Focal adhesion-independent cell migration

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Abstract

Cell migration is central to a multitude of physiological processes including embryonic development, immune surveillance and wound healing, and deregulated migration is key to cancer dissemination. Decades of investigations have uncovered many of the molecular and physical mechanisms underlying cell migration. Together with protrusion extension and cell body retraction, adhesion to the substrate via specific focal adhesions points has long been considered an essential step in cell migration. While this is true for cells moving on two-dimensional substrates, recent studies have demonstrated that focal adhesions are not required for cells moving in three dimensions, where confinement is sufficient to maintain a cell in contact with its substrate. Here, we review the investigations that have led to challenging the requirement of specific adhesions for migration, discuss the physical mechanisms proposed for cell body translocation during focal-adhesion-independent migration, and highlight the remaining open questions for the future.

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Introduction

The ability to crawl is one of the most striking cellular behaviors. Crawling migration is displayed by the vast majority of animal cells, both in culture conditions and *in vivo*, and by a number of unicellular organisms such as amoeba. A key question, which has been underlying decades of studies in search of the mechanisms driving migration, is how cells generate propelling forces and how these forces are transmitted between the cell and the substrate to effect cell body translocation.

Classical models describe cell migration as a canonical three-step process: extension of the cell leading edge in a protrusion called lamellipodium, attachment of the leading edge to the substrate and contraction of the rear with resolution of the adhesion sites. This three-step cycle was first described by Michael Abercrombie as “an attempt to see what a general physiology of cell crawling might look like” (Abercrombie 1980), and summarized a decade of observations of crawling motility in different experimental systems. Following this pioneering description, thirty years of studies dissected the molecular and physical mechanisms of each of the steps of the migration cycle (Danuser et al 2013, Gardel et al 2010, Webb et al 2002).

The conceptual framework used by Abercrombie was based on morphological observations of fibroblasts migrating on glass coverslips (Abercrombie 1980, Abercrombie et al 1970b), and in the following 20 years, migration studies overwhelmingly focused on cells crawling on 2-dimensional (2D) substrates. Yet, most cells *in vivo* migrate in three-dimensional (3D) confinement. As investigations of cell migration in 3D environments expanded over the last two decades, it became increasingly clear that while some aspects of the Abercrombie three-step cycle are conserved in 3D, the mechanisms underlying migration can also significantly differ from their 2D counterparts (Even-Ram & Yamada 2005, Friedl & Brocker 2000). One fundamental difference is the requirement for substrate attachment. Indeed, while on a 2D substrate, in the absence of attachment, Brownian motion would preclude sustained contact between the cell and the substrate and thus make effective force transmission impossible, in 3D, confinement counteracts Brownian motion and secures surface contact (Figure 1) (Friedl et al 2001). This realization led many investigators to question whether specific attachment points, usually mediated by

integrin-based focal adhesions on 2D substrates, were required for cell propulsion in 3D. Recently, several papers unambiguously showed that in 3D confinement, migration is possible without the formation of integrin-based attachments and started exploring conditions where this type of movement could occur *in vivo* (Bergert et al 2015, Lammermann et al 2008, Liu et al 2015, Ruprecht et al 2015). At the same time, biophysical studies proposed several mechanisms for how propelling forces could be generated during specific adhesion-independent migration, and what the magnitude of such forces would be (Bergert et al 2015, Hawkins et al 2009, Hawkins et al 2011, Tozluoglu et al 2013). In the past 8 years, adhesion-independent migration has emerged as a possibly common, though still poorly understood, migration mode.

In this article, we summarize our current understanding of cell migration in the absence of focal adhesions. We first summarize the studies that led up to questioning the requirement for specific adhesions in 3D migration. We then review the different contexts where adhesion-independent migration has been reported and discuss under which conditions migration without focal adhesions is likely to arise. We present the different physical mechanisms proposed for force generation and transmission during this migration mode. Finally, we compare focal adhesion-independent migration to adhesive migration and discuss to what extent they should be considered distinct migration modes.

Challenging the requirement of specific adhesions for migration

Force generation during adhesive migration

Historically, the key observation that underlied hypotheses for force generation in the Abercrombie model was that particles placed on the dorsal leading edge of a cell migrating in 2D undergo retrograde movement from the tip of the lamellipodium to its base (Abercrombie et al 1970a). At this time neither the nature of the force that drives the particles backward, nor the receptors mediating cell-particle adhesions were known. Nevertheless the authors hypothesized that the same force that drives the particles backward might move the cell forward - provided the lamellipodium is anchored to the substrate. The following decades revealed that the lamellipodium is

generated, maintained and dynamized by treadmilling arrays of actin filaments. These generate a continuous retrograde flow of material. As actin polymerizes at the tip of the cell (Glacy 1983), the force of monomer addition at the leading edge of the plasma membrane drives filaments towards the cell center and thereby generates the potential force to drive surface receptors backwards – leading to retrograde particle movement and forward translocation of the lamellipodium. A complementary force component originates from myosin II-mediated actomyosin contraction behind the lamellipodium, which generates pulling forces further driving actin filaments towards the cell center (Henson et al 1999, Medeiros et al 2006).

These actomyosin-generated intracellular forces are coupled to the substrate via transmembrane receptors, mainly of the integrin family. The coupling does not happen in a direct interaction. Instead there is a hierarchy of “clutch” molecules, which form a sliding interface between actin and the substrate-bound integrin. The clutch concept, which was introduced by Mitchison, Kirschner (Mitchison & Kirschner 1988) and Forscher (Suter et al 1998) was not only a useful analogy, but also turned out to be reflected in the molecular organization of the adhesion complexes, where the components between actin and integrin are horizontally stratified (Kanchanawong et al 2010) and move differentially when traction forces are exerted on the adhesion point (Case & Waterman 2015, Hu et al 2007). Actin and adhesion dynamics must be precisely coordinated to ensure effective force transmission, and multiple molecular and mechanical feedback loops contributing to this coordination have been identified (reviewed in (Gardel et al 2010)). This exquisite level of understanding shows that today, the mechanistic basis of crawling adhesive migration is a very mature field.

Mesenchymal versus amoeboid motility

The mechanisms of cell migration within Abercrombie’s three-step-cycle framework have mostly been investigated in mesenchymal and epithelial cells. This is somewhat paradoxical, as in these cells locomotion itself is difficult to study because cell body translocation happens at a much slower timescale than leading edge dynamics. A notable exception are fast moving epithelial cells like fish keratocytes, where the cellular structure is dominated by a large lamellipodial array, while the cell body can

be seen as an annex that is dragged by the lamellipodium (reviewed in (Rafelski & Theriot 2004)). While a powerful model, keratocytes appear to represent a rather specialized migratory sub-type where locomotion is dominated by lamellipodia and adhesion dynamics. Other extensively studied migrating cells, such as fibroblasts, keratinocytes, endothelial cells, and some cancer cells, display mesenchymal morphology and actomyosin dynamics comparable to keratocytes but their locomotion is often considerably slower (Friedl et al 1998b, Pankova et al 2010). As a result, the investigations of molecular details governing force generation and transmission in these cells became partially uncoupled from the issue of how all this contributes to actual cell movement. For example, ablation of lamellipodia by interfering with the actin assembly machinery does not necessarily abolish the migratory capacity of cells (Gupton et al 2005, Suraneni et al 2012, Wu et al 2012), suggesting that rather than driving migration, the lamellipodium might act as a sensing or steering device, while the forces actually propelling the cell are generated elsewhere (Cramer 2010).

The divergence of studies of lamellipodia and adhesion dynamics, and actual cell motility is especially obvious when considering parallel investigations of the locomotion of leukocytes, which display much faster velocities than typically observed for mesenchymal cells (Friedl et al 2001). Investigations of leukocyte motility tend to be more focused on actual locomotion, because due to faster migration, morphological and molecular dynamics are easier to correlate with cellular translocation than is the case for mesenchymal cells. Migrating leukocytes are characterized by a rounded cell shape, weak cell-substrate adhesion and constant cell shape changes. These typical morphodynamic characteristics are often described as "amoeboid", because they are reminiscent of the shape changes displayed by migrating amoeba (Yumura et al 1984). Apart from amoeba and leukocytes (Friedl et al 2001, Mandeville et al 1997), various cells in developing embryos (Blaser et al 2006, Diz-Munoz et al 2010, Trinkaus 1973), and some cancer cells (Rosel et al 2008, Sahai & Marshall 2003, Wolf et al 2003, Wyckoff et al 2006) have been shown to display amoeboid-like migration *in vivo*.

Amoeboid cells usually deform independently of adhesive substrate interactions: they polarize along a front back axis even in the suspended state, and the first

investigations questioning the requirement for substrate attachments for cell movement were studies of amoeboid migration.

Amoeboid force transduction by shape change

Long before Abercrombie's observations and the discovery of the cytoskeleton, morphological studies of amoeba and lymphocytes led to the idea of gel-sol driven motility (De Bruyn 1946, Rösel von Rosenhof et al 1755). In this mechanism, a liquefied cytoplasm drives the cellular front, possibly by physical swelling while solidification and associated shrinkage retracts the back. Before integrins were discovered (Hynes 2004), several investigators hypothesized that locomotion of leukocytes in physiological 3D environments is independent of substrate adhesion (Armstrong & Lackie 1975, Brown 1982, Haston et al 1982). As the cell-substrate receptors were not known, adhesion was tested functionally, by measuring attachment of leukocytes to 2D substrates coated with extracellular matrix or to other cells like fibroblasts. Locomotion was then measured in 3D scaffolds composed of the same molecular or cellular elements. The results showed that even within environments with which the cells did not display significant adhesion, leukocytes moved effectively and it was hypothesized that traction is mediated by the extensive amoeboid shape changes of the cells, which allowed intercalation into the complex fiber geometry and could thus provide “footholds” for force generation (see Section on Force generation below).

Migration in the absence of specific adhesions

Integrin independent locomotion

The studies showing that in 3D gels lymphocytes and neutrophils migrate effectively without any measureable adhesiveness to the matrix (Armstrong & Lackie 1975, Brown 1982, Haston et al 1982), were at odds with a universal integrin-dependency of locomotion. The universality of integrins as force transducers was supported by the fact that virtually every mammalian cell has integrins on its surface. The only known exception is the erythrocyte, which lacks any migratory capacity. Furthermore, the

absolutely detrimental consequences of integrin loss for most cell lineages argued for a central importance of integrins (Bouvard et al 2001, Fassler et al 1996, Fassler & Meyer 1995, Hirsch et al 1996). Even the recirculation of the low-adhesive cells of the hematopoietic lineage was tightly controlled by integrins. Indeed one of the best-established paradigms of cellular trafficking, the leukocyte extravasation cascade, relies on integrin-mediated adhesion to the vascular endothelium as an early step of transmigration through the vessel wall (Adams & Shaw 1994, Butcher 1991, Carlos & Harlan 1994, Shimizu et al 1991, Shimizu et al 1992). However, while the indispensability of integrins in extravasation is uncontested, endothelial adhesion does not reflect actual migration but rather a localized immobilization event. And indeed, various studies had reported that interfering with the function of specific integrins reduced, but did not completely abolish migration of leukocytes and several other cell types *in vivo* (reviewed in (Friedl & Brocker 2000)).

In 1997, Malawista and de Boisleury Chevance provided a simple and elegant proof that neutrophil granulocytes are able to migrate in confinement in the absence of integrin-mediated adhesion (Malawista & de Boisleury Chevance 1997). They showed that both $\beta 2$ integrin deficiency as well as total integrin inactivation by divalent cation chelation did not impair migration if the cells were confined between two closely adjacent glass coverslips, while migration on an open planar substrate was abolished under these conditions. One year later it was shown that integrin blocking by functionally active antibodies did not affect lymphocyte migration in 3D collagen gels (Friedl et al 1998a). To settle the question *in vivo*, Lämmermann et al used a genetic approach to knock out all integrins on leukocytes and formally proved that *in vivo* interstitial migration of dendritic cells can occur in the absence of integrins (Lämmermann et al 2008). These findings were substantiated in several other physiological settings of 3D interstitial leukocyte migration, including neutrophil granulocyte migration in the skin (Lämmermann et al 2013) and lymphocyte migration in lymphatic organs (Woolf et al 2007). Despite such clean examples the generality of integrin independence is not at all settled and it has been shown that under inflammatory conditions T cell migration in the skin seems to rely on integrin mediated adhesion (Overstreet et al 2013).

How wide-spread is the ability to display integrin independent migration is unclear. In culture, a number of cell lines including zebrafish early progenitors, cancer cells and cultured fibroblasts, have recently been shown to effectively migrate in the absence of integrins or talins, and on non-adhesive substrates, as long as the cells were provided with sufficient confinement (Bergert et al 2015, Liu et al 2015, Ruprecht et al 2015). *In vivo*, zebrafish primordial germ cells, which during early embryogenesis migrate in cellular environments with little extracellular matrix do not rely on integrin-mediated adhesions. Instead, these cells employ cadherin-based interactions with neighboring cells to transduce forces (Kardash et al 2010). Cadherin-based interactions can be viewed as another type of specific adhesions. An interesting example where non-specific substrate interactions *in vivo* have been proposed, are Schwann Cells, which have recently been shown to migrate in an integrin-independent manner along blood vessels during axonal regeneration (Cattin et al 2015).

Demonstrating integrin independence

Although an increasing number of studies clearly prove that some cells can migrate in the absence of integrins, it is usually more difficult to demonstrate that such cells do indeed not employ integrins to migrate *in vivo*, or if integrin mediated adhesion is merely dispensable for their locomotion.

Some evidence can be provided by measurements of cell velocity. When all integrins (or the universal integrin co-factor Talin) were deleted in dendritic cells, these cells did not show any significant change in migratory speed when migrating in an *in vivo* interstitium and in different fibrillar 3D matrices (Lammermann et al 2008). The same was true when dendritic cells (Renkawitz et al 2009) or cancer Walker carcinosarcoma cells (Bergert et al 2015) were confined under a layer of agarose. While such observations strongly suggest that focal adhesions, if present, do not play a major role in migration, they do not constitute unequivocal proof.

Importantly, measurements of cell velocities alone can be misleading, as some cells appear to dispose of adaptive mechanisms to compensate for changes in substrate adhesiveness. For example, integrin-deficient dendritic cells confined under agarose migrate at the same speed as their wild type counterparts; to do so, the integrin-

deficient cells compensate for inefficient force transduction and slippage between actin flows and the substrate by significantly increasing the rate of actin polymerization at the leading edge (Renkawitz et al 2009). Such compensation mechanisms are also observed in other cell types (Barnhart et al 2011) and pose a significant challenge when studying the potential involvement of adhesion receptors in migration. To allow for clear conclusions any loss of function approach has to be complemented by precise monitoring of actomyosin dynamics, which is notoriously difficult in 3D and *in vivo*.

The lack of specific adhesion involvement can also be more directly tested by monitoring fluorescently tagged focal adhesions components. Not detecting adhesion spots cannot demonstrate that faint attachment points are not present. However, when fluorescent monitoring of adhesion components is coupled to placing cells on substrates treated to prevent cell attachment and on which no spreading of detached cells is observed in the absence of confinement, it can be considered a strong indication that cells display adhesion-independent migration. Furthermore, cell shape can also be used as a readout of substrate adhesiveness, as non-adhering cells contact the substrate with a zero contact angle (Figure 2) (Evans 1992). While precise monitoring of fluorescently tagged focal adhesion components and cell-substrate contact angles can be readily achieved in culture, it is significantly more complex *in vivo* and in 3D matrices. For instance, in such thick samples adhesion points are difficult to visualize due to the detrimental effect of cytoplasmic background and to the complexity of cell shapes in such environments (Kubow & Horwitz 2011).

In summary, demonstrating that any specific migratory cell type does not use specific adhesions remains difficult, particularly in 3D matrices and *in vivo*. Simple velocity measurements can be confounded by cellular functional plasticity. To be conclusive, such studies require a combination of approaches, including measurements of cell velocities, of actin dynamics, as well as monitoring of adhesion components and of cell shape dynamics.

Conditions favoring focal adhesion-independent migration

Though actual adhesion-independence is difficult to prove, particularly *in vivo*, cells with weak substrate attachments contact the substrate with low contact angles and thus generally display the rounded morphology typical of amoeboid-like migration (Figure 2). Many migratory cell types can switch between mesenchymal and amoeboid migration modes, and conditions favoring mesenchymal-to-amoeboid transitions are thought to generally favor low-adhesion migration modes. These conditions include cell intrinsic factors: low levels of adhesion molecules, such as integrins, and high actomyosin contractility; and extrinsic factors: substrates not favoring cell adhesion, and strong confinement (Figure 3).

Cell intrinsic factors favoring adhesion-independent migration

Low levels, absence, or functional inactivity of integrins is the most obvious cell-intrinsic factor favoring non-adhesive migration. As highlighted in section III, a number of cell types including dendritic cells (Lammermann et al 2008), neutrophils (Malawista & de Boisleury Chevance 1997), lymphocytes (Friedl et al 1998a), Schwann cells (Cattin et al 2015) and some cancer cells (Bergert et al 2015, Liu et al 2015), maintain the ability to migrate in confinement upon treatments interfering with integrin function. However, due to the difficulty to truly demonstrate the lack of involvement of integrins, it remains unclear if cells actually modulate integrin expression, function and localization during migration *in vivo*.

High actomyosin contractility is another factor favoring amoeboid-like motility, as extensively reviewed elsewhere (Friedl 2004, Lammermann & Sixt 2009, Madsen & Sahai 2010, Pankova et al 2010, Wolf & Friedl 2006). In addition to being key in generating propelling forces for amoeboid migration, which relies on cytoplasmic flows generated by strong contractions at the cell rear, cortical contractility favors rounded cell shapes and could therefore directly counteract adhesion. Consistently, myosin IIA knockout in T-cells leads to impaired migration and increased cell spreading (Jacobelli et al 2010). Conversely, increased cortical contractility favors rounded amoeboid-like shape and non-adhesive migration in a number of cultured cell types (Bergert et al 2015, Liu et al 2015). Interestingly, increasing contractility by application of serum, or of Lysophosphatidic Acid (LPA), a serum phospholipid known to activate cortical contractility via the Rho/Rock pathway (Mills &

Moolenaar 2003), has been shown to induce adhesion-independent migration of isolated early zebrafish progenitors in confinement (Ruprecht et al 2015). The effect of contractility on cell velocity is less consistent. In T-cells migrating in non-adhesive micro-channels, inhibition of Myosin activity with blebbistatin has been shown to lead to decreased cell velocity (Jacobelli et al 2010). However, highly contractile cancer cells are not faster or penetrate further when invading dense collagen gels (Lautscham et al 2015). More systematic experimental investigations will be required to address the exact role of actomyosin contractility in triggering and driving adhesion independent migration.

Cell extrinsic factors favoring adhesion-independent migration

Modulating cell adhesiveness extrinsically, by placing cells in non-adhesive environments, is another obvious factor favoring non-adhesive migration. HL-60 neutrophil-like cells, which migrate in a mesenchymal fashion on fibronectin-coated substrates, switch to amoeboid-like and apparently adhesion-independent migration when placed in confinement between two non-adhesive gels (Yip et al 2015). Decreasing substrate adhesiveness also induces rapid migration in mesenchymal cells, such as fibroblasts, epithelial cells and a variety of different cancer cells, in 2D confinement between two non-adhesive glass plates (Liu et al 2015). Similarly, reducing cell-substrate attachment by using substrates with non-adhesive coatings has been shown to increase the migration velocity of isolated zebrafish progenitor cells migrating in confinement (Ruprecht et al 2015). While these observations suggest that low adhesiveness is a positive factor favoring rapid adhesion-free migration, some level of cell-substrate interactions is required. Indeed, cancer Walker carcinosarcoma cells, which migrate effectively when confined in channels coated with various non-adhesive molecules, cannot migrate in channels coated with Polyethylene-glycol (PEG) because the low friction provided by PEG is not sufficient to transmit friction forces between the cell and the channel walls (Bergert et al 2015). In contrast, other cultured cell types migrate when confined between two PEG-coated surfaces (Liu et al 2015); this could be due to cell-specific differences. It could also be due to the fact that the drag experienced by a cell migrating in a channel is substantially larger than for a cell confined between 2D surfaces, thus migration in a channel should require stronger traction forces for effective locomotion. What kind of substrates are

permissive for adhesion-independent migration and to what extent these are cell-type dependent is an important open question for future studies.

Finally, increasing levels of confinement has been shown to favor adhesion-independent migration in many cell types (Bergert et al 2015, Lammermann et al 2008, Lautscham et al 2015, Liu et al 2015, Mills & Moolenaar 2003, Renkawitz & Sixt 2010, Ruprecht et al 2015). Physical confinement seems to be sufficient to switch neutrophils to an integrin-independent motility mode, in which the presence of integrins decreases cell speed and increases traction forces (Toyjanova et al 2015). Confinement has been shown to induce cytoskeletal alterations, which allows breast cancer cells to migrate in an adhesion-independent manner; interestingly this change in migration mode depends on microtubules (Balzer et al 2012). In summary, confinement, which is essential for maintaining cell-substrate attachment during adhesion-free migration (Figure 1), also appears to favor transitions towards low-adhesive migration modes (Figure 3). However, too strong confinement has been observed to decrease and even stall migration (Harada et al 2014, Jacobelli et al 2010, Mak et al 2013, Malboubi et al 2015, Wolf et al 2013, Yip et al 2015)(Jacobelli et al 2010, Yip et al 2015). A possible reason is that factors such as cell stiffness and the volume of the nucleus could interfere with locomotion when cells are placed in strong confinement (Davidson et al 2015, Harada et al 2014, Krause & Wolf 2015, Lautscham et al 2015, Mak et al 2013, Malboubi et al 2015).

Which factors are modulated when cells resort to adhesion-independent migration *in vivo* remains to be investigated. Similarly to plasticity in other aspects of cell migration, such as the type of protrusion formed or the requirement for matrix metalloproteases for 3D migration (Bergert et al 2012, Friedl & Wolf 2010, Renkawitz & Sixt 2010), plasticity in adhesive engagement provides an important advantage for cells migrating through changing environments *in vivo*. This is particularly true for cells of the hematopoietic lineage and metastatic cancer cells, which are both capable of infiltrating diverse tissues. Finally, such plasticity could also be favoured during early development, where cells often encounter cell-rich environments with little assembled extra-cellular matrix. Careful experimental studies will be needed to unveil to what extent adhesion-independent migration contributes to

these various processes. Further investigation will also be necessary to reveal if this plasticity is cell intrinsic and therefore cell type specific, or if it is induced by the cellular environment.

Force generation during migration without focal adhesions

Several physical mechanisms have been proposed for force transmission between cell and substrate during migration without focal adhesions. Some of them remain purely theoretical while others have been to some extent tested experimentally. Below we highlight the main models of force transmission proposed.

Cell migration by swimming

It has been proposed that some cells may be able to migrate by swimming, i.e. generating propelling forces by coupling shape deformations to the surrounding fluid via hydrodynamic interactions. If experimentally confirmed, this would be the most extreme mode of adhesion-independent migration, since it does not require any solid substrate at all.

The requirements for swimming-based propulsion at the cellular scale are fundamentally different from the swimming mechanics we intuitively understand from our swimming pool experience. This is because microscopic objects of the size of cells live in a world at low Reynolds numbers (Re), where inertial forces have no effect on their motion (Dusenbery 2009, Purcell 1977). Re is the ratio of inertial to viscous forces that are typically experienced by a moving object; it is a function of the object's velocity, size, and density, as well as of the surrounding fluid's viscosity. Macroscopic objects such as animals operate at high Re , in which inertial forces dominate over viscous resistance, meaning that a thrust at a given time will propel such an object for a non-negligible time period before it is stalled by viscous resistance. In contrast, microscopic objects, such as cells (and, interestingly, some very macroscopic objects such as the earth mantle), operate at low Re , meaning that a thrust propels them only as long as it is exerted (Purcell 1977). Practically, this means that in order to move forward, a cell must constantly exert propelling forces. Another

consequence of negligible inertia at low Re is that reciprocal shape change, where a swimmer goes through cycles of deformation followed by its exact reversal, would not propel an object as every step forward would be followed by the same step backward¹. As a result, effective cellular swimming can not be achieved by cyclic movements like those of a human diver, but only by non-cyclic deformations, such as the corkscrew rotation of flagella (Blair 1990, Eisenbach 1990), asymmetric beating of cilia (Holwill et al 1995), or other asymmetric movements at the cell surface (Elgeti et al 2015).

A recent computational model described what is essentially a swimming mechanism for the migration of blebbing cells (Lim et al 2013). The cells are described as shells of cortex under tension attached to a membrane and interact with their environment only through hydrodynamic forces. This model predicts that cell body displacement can be achieved through blebbing even for a cell freely floating in medium. The key mechanistic basis of this movement is that the cell shape changes displayed by the blebbing cell in this model are not reciprocal between bleb expansion and bleb retraction: the expanding bleb displays a roughly hemispherical shape, while the bleb neck spreads during retraction, leading to a more distended shape (Figure 4a). This results in the cell behaving like a Purcell swimmer, with maximum displacement being displayed during bleb expansion. While an interesting model, it is unclear to what extent this mechanism contributes to blebbing motility, which has mostly been observed for cells in confinement, where other forces resulting from the cell's interaction with the substrate could dominate over swimming-based propulsion. It is unclear how much force would be generated by a bleb-driven swimming mechanism, and these forces would strongly depend on the asymmetry in bleb shape between expansion and retraction. However, bleb shape does not always significantly differ between expansion and retraction (Tinevez et al 2009), and non-adherent blebbing cells are usually not reported to move in suspension (Bergert et al 2012, Ruprecht et al 2015).

Interestingly, it has been reported that *Dictyostelium* amoeba and human neutrophils can effectively move towards a chemoattractant while placed in suspension in a Ficoll

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¹ Purcell called this principle the scallop theorem, because for similar reasons, a microscopic scallop at low Re would not be able to display forward motion (Purcell 1977).

gradient (Barry & Bretscher 2010). These observations suggest that these cell types can move by swimming. However, while lateral protrusions moving backwards could drive such swimming motion (Bae & Bodenschatz 2010), cell shape analysis does not appear to support this hypothesis and the cellular and physical mechanisms of their movement remain unclear (Howe et al 2013).

To summarize, while swimming motion is in principle a plausible alternative to crawling motility, it has at this point received little experimental support and its potential contribution to adhesion-free migration in confined environments has not been investigated.

Cell-substrate intercalations based force transmission

A more commonly proposed mechanism for migration without specific substrate attachment points is based on the intercalation of protrusions forming at the sides of the cell into gaps and discontinuities of the substrate (Figure 4b) (Charras & Paluch 2008, Renkawitz & Sixt 2010, Schmidt & Friedl 2010). Early descriptions of this mechanism were proposed following the observation that lymphocytes and neutrophils migrating through a 3D matrix extend lateral pseudopods that insert themselves into “footholds” in the matrix (Haston et al 1982, Mandeville et al 1997). These insertions led to matrix distortion, suggesting that forces were exerted on the footholds (Mandeville et al 1997). Later on, studies of cancer cell migration in 3D showed that treatments that interfere with the ability of the cells to rely on mesenchymal-based migration and matrix proteolysis, can trigger rounded cell shapes, suggestive of low substrate adhesion, and amoeboid-like migration with formation of non-polarized blebs around the cell body (Sahai & Marshall 2003, Wolf & Friedl 2006). The exact mechanisms of this poorly polarized migration mode are incompletely understood, however the intercalation of lateral protrusions into gaps in the matrix is a plausible possibility (Tozluoglu et al 2013). A recent investigation of Schwann cell migration during peripheral nerve repair has shown that these cells, which appear to migrate without using focal adhesions through a densely packed tissue, form multiple lateral bleb-like protrusions; these lateral blebs could contribute to generating protrusive forces by forming interdigitations with neighboring cells (Cattin et al 2015).

Though the model is conceptually attractive, it has not been demonstrated as of yet that interdigitation of lateral protrusions with matrix gaps is sufficient to propel a cell forward in a 3-dimensional matrix. Furthermore, how much force can be generated via such a mechanism has not been experimentally investigated. A recent computational model has explored the theoretical requirements for rapid cell migration for different levels of cell contractility and protrusivity, adhesiveness and matrix geometries (Tozluoglu et al 2013). This study shows that in a discontinuous 3D environment, inter-digitation of lateral protrusions into matrix gaps is the most effective of the migration modes explored by the model in this context. Interestingly, in the inter-digitation migration mode, fastest migration is achieved in the absence of specific substrate attachment, as adhesion can stall lateral protrusion dynamics and, as a result, slow down migration. While the model was not directly used to fit experimental data, the shapes and protrusion dynamics of the simulated cells are consistent with the shape dynamics of cancer cells migrating *in vivo* (Madsen et al 2015).

To summarize, a number of observations and theoretical considerations suggest that intercalation of lateral protrusions into gaps of a discontinuous 3D environment could allow cells to exert sufficient traction forces for confined migration. However, such a mechanism cannot explain cell movement in 3D environments with smooth surfaces, like microfabricated channels and microcapillaries (Liu et al 2015, Weigert et al 2013), or in environments *in vivo* where protrusion intercalation between topographical surface features is not observed (Ruprecht et al 2015).

“Pushing-off the walls”: chimneying force transmission

An alternative mechanism, termed “chimneying” in reference to a climbing technique based on pushing off opposing rock faces, has been proposed to account for migration in confinement without requiring a discontinuous substrate (Figure 4c). Chimneying motility was, to our knowledge, initially proposed to account for the motility of human leukocytes in the absence of divalent cations (and thus unable to form specific adhesions) when confined between a slide and a coverslip (Malawista & de Boisfleury Chevance 1997). The same mechanism was then hypothesized to drive

confined motility of leukocytes isolated from patients with Leukocyte Adhesion Deficiency, a condition that affects integrin $\beta 2$ function and the ability of white blood cells to form substrate attachments (Malawista et al 2000). To achieve chimneying migration, the cell has to be sufficiently confined (Malawista et al 2000), so that the cell can exert significant lateral pushing forces to wedge the cell body into place; protrusion expansion that extends the cell forward, can then effect cell body translocation (Charras & Paluch 2008, Paluch & Raz 2013, Renkawitz & Sixt 2010).

Several quantitative models have been developed to explore chimneying cell migration mechanisms. A physical model using the active gel theory (Prost et al 2015) to describe cytoskeleton behavior, investigated movement driven by a polymerizing actin network confined in a channel (Hawkins et al 2009). In this model, actin polymerizing against the membrane on the sides of the confined cell exerts pushing forces on the channel wall. The polymerization pressure leads to a build up of viscous friction between the cell and the channel walls. A denser actin meshwork at the cell rear, which could correspond to the cell nucleus, behaves like an elastic wall preventing counter-productive retrograde flow. In this model, actin polymerization against channel walls and basic viscoelastic properties of actin networks are sufficient to generate forward motion without any specific attachment between the cell and the substrate. This model has not been directly tested. However, it predicts a pressure gradient on the channel walls with a higher pressure and thus closer contact with the channel at the rear of the cell. Such a pressure gradient is consistent with Interference-Reflection-Imaging, which visualizes how close a cell is to its substrate, of dendritic cell migrating in microchannels (Hawkins et al 2009), but not necessarily of other cell types, such as cancer Walker carcinosarcoma cells migrating in confinement (Bergert et al 2015).

Interestingly, a recent experimental study of HL60 neutrophil-like cells migrating confined in micro-channels, reported that under these conditions, the cells display two distinct actin networks at the leading edge: a network growing against the free membrane at the cell front, and behind the leading edge, a network polymerizing perpendicularly to the channel walls (Wilson et al 2013). This second network could exert pressure on the walls and thus contribute to friction build up, as proposed in (Hawkins et al 2009). However, pushing forces could also result from intracellular

pressure. Another experimental study investigating HL60 neutrophil-like cells in confinement has recently quantified such pressure-generated pushing forces (Yip et al 2015). Cells were confined between two polyacrylamide gel layers coated with Pluronic F127, which prevents the formation of specific cell adhesions. Under these conditions, HL60 cells switch from the lamellipodia-driven motility they typically display to amoeboid migration, with rounded shapes and leading edge bleb formation. By tracking beads embedded in the polyacrylamide gels, the authors could show that the cells generated mainly normal stresses on the walls, and that these pushing stresses were on the order of 200-400 Pa. Interestingly, the authors also observed that cell velocities are highest at an intermediate level of confinement, while too high confinement stalls migration (Yip et al 2015). They account for this observation in the context of the model described in (Lim et al 2013). In this model, increasing confinement increases intracellular pressure, which causes larger leading edge blebs and, as a result, higher cell velocity. For excessive confinement though, intracellular pressure is so large that spontaneous blebbing occurs at the rear of the cell, abolishing cell polarity and stalling directional migration (Lim et al 2013). While the model predicts the observed effect of confinement on cell velocity (Yip et al 2015), other factors such as a stalling effect of the nucleus could also cause the observed cell stalling at high confinement (Allena et al 2015, Davidson et al 2015, Krause & Wolf 2015).

Flow-friction driven force transmission

Finally, mechanisms based on non-specific friction between the cell and the substrate have also been proposed to account for adhesion-independent migration (Hawkins et al 2011, Ruprecht et al 2015). There, intracellular forces generated by the cytoskeleton, such as flows of the actomyosin cortex, are transmitted to the substrate via non-specific friction (Figure 4d). A recent study investigating the mechanics of non-adhesive cell migration in confinement has shown that the shape and cortex dynamics displayed by Walker carcinosarcoma cells migrating in microchannels are well accounted for by a friction-based mechanism (Bergert et al 2015). Using a microfluidics device, Bergert et al also provided direct measurements of cell-substrate friction, which allowed them to measure the forces exerted by the migrating Walker cells on the substrate (discussed in the next section).

The molecular origin of non-specific friction has not been experimentally investigated. Friction could result from interactions between molecules at the cell surface and the substrate. In principle, any molecule with an extracellular domain and directly or indirectly coupled to the actin cortex could contribute. Transient weak interactions of such cell surface molecules with the substrate will necessarily occur if the cell is close enough to the substrate, and such friction would thus be favored by strong confinement. Friction-generating molecular components have not been experimentally characterized, however proteins such as cadherins, or the extracellular glycocalyx matrix are possible candidates (Friedl & Brocker 2000). Interestingly, friction could also arise from purely hydrodynamic interactions mediated by a thin layer of fluid remaining between the cell and the substrate. This type of friction is analogous to lubricated friction, well studied in engineering, and would be expected to be lower than a friction mediated by molecular interactions. Unveiling the microscopic origin of non-specific friction will be an important question for future studies.

Comparing the mechanics of focal adhesion-dependent and -independent migration

Depending on the mechanism of force generation, adhesion-independent migration differs more or less fundamentally from focal-adhesion based migration. Force generation mechanisms based on swimming, lateral protrusion intercalation or chimneying rely on substrate coupling mechanisms completely distinct from traction forces exerted on focal adhesions. In contrast, friction-based force generation shares common characteristics with force generation in adhesive cells. This is particularly true of forces exerted by non-specific coupling of molecules at the cell surface with the substrate, which mostly differs from focal adhesions by the strength and lifetime of the molecular interactions involved. Importantly, in most studied cases, such non-specific attachments are not sufficient to maintain a cell in contact with the substrate in 2D, whereas even weak integrin-based attachments can do so (Renkawitz & Sixt 2010). Hence, migration based on non-specific cell-substrate interactions is usually restricted to cells in 3D confinement. A notable exception is *Dictyostelium*

discoideum, which migrates efficiently in 2D but does not appear to rely on specific adhesion molecules (Friedl et al 2001, Loomis et al 2012, Weber et al 1995). Another fundamental distinctive feature of friction-based migration is that no (for lubricated friction) or very little (for non-specific-interactions-based friction) force is required to detach the rear of the cell. In contrast, trailing edge detachment has been shown to be limiting cell velocity in adhesion-based migration (Gupton & Waterman-Storer 2006). This is amplified by the fact that adhesion complexes grow with increasing traction forces (Choi et al 2008, Riveline et al 2001). As a result, excessive adhesion slows down migration as trailing edge detachment becomes increasingly difficult, and cell velocity as a function of substrate adhesion strength follows a Bell curve relationship, with optimal speed being achieved for intermediate adhesion and high adhesion actually stalling migration (Gupton & Waterman-Storer 2006, Palecek et al 1997, Zaman et al 2006). In contrast, increasing non-specific friction, is not expected to stall migration and theoretical modeling predicts that cell velocity should reach a plateau above a given friction magnitude (Bergert et al 2015). More quantitative studies systematically investigating the effect of friction on cell migration will be required to address these questions experimentally. Identifying the mechanistic and molecular origin of friction will be necessary to truly understand the differences between friction-based to adhesive migration.

Another important difference between adhesive and non-adhesive migration is the magnitude and distribution of the traction forces produced by the cell on the substrate. In adhesive migration, cells typically exert stresses (force per unit area) in the hundreds of Pascals, with peaks in the *kPa* range on strong focal adhesions (Balaban et al 2001, Dembo & Wang 1999, Gardel et al 2008). So far, only few studies have attempted to measure forces in non-adhesive migration. Yip et al analyzed the forces generated by neutrophil-like HL60 cells migrating between two layers of non-adhesive polyacrylamide gel with embedded tracer particles. These traction force measurements reported that the stresses generated by the cells on the gel were dominated by normal stresses over 500 *Pa*, pushing on the gel, consistent with a chimneying mechanism (Yip et al 2015). The forces in the plane of the gel were of lower magnitude, but could reach a few 100 *Pa*. In contrast, traction force measurements of confined Walker carcinosarcoma cells did not detect any stresses of magnitude comparable to those exerted by adhesive cells, and direct friction

measurements coupled to a quantification of actomyosin flows during migration revealed that the forces exerted by these non-adhering cells were on the order of 1 *Pa* or lower (Bergert et al 2015). Interestingly, in both studies, the forces exerted in the plane of the substrate by non-adhesive cells were distributed so as to expand the substrate (Bergert et al 2015, Yip et al 2015). This force distribution is inverted compared to what is typically observed for adhesive cells, which tend to contract the underlying substrate (Schwarz & Safran 2013). This distinction might have implication for cell-cell interactions, as the distribution of the forces exerted by a cell determines the pattern of substrate deformations it generates while it migrates, which in turn could be sensed and influence the migration of neighboring cells (Bergert et al 2015, Schwarz & Safran 2013).

The observation that the stresses exerted by non-adhesive migrating cells on their substrate can be in the Pascal range (Bergert et al 2015) raises the question of why adhesive cells exert stresses orders of magnitude higher. Importantly, the total force exerted on the substrate is not substantially changed: balance of forces at low Reynolds number requires that the sum of the forces exerted by a migrating cell exactly compensates the forces opposing migration. These forces are the viscous drag of the surrounding fluid, which is very low in 2D and in confinement between 2D surfaces and becomes higher for tighter confinement in microchannels or matrices, and, for adhesive cells, the resistance of the trailing edge to detachment. The observation that non-adhesive cells can migrate in tight confinement indicates that the low stresses they exert are sufficient to counteract even high viscous drag. The large stresses exerted on specific adhesions could thus be mostly employed to detach the trailing edge (Renkawitz & Sixt 2010). They could also become essential for cells migrating against flow, such as leukocytes migrating along blood vessels prior to extravasation, where the drag is substantially higher (Auffray et al 2007, Bartholomaeus et al 2009, Phillipson et al 2006). Furthermore, the strong substrate deformations induced by integrin-based adhesions on soft substrates can be used by cells to sense substrate stiffness and are essential for durotaxis, i.e. migration up stiffness gradients, displayed by many adherent cells (Plotnikov & Waterman 2013). Whether non-adherent cells are able to sense substrate stiffness by other means, e.g. via pushing forces, has not yet been investigated.

Taken together, even though quantitative data on the forces exerted by cells migrating in a non-adhesive manner are scarce, they suggest that the mechanics of cell body translocation could substantially differ from adhesion-based migration. A systematic investigation of force distributions during non-adhesive migration, particularly *in vivo*, though made challenging by the low magnitude of the forces involved, will be required to clarify to what extent adhesive and non-adhesive migration constitute two mechanically distinct crawling locomotion modes.

Future challenges and open questions

Migration in the absence of focal adhesions has emerged over the past decade as a possibly common alternative to adhesive migration for cells in 3D confinement. Several recent experimental studies have unambiguously shown that migration without integrin-based adhesions is possible in a variety of confined environments, both in culture and *in vivo*. Understanding to what extent this migration mode is spontaneously adopted by cells migrating during physiological or pathological processes *in vivo* remains a key open question for the future.

Another key open question is how propelling forces are generated during adhesion-independent migration. Several physical mechanisms have been proposed and some of them, such as cell-substrate intercalation and friction-based force transmission, are supported by experimental observations. It is conceivable that different cell types rely on different force generation mechanisms, or that different types of environments favor distinct modes of force generation. Some mechanisms could act in synergy. For instance, surface irregularities might enhance cell-substrate friction, however, large irregularities could also contribute to cell-substrate intercalation mechanism. Truly understanding the non-adhesive cell motion will require systematic studies of the forces exerted by non-adhesive cells and of the microscopic and molecular origin of these forces. Such an enterprise will necessitate a combination of cell biology, intra-vital microscopy, biophysics, and engineering of microenvironments mimicking *in vivo* contexts in a controlled manner to allow for precise measurements of forces and cell dynamics.

Finally, it would be interesting to investigate non-adhesive migration from an evolutionary perspective. Because it does not require any specific molecular interactions, it is tempting to speculate that non-adhesive migration represents an ancestral crawling migration mechanism. If this were the case, it raises the question of whether integrin-based adhesions have evolved to enable more efficient migration, or if other functions, such as substrate sensing, were key to their evolution.

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Figure 1

Figure 2

a Mesenchymal migration

Key molecules:
Talin, Integrin, Actin and other well
studied molecules

Cell velocity:
slow: $\sim 0.1 - 0.5 \mu\text{m}/\text{min}$

Well studied substrate adhesion

Commonly utilised by:
fibroblasts
keratinocytes
endothelial cells
cancer cells

b Amoeboid migration

Key molecules:
Actin, Myosin and unidentified
force generating molecules

Cell velocity:
fast: $\sim 5 - 20 \mu\text{m}/\text{min}$

Substrate interaction poorly understood

Commonly utilised by:
cells of the hemapoietic lineage
(lymphocytes, dendritic cells, T-cells)
cancer cells
cells in developing embryos

Figure 3

Figure 4

a Swimming migration

b Cell-substrate intercalation

c Chimneying force transmission

d Flow-friction-driven force transmission

Figure legends

Figure 1. Requirements for migration on 2D substrates versus 3D confinement

(a) Migration on a 2D surface: cells rely on substrate anchoring (purple dots) to counter Brownian motion. (b) In 3D environments, confinement ensures sustained contact between the cell and the substrate and counters Brownian motion; specific attachment points are thus not required. 3D confinement is achieved e.g. for cells migrating through a 3D matrix or in a microfabricated microchannel, or a microcapillary *in vivo*.

Figure 2. Mesenchymal versus amoeboid migration.

Overview of mesenchymal and amoeboid migration. Key molecules required for migration, cell velocities and typical cell shapes are highlighted. (a) Mesenchymal migration can be achieved on a flat 2D surface. Cells typically display a flat, spread-out morphology due to substrate anchoring (purple dots), resulting in a high contact angle between the cell and the substrate. (b) Amoeboid migration often requires 3D confinement. Cells are round and do not adhere or adhere only weakly to their substrate via unknown molecules, resulting in low contact angles between the cell and the substrate.

Figure 3. Conditions favoring non-adhesive migration.

Transitions between adhesive and non-adhesive migration can be favored by several cellular and environmental parameters. Low levels of substrate adhesion, high actomyosin contractility activation and high levels of confinement tend to favor adhesion-independent migration. These different parameters are not necessarily independent (e.g. high contractility can reduce adhesiveness), which makes understanding transitions between adhesive and non-adhesive migration a complex, multi-parameter problem.

Figure 4. Force generation mechanisms during adhesion-independent migration.

Schematic representation of different physical mechanisms of force generation and transmission during migration in the absence of focal adhesions. The pink line represents the actomyosin cortex, with dark pink showing strong cortex contractility

and light pink a weak cortex. (a) Intercalation of lateral protrusions into substrate gaps could serve as footholds to drive cell migration. This type of migration could be particularly effective in 3D matrices and in crowded inhomogeneous environments *in vivo* (Tozluoglu et al 2013). (b) Chimneying migration of cells in confinement. Lateral pushing forces against the surrounding substrate keep the cell body in place, allowing for high cortical contractility at the rear and protrusion expansion at the front to drive locomotion (Hawkins et al 2009). (c) During flow-friction driven migration, forces generated by contractile flows of the actomyosin cortex are transmitted to the substrate via non-specific friction (Bergert et al 2015). The molecular origin of friction is not known. (d) Swimming migration of blebbing cells. Asymmetric shape deformations during bleb expansion and bleb retraction combined with hydrodynamic interactions with the surrounding fluid could lead to cell locomotion (Lim et al 2013).

References:

- Abercrombie M. 1980. The Croonian Lecture, 1978: The Crawling Movement of Metazoan Cells. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 207: 129-47
- Abercrombie M, Heaysman JE, Pegrum SM. 1970a. The locomotion of fibroblasts in culture. 3. Movements of particles on the dorsal surface of the leading lamella. *Exp Cell Res* 62: 389-98
- Abercrombie M, Heaysman JE, Pegrum SM. 1970b. The locomotion of fibroblasts in culture. I. Movements of the leading edge. *Exp Cell Res* 59: 393-8
- Adams DH, Shaw S. 1994. Leucocyte-endothelial interactions and regulation of leucocyte migration. *Lancet* 343: 831-6
- Allena R, Thiam H, Piel M, Aubry D. 2015. A mechanical model to investigate the role of the nucleus during confined cell migration. *Comput Methods Biomech Biomed Engin* 18 Suppl 1: 1868-9
- Armstrong PB, Lackie JM. 1975. Studies of intercellular invasion in vitro using rabbit peritoneal neutrophil granulocytes (PMNS). I. Role of contact inhibition of locomotion. *J Cell Biol* 65: 439-62
- Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, et al. 2007. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 317: 666-70
- Bae AJ, Bodenschatz E. 2010. On the swimming of Dictyostelium amoebae. *Proc Natl Acad Sci U S A* 107: E165-6
- Balaban NQ, Schwarz US, Riveline D, Goichberg P, Tzur G, et al. 2001. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat Cell Biol* 3: 466-72

- Balzer EM, Tong Z, Paul CD, Hung WC, Stroka KM, et al. 2012. Physical confinement alters tumor cell adhesion and migration phenotypes. *FASEB J* 26: 4045-56
- Barnhart EL, Lee KC, Keren K, Mogilner A, Theriot JA. 2011. An adhesion-dependent switch between mechanisms that determine motile cell shape. *PLoS Biol* 9: e1001059
- Barry NP, Bretscher MS. 2010. Dictyostelium amoebae and neutrophils can swim. *Proc Natl Acad Sci U S A* 107: 11376-80
- Bartholomaeus I, Kawakami N, Odoardi F, Schlager C, Miljkovic D, et al. 2009. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. *Nature* 462: 94-8
- Bergert M, Chandradoss SD, Desai RA, Paluch E. 2012. Cell mechanics control rapid transitions between blebs and lamellipodia during migration. *Proc Natl Acad Sci U S A* 109: 14434-9
- Bergert M, Erzberger A, Desai RA, Aspalter IM, Oates AC, et al. 2015. Force transmission during adhesion-independent migration. *Nat Cell Biol* 17: 524-9
- Blair DF. 1990. The bacterial flagellar motor. *Semin Cell Biol* 1: 75-85
- Blaser H, Reichman-Fried M, Castanon I, Dumstrei K, Marlow FL, et al. 2006. Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. *Dev Cell* 11: 613-27
- Bouvard D, Brakebusch C, Gustafsson E, Aszodi A, Bengtsson T, et al. 2001. Functional consequences of integrin gene mutations in mice. *Circ Res* 89: 211-23
- Brown AF. 1982. Neutrophil granulocytes: adhesion and locomotion on collagen substrata and in collagen matrices. *J Cell Sci* 58: 455-67
- Butcher EC. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 67: 1033-6
- Carlos TM, Harlan JM. 1994. Leukocyte-endothelial adhesion molecules. *Blood* 84: 2068-101
- Case LB, Waterman CM. 2015. Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. *Nat Cell Biol* 17: 955-63
- Cattin AL, Burden JJ, Van Emmeris L, Mackenzie FE, Hoving JJ, et al. 2015. Macrophage-Induced Blood Vessels Guide Schwann Cell-Mediated Regeneration of Peripheral Nerves. *Cell* 162: 1127-39
- Charras G, Paluch E. 2008. Blebs lead the way: how to migrate without lamellipodia. *Nat Rev Mol Cell Biol* 9: 730-6
- Choi CK, Vicente-Manzanares M, Zareno J, Whitmore LA, Mogilner A, Horwitz AR. 2008. Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. *Nat Cell Biol* 10: 1039-50
- Cramer LP. 2010. Forming the cell rear first: breaking cell symmetry to trigger directed cell migration. *Nat Cell Biol* 12: 628-32
- Danuser G, Allard J, Mogilner A. 2013. Mathematical modeling of eukaryotic cell migration: insights beyond experiments. *Annu Rev Cell Dev Biol* 29: 501-28
- Davidson PM, Sliz J, Isermann P, Denais C, Lammerding J. 2015. Design of a microfluidic device to quantify dynamic intra-nuclear deformation during cell migration through confining environments. *Integr Biol (Camb)* 7: 1534-46
- De Bruyn PP. 1946. The amoeboid movement of the mammalian leukocyte in tissue culture. *Anat Rec* 95: 177-91

- Dembo M, Wang YL. 1999. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophys J* 76: 2307-16
- Diz-Munoz A, Krieg M, Bergert M, Ibarlucea-Benitez I, Muller DJ, et al. 2010. Control of directed cell migration in vivo by membrane-to-cortex attachment. *PLoS Biol* 8: e1000544
- Dusenbery DB. 2009. *Living at micro scale : the unexpected physics of being small*. Cambridge, Mass. ; London: Harvard University Press. xxx, 416 : ill. ; 25 cm. pp.
- Eisenbach M. 1990. Functions of the flagellar modes of rotation in bacterial motility and chemotaxis. *Mol Microbiol* 4: 161-7
- Elgeti J, Winkler RG, Gompper G. 2015. Physics of microswimmers--single particle motion and collective behavior: a review. *Rep Prog Phys* 78: 056601
- Evans E. 1992. Equilibrium "wetting" of surfaces by membrane-covered vesicles. *Adv Colloid Interface Sci* 39: 103-28
- Even-Ram S, Yamada KM. 2005. Cell migration in 3D matrix. *Curr Opin Cell Biol* 17: 524-32
- Fassler R, Georges-Labouesse E, Hirsch E. 1996. Genetic analyses of integrin function in mice. *Curr Opin Cell Biol* 8: 641-6
- Fassler R, Meyer M. 1995. Consequences of lack of beta 1 integrin gene expression in mice. *Genes Dev* 9: 1896-908
- Friedl P. 2004. Prespecification and plasticity: shifting mechanisms of cell migration. *Curr Opin Cell Biol* 16: 14-23
- 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
- Friedl P, Bocker EB. 2000. The biology of cell locomotion within three-dimensional extracellular matrix. *Cell Mol Life Sci* 57: 41-64
- Friedl P, Entschladen F, Conrad C, Niggemann B, Zanker KS. 1998a. 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 J Immunol* 28: 2331-43
- Friedl P, Wolf K. 2010. Plasticity of cell migration: a multiscale tuning model. *J Cell Biol* 188: 11-9
- Friedl P, Zanker KS, Bocker EB. 1998b. Cell migration strategies in 3-D extracellular matrix: differences in morphology, cell matrix interactions, and integrin function. *Microsc Res Tech* 43: 369-78
- Gardel ML, Sabass B, Ji L, Danuser G, Schwarz US, Waterman CM. 2008. Traction stress in focal adhesions correlates biphasically with actin retrograde flow speed. *J Cell Biol* 183: 999-1005
- Gardel ML, Schneider IC, Aratyn-Schaus Y, Waterman CM. 2010. Mechanical integration of actin and adhesion dynamics in cell migration. *Annu Rev Cell Dev Biol* 26: 315-33
- Glacy SD. 1983. Subcellular distribution of rhodamine-actin microinjected into living fibroblastic cells. *J Cell Biol* 97: 1207-13
- Gupton SL, Anderson KL, Kole TP, Fischer RS, Ponti A, et al. 2005. Cell migration without a lamellipodium: translation of actin dynamics into cell movement mediated by tropomyosin. *J Cell Biol* 168: 619-31
- Gupton SL, Waterman-Storer CM. 2006. Spatiotemporal feedback between actomyosin and focal-adhesion systems optimizes rapid cell migration. *Cell* 125: 1361-74

- Harada T, Swift J, Irianto J, Shin JW, Spinler KR, et al. 2014. Nuclear lamin stiffness is a barrier to 3D migration, but softness can limit survival. *J Cell Biol* 204: 669-82
- Haston WS, Shields JM, Wilkinson PC. 1982. Lymphocyte locomotion and attachment on two-dimensional surfaces and in three-dimensional matrices. *J Cell Biol* 92: 747-52
- Hawkins RJ, Piel M, Faure-Andre G, Lennon-Dumenil AM, Joanny JF, et al. 2009. Pushing off the walls: a mechanism of cell motility in confinement. *Phys Rev Lett* 102: 058103
- Hawkins RJ, Poincloux R, Benichou O, Piel M, Chavrier P, Voituriez R. 2011. Spontaneous contractility-mediated cortical flow generates cell migration in three-dimensional environments. *Biophys J* 101: 1041-5
- Henson JH, Svitkina TM, Burns AR, Hughes HE, MacPartland KJ, et al. 1999. Two components of actin-based retrograde flow in sea urchin coelomocytes. *Mol Biol Cell* 10: 4075-90
- Hirsch E, Iglesias A, Potocnik AJ, Hartmann U, Fassler R. 1996. Impaired migration but not differentiation of haematopoietic stem cells in the absence of beta1 integrins. *Nature* 380: 171-5
- Holwill ME, Foster GF, Hamasaki T, Satir P. 1995. Biophysical aspects and modelling of ciliary motility. *Cell Motil Cytoskeleton* 32: 114-20
- Howe JD, Barry NP, Bretscher MS. 2013. How do amoebae swim and crawl? *PLoS One* 8: e74382
- Hu K, Ji L, Applegate KT, Danuser G, Waterman-Storer CM. 2007. Differential transmission of actin motion within focal adhesions. *Science* 315: 111-5
- Hynes RO. 2004. The emergence of integrins: a personal and historical perspective. *Matrix Biol* 23: 333-40
- Jacobelli J, Friedman RS, Conti MA, Lennon-Dumenil AM, Piel M, et al. 2010. Confinement-optimized three-dimensional T cell amoeboid motility is modulated via myosin IIA-regulated adhesions. *Nat Immunol* 11: 953-61
- Kanchanawong P, Shtengel G, Pasapera AM, Ramko EB, Davidson MW, et al. 2010. Nanoscale architecture of integrin-based cell adhesions. *Nature* 468: 580-4
- Kardash E, Reichman-Fried M, Maitre JL, Boldajipour B, Papusheva E, et al. 2010. A role for Rho GTPases and cell-cell adhesion in single-cell motility in vivo. *Nat Cell Biol* 12: 47-53; sup pp 1-11
- Krause M, Wolf K. 2015. Cancer cell migration in 3D tissue: negotiating space by proteolysis and nuclear deformability. *Cell Adh Migr* 9: 357-66
- Kubow KE, Horwitz AR. 2011. Reducing background fluorescence reveals adhesions in 3D matrices. *Nat Cell Biol* 13: 3-5; author reply 5-7
- Lammermann T, Afonso PV, Angermann BR, Wang JM, Kastenmuller W, et al. 2013. Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature* 498: 371-5
- Lammermann T, Bader BL, Monkley SJ, Worbs T, Wedlich-Soldner R, et al. 2008. Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature* 453: 51-5
- Lammermann T, Sixt M. 2009. Mechanical modes of 'amoeboid' cell migration. *Curr Opin Cell Biol* 21: 636-44
- Lautscham LA, Kammerer C, Lange JR, Kolb T, Mark C, et al. 2015. Migration in Confined 3D Environments Is Determined by a Combination of Adhesiveness, Nuclear Volume, Contractility, and Cell Stiffness. *Biophys J* 109: 900-13

- Lim FY, Koon YL, Chiam KH. 2013. A computational model of amoeboid cell migration. *Comput Methods Biomech Biomed Engin* 16: 1085-95
- Liu YJ, Le Berre M, Lautenschlaeger F, Maiuri P, Callan-Jones A, et al. 2015. Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. *Cell* 160: 659-72
- Loomis WF, Fuller D, Gutierrez E, Groisman A, Rappel WJ. 2012. Innate non-specific cell substratum adhesion. *PLoS One* 7: e42033
- Madsen CD, Hooper S, Tozluoglu M, Bruckbauer A, Fletcher G, et al. 2015. STRIPAK components determine mode of cancer cell migration and metastasis. *Nat Cell Biol* 17: 68-80
- Madsen CD, Sahai E. 2010. Cancer dissemination--lessons from leukocytes. *Dev Cell* 19: 13-26
- Mak M, Reinhart-King CA, Erickson D. 2013. Elucidating mechanical transition effects of invading cancer cells with a subnucleus-scaled microfluidic serial dimensional modulation device. *Lab Chip* 13: 340-8
- Malawista SE, de Boisleury 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 U S A* 94: 11577-82
- Malawista SE, de Boisleury 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: 183-9
- Malboubi M, Jayo A, Parsons M, Charras G. 2015. An open access microfluidic device for the study of the physical limits of cancer cell deformation during migration in confined environments. *Microelectron Eng* 144: 42-45
- Mandeville JT, Lawson MA, Maxfield FR. 1997. Dynamic imaging of neutrophil migration in three dimensions: mechanical interactions between cells and matrix. *J Leukoc Biol* 61: 188-200
- Medeiros NA, Burnette DT, Forscher P. 2006. Myosin II functions in actin-bundle turnover in neuronal growth cones. *Nat Cell Biol* 8: 215-26
- Mills GB, Moolenaar WH. 2003. The emerging role of lysophosphatidic acid in cancer. *Nat Rev Cancer* 3: 582-91
- Mitchison T, Kirschner M. 1988. Cytoskeletal dynamics and nerve growth. *Neuron* 1: 761-72
- Overstreet MG, Gaylo A, Angermann BR, Hughson A, Hyun YM, et al. 2013. Inflammation-induced interstitial migration of effector CD4(+) T cells is dependent on integrin alphaV. *Nat Immunol* 14: 949-58
- Palecek SP, Loftus JC, Ginsberg MH, Lauffenburger DA, Horwitz AF. 1997. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* 385: 537-40
- Paluch EK, Raz E. 2013. The role and regulation of blebs in cell migration. *Curr Opin Cell Biol* 25: 582-90
- Pankova K, Rosel D, Novotny M, Brabek J. 2010. The molecular mechanisms of transition between mesenchymal and amoeboid invasiveness in tumor cells. *Cell Mol Life Sci* 67: 63-71
- Phillipson M, Heit B, Colarusso P, Liu L, Ballantyne CM, Kubes P. 2006. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med* 203: 2569-75

- Plotnikov SV, Waterman CM. 2013. Guiding cell migration by tugging. *Curr Opin Cell Biol* 25: 619-26
- Prost J, Jülicher F, Joanny JF. 2015. Active Gel Physics. *Nature Physics* 11: 111-17
- Purcell EM. 1977. Life at low Reynolds number. *American Journal of Physics* 45: 3-11
- Rafelski SM, Theriot JA. 2004. Crawling toward a unified model of cell mobility: spatial and temporal regulation of actin dynamics. *Annu Rev Biochem* 73: 209-39
- Renkawitz J, Schumann K, Weber M, Lammermann T, Pflücke H, et al. 2009. Adaptive force transmission in amoeboid cell migration. *Nat Cell Biol* 11: 1438-43
- Renkawitz J, Sixt M. 2010. Mechanisms of force generation and force transmission during interstitial leukocyte migration. *EMBO Rep* 11: 744-50
- Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, et al. 2001. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J Cell Biol* 153: 1175-86
- Rosel D, Brabek J, Tolde O, Mierke CT, Zitterbart DP, et al. 2008. Up-regulation of Rho/ROCK signaling in sarcoma cells drives invasion and increased generation of protrusive forces. *Mol Cancer Res* 6: 1410-20
- Rösel von Rosenhof AJ, Kleemann CFC, Fleischmann JJf. 1755. *Der monatlich-herausgegebenen Insecten-Belustigung*. pp. 621. Nürnberg: zu finden bey den Röselischen Erben, gedruckt bey Johann Joseph Fleischmann. 3 v. in 2 : col. ill., ports ; 27 cm. pp.
- Ruprecht V, Wieser S, Callan-Jones A, Smutny M, Morita H, et al. 2015. Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. *Cell* 160: 673-85
- 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: 711-9
- Schmidt S, Friedl P. 2010. Interstitial cell migration: integrin-dependent and alternative adhesion mechanisms. *Cell Tissue Res* 339: 83-92
- Schwarz U, Safran S. 2013. Physics of Adherent Cells. *Rev Mod Phys* 85: 1327
- Shimizu Y, Newman W, Gopal TV, Horgan KJ, Graber N, et al. 1991. Four molecular pathways of T cell adhesion to endothelial cells: roles of LFA-1, VCAM-1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. *J Cell Biol* 113: 1203-12
- Shimizu Y, Newman W, Tanaka Y, Shaw S. 1992. Lymphocyte interactions with endothelial cells. *Immunol Today* 13: 106-12
- Suraneni P, Rubinstein B, Unruh JR, Durnin M, Hanein D, Li R. 2012. The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. *J Cell Biol* 197: 239-51
- Suter DM, Errante LD, Belotserkovsky V, Forscher P. 1998. The Ig superfamily cell adhesion molecule, apCAM, mediates growth cone steering by substrate-cytoskeletal coupling. *J Cell Biol* 141: 227-40
- Tinevez JY, Schulze U, Salbreux G, Roensch J, Joanny JF, Paluch E. 2009. Role of cortical tension in bleb growth. *Proc Natl Acad Sci U S A* 106: 18581-6
- Toyjanova J, Flores-Cortez E, Reichner JS, Franck C. 2015. Matrix confinement plays a pivotal role in regulating neutrophil-generated tractions, speed, and integrin utilization. *J Biol Chem* 290: 3752-63

- Tozluoglu M, Tournier AL, Jenkins RP, Hooper S, Bates PA, Sahai E. 2013. Matrix geometry determines optimal cancer cell migration strategy and modulates response to interventions. *Nat Cell Biol* 15: 751-62
- Trinkaus JP. 1973. Surface activity and locomotion of *Fundulus* deep cells during blastula and gastrula stages. *Dev Biol* 30: 69-103
- Webb DJ, Parsons JT, Horwitz AF. 2002. Adhesion assembly, disassembly and turnover in migrating cells -- over and over and over again. *Nat Cell Biol* 4: E97-100
- Weber I, Wallraff E, Albrecht R, Gerisch G. 1995. Motility and substratum adhesion of Dictyostelium wild-type and cytoskeletal mutant cells: a study by RISM/bright-field double-view image analysis. *J Cell Sci* 108 (Pt 4): 1519-30
- Weigert R, Porat-Shliom N, Amornphimoltham P. 2013. Imaging cell biology in live animals: ready for prime time. *J Cell Biol* 201: 969-79
- Wilson K, Lewalle A, Fritzsche M, Thorogate R, Duke T, Charras G. 2013. Mechanisms of leading edge protrusion in interstitial migration. *Nat Commun* 4: 2896
- Wolf K, Friedl P. 2006. Molecular mechanisms of cancer cell invasion and plasticity. *Br J Dermatol* 154 Suppl 1: 11-5
- Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, et al. 2003. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J Cell Biol* 160: 267-77
- Wolf K, Te Lindert M, Krause M, Alexander S, Te Riet J, et al. 2013. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J Cell Biol* 201: 1069-84
- Woolf E, Grigorova I, Sagiv A, Grabovsky V, Feigelson SW, et al. 2007. Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. *Nat Immunol* 8: 1076-85
- Wu C, Asokan SB, Berginski ME, Haynes EM, Sharpless NE, et al. 2012. Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis. *Cell* 148: 973-87
- Wyckoff JB, Pinner SE, Gschmeissner S, Condeelis JS, Sahai E. 2006. ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. *Curr Biol* 16: 1515-23
- Yip AK, Chiam KH, Matsudaira P. 2015. Traction stress analysis and modeling reveal that amoeboid migration in confined spaces is accompanied by expansive forces and requires the structural integrity of the membrane-cortex interactions. *Integr Biol (Camb)* 7: 1196-211
- Yumura S, Mori H, Fukui Y. 1984. Localization of actin and myosin for the study of amoeboid movement in Dictyostelium using improved immunofluorescence. *J Cell Biol* 99: 894-9
- Zaman MH, Trapani LM, Sieminski AL, Mackellar D, Gong H, et al. 2006. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc Natl Acad Sci U S A* 103: 10889-94