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Mechanical modes of 'amoeboid' cell migration

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The morphological term 'amoeboid' migration subsumes a number of rather distinct biophysical modes of cellular locomotion that range from blebbing motility to entirely actin-polymerization-based gliding. Here, we discuss the diverse principles of force generation and force transduction that lead to the distinct amoeboid phenotypes. We argue that shifting the balance between actin protrusion, actomyosin contraction, and adhesion to the extracellular substrate can explain the different modes of amoeboid movement and that blebbing and gliding are barely extreme variants of one common migration strategy. Depending on the cell type, physiological conditions or experimental manipulation, amoeboid cells can adopt the distinct mechanical modes of amoeboid migration.

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Introduction

Rapid single cell crawling is commonly referred to as 'amoeboid' migration which owes its name to the protozoon *Amoeba proteus* (*amoibe*`(αμοιβή) as the Greek word for 'change', and Proteus as the Greek god of change) [1,2]. The group of amoeboid migrating cells is heterogeneous and comprises different unicellular eukaryotes and several individually migrating metazoan cell types. While these cells vary in size, compactness, and habitat, they all share one morphological feature that defines them as 'amoeboid': during locomotion they constantly change shape by rapidly protruding and retracting extensions that have been originally described as pseudopods or 'false feet'. However, different amoeboid cells employ various mechanical strategies resulting in variants of amoeboid phenotypes like contraction-based blebbing or entirely polymerization-driven gliding. Hence, the morphological definition of the term 'amoeboid' is problematic as it subsumes rather different mechanistic principles.

In this review, we distinguish different modes of amoeboid migration by dissecting components of force generation (protrusion and contraction) and force transduction (adhesiveness). We conclude that shifting the balance between these components creates distinct modes of amoeboid movement. We will primarily focus on the crawling of leukocytes in two-dimensional (2D) and three-dimensional (3D) environments and compare it with the migration of other amoeboid cells. Importantly, the discussed modes of migration are restricted to cells moving in porous environments that do not require proteolytic degradation or opening of junctions in order to be traversed [3]. Accordingly, invasion and penetration of extracellular or cellular barriers like basement membranes and epithelial or endothelial linings follow other principles that are discussed elsewhere [4,5].

Principles of force generation

Almost all forms of amoeboid migration are driven by the forces of a polarized actomyosin cytoskeleton, while other cytoskeletal elements play barely regulatory or supportive roles [6,7]. The two force-generating principles of the actin cytoskeleton are network expansion (polymerization) and network shrinkage (contraction). While only contraction can retract the cell, both principles can protrude the plasma membrane: Firstly, polymerizing actin filaments can move beads, bacteria, and virus intracellularly [8]. When expanding below the leading plasma membrane, they generate sufficient force to push out lamellipodia (flat, sheet-like) and filopodia (thin, needle-like) [9–11]. 'Pseudopodia' is the historical term describing all cellular protrusions, but nowadays, mostly refers to the finger-like protrusions of *Dictyostelium* and leukocytes. Secondly, actin-network contraction generates protrusions via hydrostatic pressure gradients. Contraction is largely dependent on type II myosins that cause tension in actin networks by sliding actin filaments past one another. Myosin II activity causes a local rise in hydrostatic pressure that can either lead to ruptures in the cortical actin network [12] or to local detachment of the plasma membrane from the cortical cytoskeleton [13,14]. In both cases, a flow of cytosol along the pressure gradient can protrude the plasma membrane and form a radially expanding membrane bleb. Once the pressure is equilibrated, bleb inflation slows down, F-actin and actin-binding proteins are recruited into the bleb and finally myosin II activity leads to bleb retraction [15•].

Balancing the protrusive forces

On the first sight, the contraction-based and polymerization-based protrusion modes appear rather exclusive —

and indeed, under some circumstances they occur in their pure form.

Entirely polymerization-driven protrusions were demonstrated in *Dictyostelium*, neutrophils, T lymphocytes, and dendritic cells upon blockade of myosin II [16,17•,18,19••,20•]. As in all these cases contractile forces were largely eliminated, the sheer force of polymerization was apparently sufficient to protrude the membrane. Remarkably, these cells were still able to migrate in low-adhesive 2D or confined environments, as we will discuss below. An interesting analogy to polymerization-driven protrusions is the extensions of nematode sperms. Instead of actin, these cells utilize polymeric filaments of major sperm protein to drive locomotion. These filaments lack inherent polarity and the orientation of polymerization is given by an intracellular pH-gradient [21,22] which makes it very unlikely that motor molecules move the filaments to generate contractile forces.

At the other end of the biophysical spectrum are cells that form actin-free membrane blebs while they are migrating. This has been described for *Dictyostelium* [20•,23], killifish deep cells [24], zebrafish primordial germ cells [25••], and several types of tumor cells (see Box 1). Importantly, blebs in migrating cells can occur polarized at the leading edge as has been demonstrated in an elaborate *in vivo* study on the blebbing migration of germ cells. Here, it was shown that sensing of guidance cues led to a local rise in free calcium at the leading edge and subsequent activation of myosin II that generated the focalized contraction to extrude polarized blebs. The 'aging' bleb subsequently became coated with an actin cortex that was ultimately retracted into the cell center, while a new bleb formed on top of the retracting one [25••].

In all cases of blebbing migration, myosin II activity was indispensable for bleb formation. Interestingly, a study utilizing *Dictyostelium* cells that constitutively moved in the blebbing mode, demonstrated that, once blebs were eliminated by the blockade of myosin II, the cells continued to locomote with actin-rich pseudopodia [20•]. Vice versa, pharmacological deceleration of actin polymerization in dendritic cells by low-dose latrunculin treatment led to an overweight of myosin II-based contraction and consequently cells continued to move while blebs formed at the leading edge (Figure 1A,B). While these switches in migration modes resulted from experimental manipulations, deep cells of killifish naturally shift between blebbing and protrusion [26•] (Figure 1C, image sequence kindly provided by Rachel Fink). Here, within one cell, protrusions alternate between phases of actin-rich pseudopods and actin-free blebs.

Together, these findings suggest that both hydrostatic and protrusive forces continuously synergize to protrude the leading plasma membrane. Hence, imbalances between these forces do not stall protrusion, but rather manifest as either the blebbing or the polymerization-driven phenotype.

Box 1 Amoeboid morphology of tumor cells.

Some tumor cell lines (e.g. A375m melanoma and LS174T colon carcinoma) show inherent amoeboid appearance [68], and mesenchymal-type cell lines (e.g. HT-1080 fibrosarcoma and MDA-MB-231 mammary carcinoma) can switch to amoeboid morphology after protease inhibition [68–70]. While the mesenchymal mode is promoted by signals that activate the small GTPase Rac, which triggers actin polymerization, the amoeboid mode results from enhanced contractility. Here, activation of the small GTPase RhoA leads to ROCK-dependent myosin light chain phosphorylation and myosin II activation [68]. Thus, Rac and RhoA signaling have opposing effects and reciprocally dampen each others activity [71••]. Regulatory proteins that favor the amoeboid mode either activate RhoA/ROCK signaling [72,73] or inhibit Rac activation [71••]. Proteins that act antiamoeboid activate Rac signaling [71••,74,75].

As the formation of blebs often coincides with the amoeboid phenotype and enhanced invasion or migration, blebbing movement has been widely promoted as a tumor cell migration strategy [60,76]. However, how tumor cells generate traction for migration has not been thoroughly investigated so far and also the question if protease-free tumor cell migration is possible under physiological conditions remains controversial [77]. Traction by cellular blebs as a movement strategy seems unlikely as most tumor cells form blebs in an uncontrolled fashion in all directions. It is possible that blebbing tumor cells produce traction by residual adhesive protrusions. This would be in line with impaired migration of single amoeboid tumor cells upon integrin blockade [68,70,71••], but contradicts reports suggesting that amoeboid tumor cell migration is integrin-independent [78,79]. These discrepancies might be owing to different experimental design (e.g. type of 3D matrix) and the heterogeneity in migration strategies of the different tumors. A further problem might be that studies of tumor invasion are sometimes put on the same level with interstitial migration, although these processes differ markedly. During interstitial migration the whole cell body is surrounded by a fibrillar matrix scaffold. By contrast, the cell body of an invasive cell cannot exert forces against surrounding fibers, as they barely overlie the gel or basement membrane. Hence, pushing forces will lift up the cell body owing to the lacking supportive scaffold at the back of the cell and pulling forces become essential to allow invasion. Consequently, integrins are required for invasive processes as observed for leukocytes, but dispensable for interstitial movement [19••,53,80,81] (TL and MS, unpublished observations).

Principles of force transduction

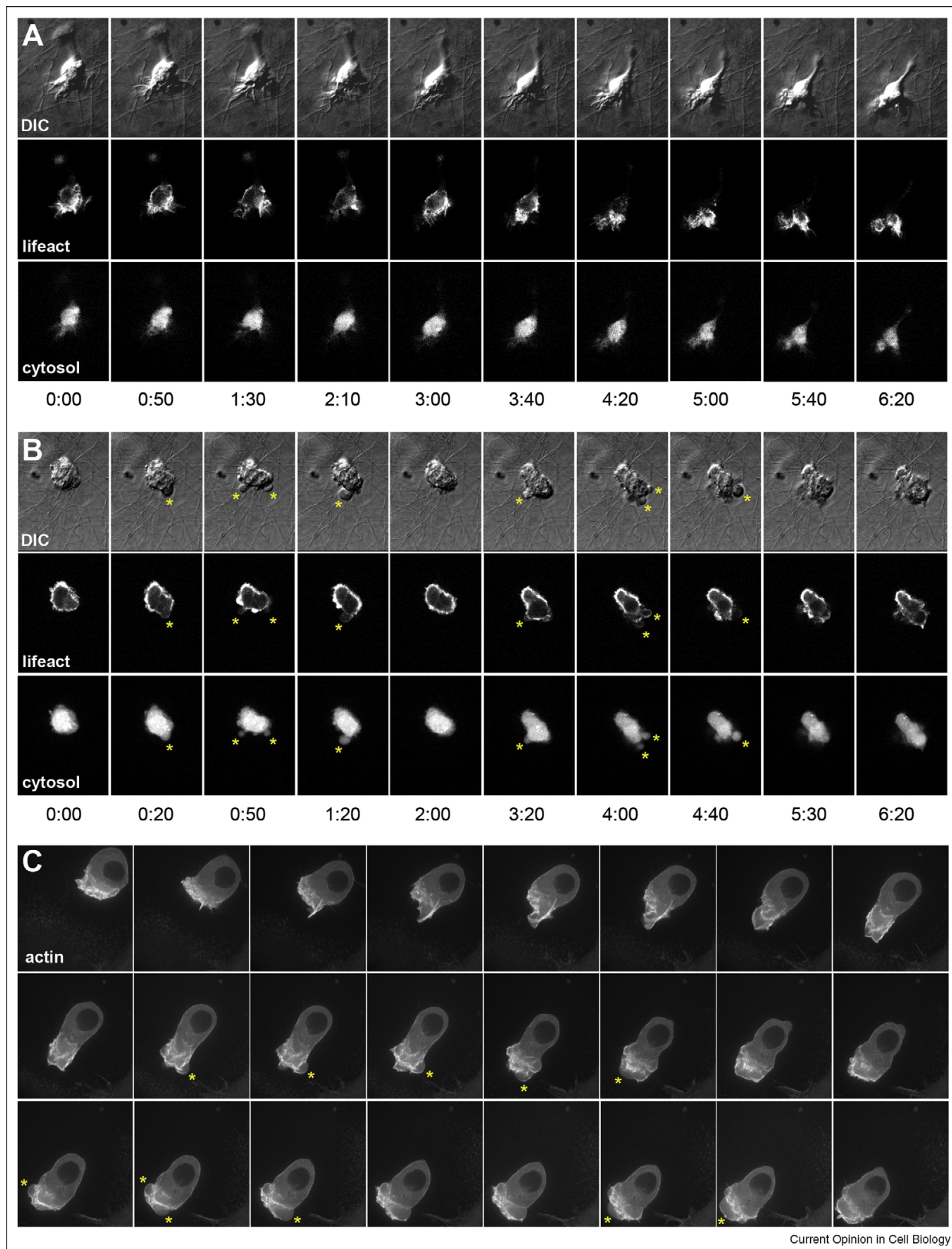
Intracellular forces can deform the cell body, but will only drive locomotion once they are transmitted to the environment. As the preconditions for force transduction vary fundamentally with the geometry of the cellular surrounding, we will separately discuss migration in 2D and 3D environments.

Migration on 2D surfaces

The role of adhesion

At the size scale of cells, Brownian motion rules over gravity, meaning that the weight of the cell is not sufficient to maintain surface contact. Hence, cells

Figure 1



migrating over 2D substrates require adhesion receptors to anchor them to the surface (Figure 2I–III). Although adhesive forces of amoeboid cells are generally considered to be low compared to mesenchymal or epithelial cells, they still cover a rather broad range. Extreme examples of surface anchoring are intravascularly crawling leukocytes that are sufficiently adherent to resist the shear forces of the blood stream [27,28•,29]. Surface anchoring is a prerequisite for migration in 2D, but it solely immobilizes the cell [30]. Actual locomotion requires membrane-parallel traction forces against the direction of movement (retrograde forces).

How can a protruding membrane generate retrograde forces? In cells moving with actin-rich protrusions, actin polymerization and actomyosin contraction synergistically generate retrograde forces (Figure 2I): by expanding against the mechanical resistance of the plasma membrane (or the adjacent extracellular environment), actin filaments push themselves backward, while protruding the membrane. Contractility in posterior areas supports this retrograde movement of the actin cortex, enhancing the retrograde force that can be coupled to the substrate via transmembrane receptors [31]. The resulting pulling forces are measurable as rearward-pointing substrate deformations at the leading edge of aggregation-prone *Dictyostelium* cells [32•]. However, such forces could not be detected in vegetative *Dictyostelium* cells [33] and neutrophils [34]. The reasons for this failure might be twofold. First, the traction forces required to move low-adhesive cells are small (see next section). Second, amoeboid cells lack the focalized adhesion structures of mesenchymal and epithelial cells. While for amoeba and nematode sperms the nature and molecular composition of the adhesion structures is debated [35–38], it is well established for amoeboid leukocytes that integrins are diffusely distributed on the plasma membrane [39,40]. The resulting diffusively distributed pattern of rearward-pointing force vectors might fall under the limit of detection of conventional traction-force microscopy.

In contrast to numerous studies demonstrating such polymerization-driven locomotion, few studies describe leading edge bleb formation of cells moving on 2D substrates as demonstrated in *Dictyostelium* [20•,23] (Figure 2III). As a newly formed and protruding bleb is free of actin filaments that generate retrograde forces, it is entirely unclear whether and how blebs might transduce traction forces onto the substrate: once the bleb adhered to the substrate, the anterograde forces of the expanding bleb would rather push the cell body backward than pull it forward. One mechanically feasible scenario is similar to polymerization-based motility: once the 'aging' bleb is coated with cortical actin, this actin shell contracts and retracts into the cell body, while a new bleb can form at the leading edge. The retrograde movement of this actin shell might be used to transmit force onto the substrate via transmembrane receptors. Alternatively, it is possible that, like in killifish deep cells, pseudopodial and blebbing phases co-occur.

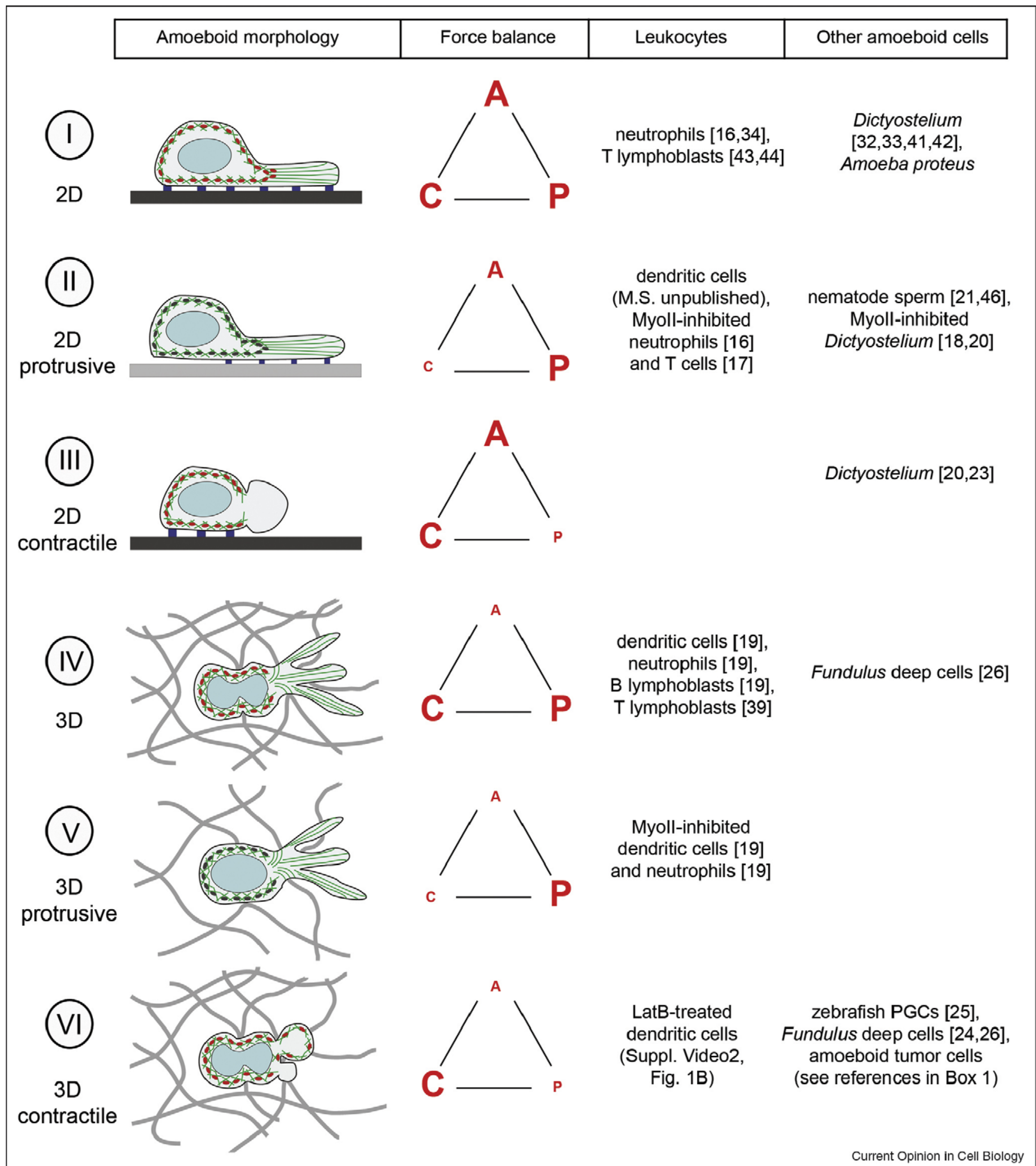
Negotiating adhesion and contraction

One important side effect of substrate anchoring is that adhesions need to be disassembled at the trailing edge. This is possible by dynamic regulation of adhesion receptors like integrins that can be locally switched off and thereby release the substrate at the cell rear. In addition, myosin II-dependent contraction forces at the trailing edge are required to mechanically support deadhesion and retraction, which is measurable as high forward traction stresses at the trailing edge of *Dictyostelium* [32•,33,41] and neutrophils [34] (Figure 2I). Accordingly, inhibition of myosin II activity during migration on high-adhesive surfaces led to reduced velocities in *Dictyostelium* [42] and caused an elongated morphology of neutrophils [16] and T lymphocytes [43,44] owing to impaired tail retraction.

On low-adhesive surfaces *Dictyostelium* [18], neutrophils [16] and T lymphocytes [17•] crawled independently of myosin II and without signs of elongation (Figure 2II). These findings demonstrate that low adhesiveness 'spares' the cell contractile work to detach the back

(Figure 1 Legend) Both actin polymerization and internal hydrostatic pressure contribute to cellular protrusions in individual cells. **(A** and **B)** Dendritic cell (DC) chemotaxis through a three-dimensional collagen lattice recorded with spinning-disc confocal microscopy (min:s). Polarized DCs migrate toward the higher CCL19 concentration at the image bottom. The upper row shows a differential interference contrast (DIC) image sequence, the middle row the corresponding fluorescence of lifeact:GFP which visualizes actin dynamics and the lower row fluorescence of tetramethylrhodamine for visualizing the cytoplasm. See also supplemental videos 1 and 2. (A) Normal wild-type DC migration is characterized by a thin band of actin around the cell body and prominent actin-rich pseudopodia at the cell front indicating actin-network expansion as the propulsive force. (B) Treatment of wild-type DCs with 150 nM latrunculin B depolymerizes the dynamic actin filaments of the leading edge, while cortical actin remains largely intact. A thin band of actin surrounds the cell body, while short-lived blebs (yellow asterisks) form primarily at the cell front. Thus, in the absence of actin polymerization, contraction-based internal hydrostatic pressure generates protrusive blebs. This indicates that both forces, actin polymerization and internal pressure, contribute to cellular protrusions during normal DC migration. If bleb formation produces traction for migration or if it is only an epiphenomenon remains to be shown. (C) This sequence shows a deep cell migrating through the subepithelial space of the yolk sac of an intact killifish embryo. The *Fundulus heteroclitus* embryo was injected with a GFP-actin plasmid at the two-cell stage. The cell starts migrating with a structured lamellipodium at the front and a thin band of actin around the entire periphery. In the course of migration, phases of actin-rich protrusions alternate with phases of bleb formation (yellow asterisks). Free of organized cortical actin, the cytoplasm protrudes beyond the existing cortex. Once the bleb forms, the fluorescent line of actin is seen to disappear, and a new cortical array is established as the bleb spreads. The image sequence was reproduced by courtesy of Rachel Fink, Mount Holyoke College, South Hadley, MA. Reproduced, with permission, from [26•], © 2007 Rachel Fink. All rights reserved. Reprinted under license from The American Society for Cell Biology.

Figure 2



The force-relationship between adhesion, contraction and polymer-network expansion determines the 'amoeboid' phenotype. The three major forces in cell migration are adhesion (A), contraction (C) and polymer-network expansion (P). Cell forward locomotion results from their balanced interplay (indicated by the red-lettered triangles). **(I–VI)** 'Amoeboid' crawling comprises various migration modes which differ in their primary driving forces, but are all variants of the same scheme. The amoeboid morphology of a specific cell type is determined by its inherent adhesive, contractile, and polymerizing equipment. Apart from that, individual cells (e.g. leukocytes) can switch between amoeboid modes after genetic or pharmacological interference with adhesion, contraction or polymer-network expansion. (I–III) Amoeboid cell movement on two-dimensional (2D) surfaces requires adhesion to transduce internal contractile forces onto the substratum. (I) A polymerizing network (green, in most cases actin) 'pushes' the membrane

and thereby makes migration energetically more effective. However, the fact that cells still had to be sufficiently adhesive to stay confined to the surface raises the question how the tail could be retracted at all. For the motor-free nematode sperms it has been suggested that rearrangement and disassembly of the polymer network at the posterior part creates sufficient shrinkage forces to disrupt adhesion sites and cause rear retraction [45,46]. Although this has not been proven, similar mechanisms might shrink actin networks in the absence of contractility [21]. Another potential tail retraction force is mediated by membrane tension. Plasma membranes display low elasticity and tensile forces quickly equilibrate over the cell surface. A nonextendable membrane bag implies that an expanding leading edge directly pulls on the trailing edge and causes retraction [47]. Together, on 2D substrates, adhesion and contraction have to be carefully balanced in order to allow effective locomotion. This principle is not unique to amoeboid cells and has also been demonstrated for migrating epithelial cells [48].

Migration in 3D environments

The role of adhesion

While surface anchoring is indispensable for migration on 2D substrates, this might change when cells are embedded in a 3D context. Once the cell is tightly surrounded by fibrils or surfaces, this confinement sufficiently immobilizes it and surface anchoring might become dispensable (Figure 2IV–VI). Hence, the transmission of traction forces alone might be sufficient to move the cell.

Early descriptive studies already favored a nonadhesive migration mode of leukocytes through 3D matrices [49,50]. After integrins were identified, it was widely assumed that leukocytes also employ them for locomotion. However, integrin-blocking studies in collagen gels were inconclusive and suggested leukocyte migration to be either dependent [51–53] or independent [39] of integrins. *In vivo* migration studies were limited and showed partial reduction (30%) in speed of neutrophils moving through mesenteric tissue after blocking α2 integrin function [54] and integrin-independent lymphocyte migration in the lymph node [55•]. Recently, it was shown that genetic depletion of all 24 possible integrin heterodimers did not alter migration velocities of mouse dendritic cells, neutrophils and B cells in collagen gels and dendritic cell migration in skin and lymph nodes *in vivo* [19••]. This study ultimately demonstrated that integrins are dispensable for interstitial migration, but also showed that the same cells strictly depended on force transduction via integrins when migrating on 2D substrates.

A similar principle was demonstrated using artificially confined environments: neutrophils depended on integrins when migrating on planar surfaces, but performed integrin-independent 'chimneying' between two closely adjacent glass slides [56]. Here, the cells that were jammed between two surfaces were forced into substrate contact. This makes surface anchoring dispensable, but leaves open the question how traction forces are transduced to drive forward locomotion. A recently proposed theoretical model demonstrated that the sheer force of actin filaments polymerizing and thereby pushing perpendicularly against inert surfaces is sufficient to allow locomotion in the absence of force coupling [57•] (Figure 2V). A fibrillar scaffold with its complex surface texture might provide even better conditions to generate traction as the cells can insert protrusions into 'footholds', which provides physical anchorage to transduce traction forces without adhering to the substrate [58].

The role of contraction

While such locomotion modes do only rely on actin polymerization, it was shown that migration of dendritic cells within tube-like microchannels is accelerated by myosin II-driven contractile forces [59]. Along the same line, T cells squeezed between an inert surface and an agarose layer require myosin II-based contraction for fast, nonadhesive motility, but after depletion of myosin II still migrate with reduced speed in an adhesion-dependent

(Figure 2 Legend Continued) forward. Myosin II (MyoII, red ellipses)-based contraction behind the leading edge produces traction underneath the adhesion points (blue). On high adhesive surfaces (black thick line), actomyosin contraction at the back is required to detach the cell from the substrate. Rear end contraction is not necessary when cells migrate on low adhesive substrates (gray thick line) (II). When the contractile function of myosin II is defect (black ellipses), actin polymerization alone can produce traction under adhesion points as has been shown in *Dictyostelium*. Migration without contraction postulates cell retraction either by membrane tension or by polymer-network disassembly (see text). (III) Myosin II-based contraction alone can generate internal hydrostatic pressure to bulge out plasma membrane. The forming bleb is first cytoplasm-filled and devoid of actin, but fills with actin and myosin II during retraction. Even though blebs have been observed during 2D migration, it is still unclear if they can transduce traction on the surface. This would require adhesive interactions between the bleb and the substrate which have so far not been shown. (IV–VI) Three-dimensional (3D) and confined environments enable migrating cells to exert perpendicular forces between at least two surfaces, which are not possible on 2D substrates. As 3D migration does not require adhesion-mediated traction, perpendicular forces might act as fixation points. (IV) Amoeboid movement in interstitial fibrillar networks (gray) requires contraction only when cells have to squeeze the nucleus (light blue) through narrow pores. (V) In less dense networks this deforming contraction is not necessary. Here, amoeboid migration is solely driven by polymer-network expansion. Front-to-back gradients of internal stiffness (gel–sol gradients) might additionally facilitate this movement, but have so far not been shown. (VI) Contraction-based increase in internal hydrostatic pressure and directed bleb formation can protrude the leading edge. Migration of zebrafish primordial germ cells (PGCs) serves as good example. However, it still remains to be shown how short-lived blebs (first actin-devoid, then actin-filled and myosin II-filled during retraction) generate traction on the environment. LatB: latrunculin B (actin-depolymerizing agent).

manner [17•]. These findings strongly argue for an additional role of hydrostatic forces to drive migration in confined environments. The most extreme form of hydrostatic migration might be entirely bleb-driven movement (Figure 2VI). Here, membrane blebs could expand into preformed pores of a 3D substrate where they subsequently become inflated with cytoplasm, and thereby, immobilized. This immobilization might provide the counter-force required to retract the trailing edge and move forward [60]. Bleb-driven force transduction is extremely attractive, as it would be independent of both actin treadmilling and force coupling. However, this concept has still not been experimentally confirmed (see also Box 1).

In dense collagen gels, an interesting nonadhesive variant of the tail retraction problem became apparent: Upon blockade of myosin II, the rigid nucleus became stuck in the fibrillar meshwork, whereas the leading edge continued to migrate, causing dramatic cell elongation [19••]. Thus, regulation of RhoA activation and actomyosin contractility at the trailing edge is necessary to deform and propel the nucleus through narrow pores [19••,61] (Figure 2IV). In sparse gels that display large pore sizes, leukocytes can reach normal peak velocities upon myosin II blockade. Together with the finding that this migratory mode was independent of integrins, this suggested that within confined environments amoeboid movement can be entirely driven by actin polymerization [19••] (Figure 2V). Accordingly, coordination of actin flow rather than force coupling was shown to be the most essential factor that determines interstitial migration [62•].

Conclusion

Although 'amoeboid' migration is often referred to as a mechanistically well-defined concept, it is important to stress that this term is not more than a morphological description that subsumes a heterogeneous spectrum of biophysical migration modes that is neither thoroughly understood nor clearly distinguishable from other migratory modes. For example, *Dictyostelium* cells can adopt a migration mode that closely resembles gliding keratocytes [63]. However, owing to their constant shape, keratocytes do not fall into the amoeboid category [47]. Here, terminology harbors the danger that migrating cells with roundish shape are categorized as 'amoeboid' instead of questioning mechanistic aspects of their cytoskeletal dynamics. In our rather schematical contemplation on actomyosin mechanics, we did neither consider regulatory aspects of actomyosin nor supportive roles of other cytoskeletal elements like actin-crosslinkers, microtubules, vimentin, and septins [64–67]. Nevertheless, understanding these factors in well-defined amoeboid model cells will be essential and will shed further light on the diverse migration mechanics that we summarized here.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ceb.2009.05.003.

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