on one side and ‘gripped’ on the other, locally increasing the polymerization rate on the slippery areas to maintain directionality (Fig 2E). These findings illustrate the enormous plasticity in the migratory strategies of leukocytes, that allow them to adapt to their local environment without losing time accommodating to the tissue.

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REFERENCES

- Bajenoff MJ, Egen GL, Koo YJ, Laugier P, Brau F, Glaichenhaus N, Germain RN (2006) Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* **25**: 989–1001
- Bardi G, Niggli V, Loetscher P (2003) Rho kinase is required for CCR7-mediated polarization and chemotaxis of T lymphocytes. *FEBS Lett* **542**: 79–83
- Blaser H, Reichman-Fried M, Castanon I, Dumstrei K, Marlow FL, Kawakami K, Solnica-Krezel L, Heisenberg CP, Raz E (2006) Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. *Dev Cell* **11**: 613–627

- Boissonnas A, Fetler LI, Zeelenberg S, Hugues S, Amigorena S (2007) *In vivo* imaging of cytotoxic T cell infiltration and elimination of a solid tumor. *J Exp Med* **204**: 345–356
- Charras G, Paluch E (2008) Blebs lead the way: how to migrate without lamellipodia. *Nat Rev Mol Cell Biol* **9**: 730–736
- Charras GT, Yarrow JC, Horton MA, Mahadevan L, Mitchison TJ (2005) Non-equilibration of hydrostatic pressure in blebbing cells. *Nature* **435**: 365–369
- Charras GT, Hu CK, Coughlin M, Mitchison TJ (2006) Reassembly of contractile actin cortex in cell blebs. *J Cell Biol* **175**: 477–490
- Chen WT (1981) Mechanism of retraction of the trailing edge during fibroblast movement. *J Cell Biol* **90**: 187–200
- Chiarugi P, Giannoni E (2008) Anoikis: a necessary death program for anchorage-dependent cells. *Biochem Pharmacol* **76**: 1352–1364
- Cramer LP (1997) Molecular mechanism of actin-dependent retrograde flow in lamellipodia of motile cells. *Front Biosci* **2**: d260–d270
- Cukierman E, Pankov R, Stevens DR, Yamada KM (2001) Taking cell-matrix adhesions to the third dimension. *Science* **294**: 1708–1712
- Danuser G (2009) Testing the lamella hypothesis: the next steps on the agenda. *J Cell Sci* **122**: 1959–1962
- Fink RD, Trinkaus JP (1988) Fundulus deep cells: directional migration in response to epithelial wounding. *Dev Biol* **129**: 179–190
- Fournier MF, Sauser R, Ambrosi D, Meister JJ, Verkhovsky AB (2010) Force transmission in migrating cells. *J Cell Biol* **188**: 287–297
- Franitza S, Alon R, Lider O (1999) Real-time analysis of integrin-mediated chemotactic migration of T lymphocytes within 3-D extracellular matrix-like gels. *J Immunol Methods* **225**: 9–25
- Friedl P, Weigelin B (2008) Interstitial leukocyte migration and immune function. *Nat Immunol* **9**: 960–969
- Friedl P, Noble PB, Zanker KS (1995) T lymphocyte locomotion in a three-dimensional collagen matrix. Expression and function of cell adhesion molecules. *Immunol J* **154**: 4973–4985
- Friedl P, Entschladen F, Conrad C, Niggemann B, Zanker KS (1998) CD4+ T lymphocytes migrating in three-dimensional collagen lattices lack focal adhesions and utilize beta1 integrin-independent strategies for polarization, interaction with collagen fibers and locomotion. *Eur Immunol* **28**: 2331–2343
- Friedl P, Borgmann S, Bocker EB (2001) Amoeboid leukocyte crawling through extracellular matrix: lessons from the *Dictyostelium* paradigm of cell movement. *J Leukoc Biol* **70**: 491–509
- Galbraith CG, Yamada KM, Galbraith JA (2007) Polymerizing actin fibers position integrins primed to probe for adhesion sites. *Science* **315**: 992–995
- Gupton SL, Waterman-Storer CM (2006) Spatiotemporal feedback between actomyosin and focal-adhesion systems optimizes rapid cell migration. *Cell* **125**: 1361–1374
- Haston WS (1987) F-actin distribution in polymorphonuclear leucocytes. *J Cell Sci* **88**: 495–501
- Haston WS, Shields JM, Wilkinson PC (1982) Lymphocyte locomotion and attachment on two-dimensional surfaces and in three-dimensional matrices. *J Cell Biol* **92**: 747–752
- Hawkins RJ, Piel M, Faure-Andre G, Lennon-Dumenil AM, Joanny JF, Prost J, Voituriez R (2009) Pushing off the walls: a mechanism of cell motility in confinement. *Phys Rev Lett* **102**: 058103
- Henson JH, Svitkina TM, Burns AR, Hughes HE, MacPartland KJ, Nazarian R, Borisy GG (1999) Two components of actin-based retrograde flow in sea urchin coelomocytes. *Mol Biol Cell* **10**: 4075–4090
- Hu K, Ji L, Applegate KT, Danuser G, Waterman-Storer CM (2007) Differential transmission of actin motion within focal adhesions. *Science* **315**: 111–115
- Jacobelli J, Bennett FC, Pandurangi P, Tooley AJ, Krummel MF (2009) Myosin-IIA and ICAM-1 regulate the interchange between two distinct modes of T cell migration. *Immunol J* **182**: 2041–2050
- Jurado C, Hsnerick JR, Lee J (2005) Slipping or gripping? Fluorescent speckle microscopy in fish keratocytes reveals two different mechanisms for generating a retrograde flow of actin. *Mol Biol Cell* **16**: 507–518
- Kardash E, Reichman-Fried M, Maitre JL, Boldajipour B, Papisheva E, Messerschmidt EM, Heisenberg CP, Raz EA (2010) Role for Rho GTPases and cell-cell adhesion in single-cell motility *in vivo*. *Nat Cell Biol* **12**: 47–53
- Keller H, Rentsch P, Hagmann J (2002) Differences in cortical actin structure and dynamics document that different types of blebs are formed by distinct mechanisms. *Exp Cell Res* **277**: 161–172
- Lai FP, Szczodrak M, Block J, Faix J, Breitsprecher D, Mannherz HG, Stradal TE, Dunn GA, Small JV, Rottner K (2008) Arp2/3 complex interactions and actin network turnover in lamellipodia. *EMBO J* **27**: 982–992
- Lammermann T, Sixt M (2009) Mechanical modes of ‘amoeboid’ cell migration. *Curr Opin Cell Biol* **21**: 636–644
- Lammermann T *et al* (2008) Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature* **453**: 51–55
- Lin CH, Forscher P (1995) Growth cone advance is inversely proportional to retrograde F-actin flow. *Neuron* **14**: 763–771
- Lin CH, Espreafico EM, Mooseker MS, Forscher P (1997) Myosin drives retrograde F-actin flow in neuronal growth cones. *Biol Bull* **192**: 183–185
- Lindbom L, Werr J (2002) Integrin-dependent neutrophil migration in extravascular tissue. *Semin Immunol* **14**: 115–121
- Luster AD, Alon R, von Andrian UH (2005) Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol* **6**: 1182–1190
- Malawista SE, de Boisfleury Chevance A (1997) Random locomotion and chemotaxis of human blood polymorphonuclear leukocytes (PMN) in the presence of EDTA: PMN in close quarters require neither leukocyte integrins nor external divalent cations. *Proc Natl Acad Sci USA* **94**: 11577–11582
- Malawista SE, de Boisfleury Chevance A, Boxer LA (2000) Random locomotion and chemotaxis of human blood polymorphonuclear leukocytes from a patient with leukocyte adhesion deficiency-1: normal displacement in close quarters via chimneying. *Cell Motil Cytoskeleton* **46**: 183–189
- Massberg S *et al* (2007) Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell* **131**: 994–1008
- Medeiros NA, Burnette DT, Forscher P (2006) Myosin II functions in actin-bundle turnover in neuronal growth cones. *Nat Cell Biol* **8**: 215–226
- Mitchison T, Kirschner M (1988) Cytoskeletal dynamics and nerve growth. *Neuron* **1**: 761–772
- Mogilner A, Oster G (2003) Polymer motors: pushing out the front and pulling up the back. *Curr Biol* **13**: R721–R733
- Munevar SY, Wang L, Dembo M (2001) Distinct roles of frontal and rear cell-substrate adhesions in fibroblast migration. *Mol Biol Cell* **12**: 3947–3954
- Nemethova M, Auinger S, Small JV (2008) Building the actin cytoskeleton: filopodia contribute to the construction of contractile bundles in the lamella. *J Cell Biol* **180**: 1233–1244
- Oliver T, Lee J, Jacobson K (1994) Forces exerted by locomoting cells. *Semin Cell Biol* **5**: 139–147
- Paluch E, Piel M, Prost J, Bornens M, Sykes C (2005) Cortical actomyosin breakage triggers shape oscillations in cells and cell fragments. *Biophys J* **89**: 724–733
- Paluch E, van der Gucht J, Sykes C (2006) Cracking up: symmetry breaking in cellular systems. *J Cell Biol* **175**: 687–692
- Redd MJ, Kelly G, Dunn G, Way M, Martin P (2006) Imaging macrophage chemotaxis *in vivo*: studies of microtubule function in zebrafish wound inflammation. *Cell Motil Cytoskeleton* **63**: 415–422
- Renkawitz J, Schumann K, Weber M, Lammermann T, Pflücke H, Piel M, Polleux J, Spatz JP, Sixt M (2009) Adaptive force transmission in amoeboid cell migration. *Nat Cell Biol* **11**: 1438–1443
- Sahai E, Marshall CJ (2003) Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat Cell Biol* **5**: 711–719
- Sanz-Moreno V, Gadea G, Ahn J, Paterson H, Marra P, Pinner S, Sahai E, Marshall CJ (2008) Rac activation and inactivation control plasticity of tumor cell movement. *Cell* **135**: 510–523
- Schmidt S, Friedl R (2010) Interstitial cell migration: integrin-dependent and alternative adhesion mechanisms. *Cell Tissue Res* **339**: 83–92
- Smith A, Carrasco YR, Stanley P, Kieffer N, Batista FD, Hogg N (2005) A talin-dependent LFA-1 focal zone is formed by rapidly migrating T lymphocytes. *J Cell Biol* **170**: 141–151
- Vallotton P, Small JV (2009) Shifting views on the leading role of the lamellipodium in cell migration: speckle tracking revisited. *J Cell Sci* **122**: 1955–1958
- Vicente-Manzanares M, Choi CK, Horwitz AR (2009) Integrins in cell migration—the actin connection. *J Cell Sci* **122**: 199–206
- Werr J, Xie X, Hedqvist P, Ruoslahti E, Lindbom L (1998) Beta1 integrins are critically involved in neutrophil locomotion in extravascular tissue *In vivo*. *J Exp Med* **187**: 2091–2096

- Werr J, Johansson J, Eriksson EE, Hedqvist P, Ruoslahti E, Lindbom L (2000) Integrin alpha(2)beta(1) (VLA-2) is a principal receptor used by neutrophils for locomotion in extravascular tissue. *Blood* **95**: 1804–1809
- Wicki A, Niggli V (2001) The Rho/Rho-kinase and the phosphatidylinositol 3-kinase pathways are essential for spontaneous locomotion of Walker 256 carcinosarcoma cells. *Int Cancer J* **91**: 763–771
- Wilson EH *et al* (2009) Behavior of parasite-specific effector CD8+ T cells in the brain and visualization of a kinesis-associated system of reticular fibers. *Immunity* **30**: 300–311
- Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, Stack MS, Friedl P (2007) Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. *Nat Cell Biol* **9**: 893–904
- Woolf E, Grigorova I, Sagiv A, Grabovsky V, Feigelson SW, Shulman Z, Hartmann T, Sixt M, Cyster JC, Alon R (2007) Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. *Nat Immunol* **8**: 1076–1085
- Xu J, Wang F, Van Keymeulen A, Rentel M, Bourne HR (2005) Neutrophil microtubules suppress polarity and enhance directional migration. *Proc Natl Acad Sci USA* **102**: 6884–6889