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PERSPECTIVES

OPINION

Blebs lead the way: how to migrate without lamellipodia

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Abstract | Blebs are spherical membrane protrusions that are produced by contractions of the actomyosin cortex. Blebs are often considered to be a hallmark of apoptosis; however, blebs are also frequently observed during cytokinesis and during migration in three-dimensional cultures and *in vivo*. For tumour cells and a number of embryonic cells, blebbing migration seems to be a common alternative to the more extensively studied lamellipodium-based motility. We argue that blebs should be promoted to a more prominent place in the world of cellular protrusions.

In most eukaryotic cells, the plasma membrane is tightly bound to the cell cortex, a layer of actin, myosin and associated proteins[1]. Myosin motor proteins maintain the cortex under tension, thereby exerting hydrostatic pressure on the cytoplasm. Occasionally, the plasma membrane separates from the cortex and cytoplasmic pressure leads to herniations of the membrane that grow into spherical protrusions called blebs (FIGS 1,2). Blebs differ from other cellular protrusions, such as lamellipodia or filopodia, in that their growth is pressure-driven, rather than due to polymerizing actin filaments pushing against the membrane (as in lamellipodia or filopodia)[2]. Initially, the bleb membrane is not supported by an actin cytoskeleton, but an actin cortex subsequently reassembles before bleb retraction.

The specific role of blebs both in physiological and pathological situations is still subject to debate. Blebbing is characteristic of the execution phase of apoptosis[3]; however, blebs are also observed in healthy cells, for example during cytokinesis and cell spreading (BOX 1). Despite numerous observations from the 'pre-molecular era' identifying blebs at the leading edge of migrating cells, blebbing motility has been eclipsed by its lamellipodial cousin. Indeed, for the last couple of decades, the lamellipodium has been considered the be-all and end-all of cell migration[2]. However, many cell types, from amoebae to embryonic cells and mammalian tumour cells, can use blebs for motility[4–7] (reviewed in REF. 8). An increasing number of recent studies point to blebbing migration as an important motility mechanism and a common alternative to lamellipodia-driven migration in three-dimensional (3D) environments. Notably, blebbing motility is essential for certain cell types during development[5], and can be used by metastatic cells to escape anti-tumour treatments[6,9]. The molecular mechanisms of blebbing migration are beginning to be unveiled, paving the way for in-depth studies of this underrated motility mode[5,10].

In this Opinion article, we review old and new observations of bleb-driven migration and present our current knowledge of the mechanisms of bleb growth. We then discuss potential mechanisms by which blebbing can be polarized and translated into movement (FIG. 3). Finally, we discuss the respective advantages of blebbing and lamellipodial motility.

Blebbing motility

The most striking examples of blebbing motility have been observed during embryonic development in amphibian[11] and fish embryos[4,12–16] (FIGS 2a, 4). Cells dissociated from amphibian embryos can also migrate using bleb-like protrusions[11,17,18]. Live studies in zebrafish[5] have unequivocally shown that primordial germ cells (PGCs) use blebs to migrate (FIG. 2a). Similar observations have been made in fixed PGCs from *Drosophila melanogaster* embryos[19]. These findings suggest that blebbing might be widely used by PGCs across species. In embryonic cells, blebs often elongate into longer tubular structures, known as lobopodia[13] (FIG. 4b). Together, these observations hint at blebbing motility as a common feature of embryonic cells.

Early studies of migration in embryos often refer to blebbing motility as amoeboid motility[12]. Indeed, various amoebae move by means of spherical or cylindrical pseudopodia that are reminiscent of blebs and lobopodia[20]. Similarly to bleb formation, amoeboid pseudopodium growth is driven by hydrostatic pressure, which is generated by myosin contraction[21]. Furthermore, in *Amoeba proteus*, the pseudopodium tip is poor in filamentous (F)-actin during its growth and rich in F-actin during retraction, which suggests that some amoebae pseudopodia are indeed bleb-like protrusions[22,23]. Blebs have been clearly identified in *Dictyostelium discoideum* cells shortly after exposure to a chemoattractant[24]. Moreover, *D. discoideum* can simultaneously use blebs and lamellipodia to migrate[7] (FIG. 2b).

Finally, tumour cells that migrate through extracellular matrix (ECM) gels or through connective tissue can use blebbing motility as an alternative to lamellipodial migration. This 'amoeboid-like' migration does not require matrix degradation and can allow the cells to escape anti-tumour treatments that rely on protease inhibitors[6,10,25] (reviewed in REF. 9) (FIG. 4a). Other tumour cells use blebs to force their way through the endothelium and invade new tissues[26]. White blood cells can migrate using bleb-like protrusions when placed in 3D matrices[27]. Strikingly, Walker carcinosarcoma cells, a lymphocytic cell line, can use blebbing motility both on 2D and 3D substrates[28–30].

The life cycle of a bleb

Most of our mechanistic knowledge of bleb formation comes from non-migrating cells, although an increasing number of studies suggest that there are similar mechanisms in motile cells. In non-motile cells, the

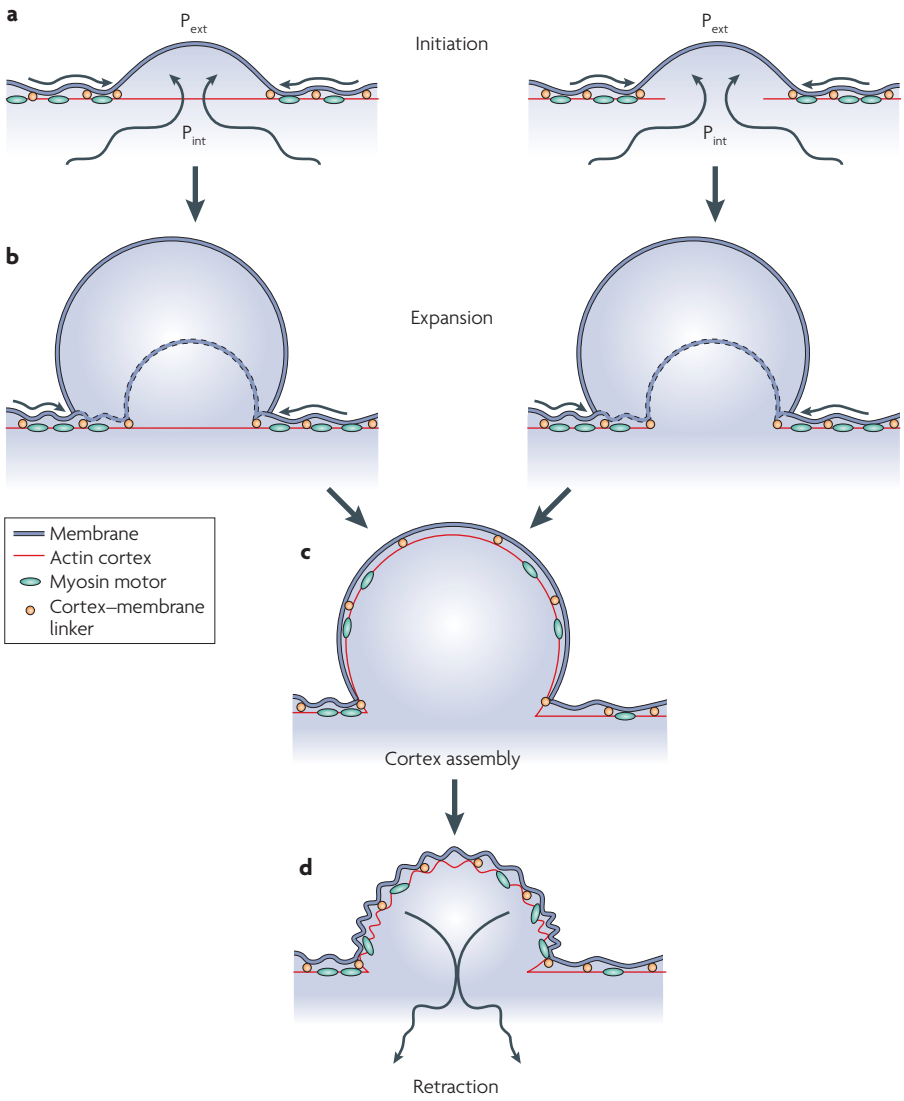

Figure 1 | **The bleb life cycle.** The bleb life cycle can be subdivided into three phases: bleb initiation (nucleation), expansion and retraction. **a** | Bleb initiation can result from a local detachment of the cortex from the membrane (left model) or from a local rupture of the cortex (right model). **b** | Hydrostatic pressure in the cytoplasm (P_{int}) then drives membrane expansion by propelling cytoplasmic fluid through the remaining cortex (left model) or through the cortex hole (right model). Concomitantly, the membrane can detach further from the cortex, increasing the diameter of the bleb base (dashed line). **c** | As bleb expansion slows down, a new actin cortex reforms under the bleb membrane. **d** | Recruitment of myosin to the new cortex is followed by bleb retraction. P_{ext}, extracellular hydrostatic pressure.

bleb life cycle can be subdivided into three phases: bleb initiation (often referred to as nucleation), bleb expansion and bleb retraction. In migrating cells, retraction does not always occur, but instead the cell body moves forward as a result of contraction at the rear[5,31]. In this section, we summarize what is known about the bleb life cycle, paying particular attention to what has also been observed during blebbing motility (reviewed in REFS 8, 32; see TABLE 1 for a list of proteins that have been identified in blebs and their potential roles in blebbing).

Bleb initiation. Two distinct mechanisms of bleb initiation have been observed experimentally: a local dissociation of the membrane from the cortex[33] or a local rupture of the actin cortex[28,34]. During migration, detachment of the membrane from the actin cortex has been observed in *Fundulus* deep cells[31], zebrafish PGCs[5] (FIG. 2a) and Walker carcinosarcoma cells[29]. Tears in the actin cortex have only been reported in fixed Walker carcinosarcoma cells[28], but they have not been thoroughly investigated in live cells during blebbing motility. Both types of initiation can occur because of localized myosin-driven contraction of the actin cortex[32,35], but any local loss of membrane-cortex adhesion (FIG. 1a,b; left model) or intra/extracellularly-induced local cortex weakening (FIG. 1a,b; right model) could achieve the same results when coupled to a uniform intracellular hydrostatic pressure (FIG. 3). Myosin activation occurs downstream of either the small GTPase RhoA and its effector Rho-kinase (ROCK), or myosin light chain kinase (MLCK). Activation of both pathways has been observed in non-motile blebbing cells[36,37] and in motile blebbing cells (mammalian tumour cells[6,10,38], *D. discoideum*[24], Walker carcinosarcoma cells[39] and amphibian and fish embryo cells[5,31]).

Bleb expansion. After initiation, the pressure that is generated by actomyosin contraction drives bleb expansion, which lasts for 5–30 seconds. Flow of cytosol into the bleb increases its volume and therefore the bleb surface area must increase. Surface area increase results from the flow of lipids into the bleb through the bleb neck and the tearing (delamination) of membrane from the actin cortex[32]. Membrane delamination has not been documented in motile blebbing cells, although it occurs in dissociated embryonic blastomeres, which produce non-polarized blebs[40]. Flow of membrane through the bleb neck into the growing bleb has been observed in *Fundulus* deep cells[41] and Walker carcinosarcoma cells[42]. Growing blebs are devoid of an actin cortex within the limit of optical resolution (both in non-motile[33,34,37] and motile cells[5,7,19,31]), but seem to possess a spectrin-based submembranous cytoskeleton that is similar to that of red blood cells[43] (TABLE 1). The maximal bleb size seems to be determined by the initial growth rate of the bleb and the time needed for the cortex to repolymerize at the bleb membrane[33]. Both bleb growth rate and maximal size are related to cortical tension (E.P., unpublished observations).

Cortex repolymerization and retraction. The last stage involves the reformation of an actomyosin cortex, followed by bleb retraction[5,29,31]. Actin cortex repolymerization probably begins as expansion slows, but the mechanisms that regulate actin nucleation in blebs remain unclear, as the two best-characterized actin nucleators, the actin-related protein-2/3 (ARP2/3) complex and the mammalian formin diaphanous (mDia1), are not detected under the membrane of blebs of filamin-deficient cells[43], which bleb profusely and are commonly used in studies of blebbing[44]. A detailed study in non-motile

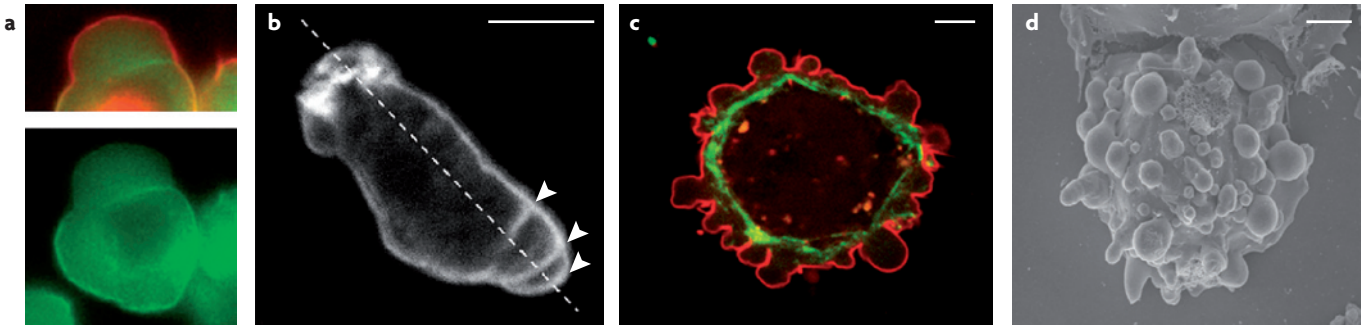

Figure 2 | **Examples of cell blebbing. a** | A zebrafish primordial germ cell (PGC) imaged in a live embryo. During blebbing motility, the PGC cell membrane (labelled by farnesylated *Discosoma* red fluorescent protein) separates from the actin cytoskeleton (labelled with actin-enhanced green fluorescent protein). The separation of the membrane from the cortex is a hallmark of blebbing. Later, an actin cortex reforms at the bleb membrane. Reproduced, with permission, from REF. 5 © (2006) Cell Press. **b** | Actin cortex of a blebbing *Dictyostelium discoideum*. The cell is migrating towards the bottom right. White arrowheads indicate successive blebs and arcs of the actin cortex at the leading edge. The actin cortex was stained with rhodamine phalloidin. Scale bar, 5 μm. Reproduced, with permission, from REF. 7 © (2006) Company of Biologists. **c** | Myosin light chain localization in a filamin-deficient melanoma cell. Myosin (in green) localizes to distinct puncta under the blebbing membrane (in red). Scale bar, 5 μm. Reproduced, with permission, from REF. 32 © (2008) Blackwell Scientific Publications. **d** | Scanning electron microscopy image of a filamin-deficient melanoma cell. Blebs can clearly be seen over the entire cell surface. Most blebs are spherical, others have elongated into lobopodia or have sprouted a side bleb. Scale bar, 5 μm.

cells has shown the sequential recruitment to the bleb membrane of F-actin membrane-linker proteins, actin, actin-bundling proteins and contractile proteins[43] (TABLE 1). Simultaneously, the actin cortex that remains at the base of the bleb is disassembled, possibly owing to the constitutive turnover of actin. All of the aforementioned proteins form a continuous rim under the bleb membrane except for myosin, which localizes to discrete spots[43] (FIG. 2c). Unless the bleb is stabilized (for example, by substrate adhesions), myosin-driven contraction mediates bleb retraction, which is slower than expansion (60–120 seconds). In migrating cells, a new bleb often forms soon after cortex repolymerization under the membrane[5,31]; such sequential bleb expansion might explain the periodicity of pseudopodia formation that is sometimes observed during amoeboid motility[45].

From blebbing to movement

For blebbing to result in cellular movement, the cells need to create blebs only at the leading edge and exert a force onto the substrate to translocate their cell body. Data are lacking on this subject. We present several mechanisms which might lead to polarized bleb formation and movement.

How do cells polarize bleb formation?

One main difference between motile and non-motile blebbing cells is that motile blebbing cells generate blebs primarily at their leading edge. The stimuli that lead to blebbing motility seem to be cell-specific; stem-cell-derived factor-1 (SDF1) triggers PGC motility[5], whereas cyclic AMP leads to *D. discoideum* movement[7]. However, it is not known how these signals trigger polarized bleb formation and, depending on the initiation mechanism, different models can be proposed. If blebs nucleate through the local detachment of the membrane from the actin cortex, polarization could result from a localized weakening of membrane–cortex attachments or a local increase in the pressure that is exerted on the membrane (FIG. 3a). This could be achieved by polarizing

Box 1 | **Blebs during apoptosis, cytokinesis, cell spreading and virus uptake**

Apoptosis

Apoptotic blebbing is the most commonly reported example of blebbing and forms one of the most spectacular features of the execution phase of apoptosis[3]. Apoptotic blebbing seems to be a direct consequence of the overactivation of myosin II. Multiple myosin-activation mechanisms have been identified, including myosin phosphorylation by myosin light chain kinase (MLCK)[59], myosin activation downstream of caspase-cleaved Rho-kinase-1 (ROCK1) in caspase-dependent apoptosis[36,60] and myosin activation downstream of ROCK2 in caspase-independent apoptosis[61]. Inhibition of any of these pathways reduces or inhibits blebbing. Although apoptotic blebbing is not in itself lethal (cells can form blebs for hours if caspases are inhibited[59]), blebbing could facilitate dispersion of fragmented DNA into apoptotic bodies[36]. One intriguing suggestion is that membrane proteins on the surface of blebs that have been separated from the cell body could serve as chemoattractants to recruit macrophages to the site of apoptosis[62].

Blebs also form during necrosis, the last stage of cell death, but these necrotic blebs are larger, do not retract and their formation does not depend on myosin contractility[63].

Cytokinesis

Blebs are commonly observed during cell division, from the onset of anaphase until late cytokinesis[64–66]. Although mitotic blebs had already been reported a century ago[67], their role in cytokinesis remains unclear. They might be a mere side-effect of tension build-up in the cortex during cell division, as it has been proposed that increased tension can directly result in bleb formation[47]. Alternatively, blebs might represent a rapid way of generating additional cortex and/or membrane surface area during cytokinesis and subsequent cell spreading[43,68].

Cell spreading

Dynamic blebbing occurs during the first minutes of cell spreading on adherent substrates, preceding lamellipodia formation[55,68]. Subsequent to the adhesion of a large bleb to the substrate, lamellipodial extension takes over, initiating a phase of rapid spreading. The role of blebs in cell spreading is not well understood.

Virus infection

Intriguingly, infection by some viruses seems to trigger blebbing in the viral target cells and inhibition of blebbing decreases infection rates[69]. The exact role of blebbing during virus uptake remains to be identified.

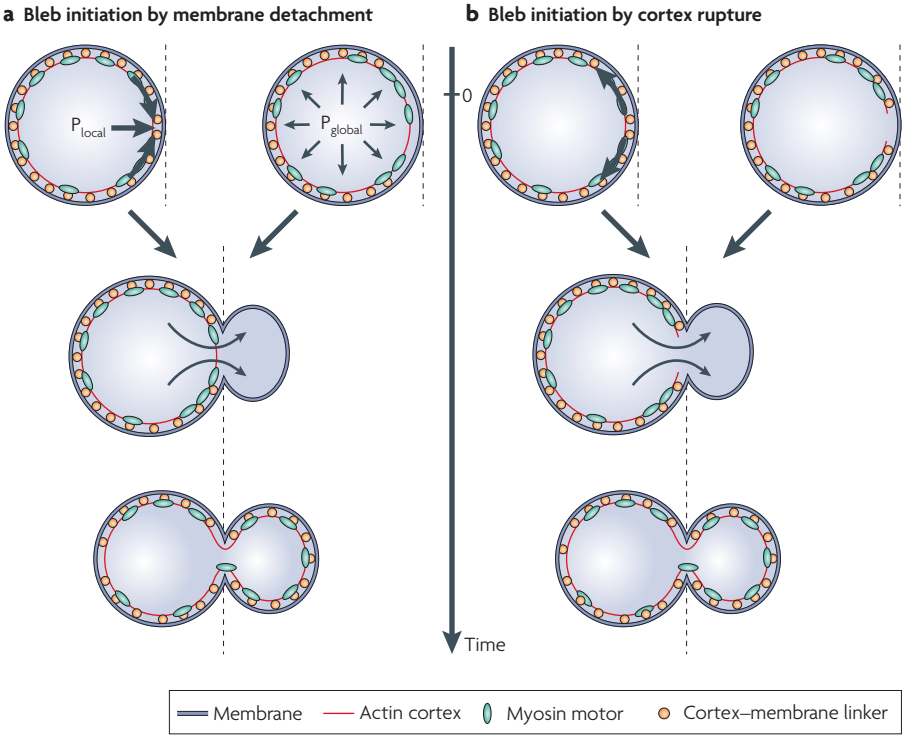

Figure 3 | **Generating polarized blebbing.** Polarized blebbing can be created either by local detachment of the membrane from the cytoskeleton or by local rupture of the actin cortex. **a** | A local contraction of the actomyosin cortex (top left panel, black arrows) can give rise to a local increase in pressure (P_{local}) when the cytoplasm is very dense. The increased pressure tears the membrane from the cortex, resulting in bleb initiation in that location. A similar result can be obtained with a global pressure (P_{global}) if cortex–membrane linkers are polarized to the rear of the cell (top right panel). A new actin cortex forms under the bleb membrane and the old cortex is disassembled, giving rise to a constriction in the cortex. The phenomenon can then reiterate. **b** | A high local stress in the actomyosin cortex (black arrows, top left panel) or a local disassembly in the cortex (top right panel) can tear the cortex. Cytoplasm flows out of the cell body through the crack, which leads to bleb expansion. A new actin cortex reforms and the phenomenon can reiterate. In both images, the dashed line shows the position of the leading edge before bleb initiation.

the distribution of the actin–membrane linker ERM (ezrin, radixin and moesin) proteins to the rear of the cell. This model is supported by observations in fixed Walker carcinosarcoma cells[46]. Alternatively, polarized blebbing could result from preferential tearing of the actin cortex at the leading edge (FIG. 3b), for example, in response to locally higher contractility close to the rupture point[34,47]. Contrary to the first model, no particular asymmetry in membrane–cortex adhesion is needed for preferential tearing at the leading edge. After membrane delamination, the old leading-edge contractile cortex is disassembled and a new cortex is then reassembled under the membrane of the growing bleb. In both models, the front of the cell is more prone to subsequent blebbing than the rear because the leading-edge cortex is always younger and more fragile. Hence, once symmetry is broken, polarization can readily be maintained.

In both models, an increased concentration of myosin at the leading edge can trigger bleb initiation (FIG. 3). Intriguingly, during blebbing motility, myosin motors localize to the front of some vertebrate cells[5,39,46] but to the rear of *A. proteus*[22]. ROCK1, a regulator of cell contractility, also localizes to the rear of motile blebbing tumour cells[10]. Pressure that is generated by cortical contractions is needed for bleb growth, so the observed differences in myosin distribution might reflect differences in cytoplasmic densities between cell types. Indeed, if cytoplasmic components (ribosomes, mitochondria, vesicles and the cytoskeleton) are tightly packed (resulting in an effective cytoplasmic mesh size of ~30nm), pressure equilibration over the whole cell is slower than the timescale of bleb growth, and active myosin motors need to be localized close to the leading edge, where the pressure increase will lead to bleb formation[48]. By contrast, if macromolecules are dilute, pressure equilibrates rapidly and active myosin motors can theoretically be localized anywhere on the cell cortex[49].

How is blebbing translated into movement? For blebbing to be translated into movement, cells need to exert a force on the ECM and translocate their mass. In lamellipodial motility, cell-body translocation is achieved by contraction of the cell rear, coupled to adhesion of the lamellipodium to the substrate[1]. As focal adhesions have not been investigated in blebbing cells, it is unknown how cell-body translocation is achieved during blebbing migration. It is clear that some form of interaction with the ECM is necessary for translocation, which could be achieved by weakly adhering to the substrate or the surrounding cells (FIG. 4c), or by applying forces on the extracellular environment perpendicular to the direction of movement (FIG. 4d,e).

In the first scenario, the cell forms weak adhesions with the substrate[50]. During bleb formation, adhesion proteins in the membrane would attach to the ECM and integrate with the assembling cortex. Then, similar to lamellipodial motility, the cell mass would move forward by contraction of the cell rear (uropod) and cell–ECM adhesions at the rear would detach[1]. Indeed, migrating blebbing Walker carcinosarcoma cells form loose contacts with the substrate[30]. In embryos, cells migrate over other cells and loose transient cell–cell contacts have been observed with a contact distance that is greater than that of adherens or tight junctions[15,51]. In PGCs, a decrease in the expression of the adhesion protein E-cadherin coincides with the onset of blebbing[52], which suggests that strong cell–cell adhesions might actually impede bleb-based migration.

The second scenario only applies when the cell is slightly compressed between two flat substrates or in a 3D network with a mesh size that is comparable to the size of the cell (FIG. 4d,e). No specific molecular adhesion to the substrate is required; the compressed cell exerts forces perpendicular to the top and bottom substrates (FIG. 4d) or ECM fibres (FIG. 4e), and squeezes itself forward — a mechanism that is known as chimneying[53]. The cycle starts with the nucleation of a bleb at the leading edge. The uropod contracts and simultaneously the bleb grows, until it makes multiple contacts with the substrate and wedges itself into place; the cycle can then resume. Such migration has been observed in a low adhesion subtype of Walker carcinosarcoma cells[30] and in neutrophils from patients

with leukocyte adhesion deficiency[53]; when plated on coverslips few cells migrate, whereas when cells are slightly squeezed between two substrates they migrate efficiently. Similarly, the migration of tumour cells with downregulated ECM adhesion proteins or matrix proteases, which push bleb-like protrusions through pores in the ECM gel[9,10], could result from non-adhesive mechanical interactions with the matrix coupled to cortical contraction (FIG. 4a,e). Further studies that focus on mechanical deformations of the ECM network will be needed to understand how the leading-edge bleb interacts with the environment.

Why do cells use blebbing motility?

Some cell types exclusively use one type of protrusion for motility (for example, lamellipodia in fish keratocytes[54] and blebs in zebrafish PGCs[5]), whereas other cells can switch between motility modes depending on their environment. What determines the type of protrusion a cell will form is not well understood. Some tumour cells preferentially migrate using pseudopodia (the mesenchymal migration mode), whereas others use blebbing motility (the amoeboid mode)[6,9]. Strikingly, protease inhibitors, which prevent mesenchymal motility, can induce a switch from mesenchymal to blebbing motility[9]. By forming blebs, the tumour cells can then squeeze themselves through pre-existing gaps in the matrix[25]. This plasticity in migration modes represents a putative escape mechanism following protease-inhibitor treatment of cancer dissemination. Neutrophils and lymphocytes can switch to blebbing migration when adhesion to the substrate is reduced[30,53]. Finally, in *D. discoideum* cells, osmolarity of the medium seems to modulate the mode of motility, with blebbing the dominant mode in low osmolarity media and lamellipodia the dominant mode in high osmolarity media[7]. Strikingly, blebs in *Fundulus* deep cells can flatten and spread as lamellipodia, possibly because of locally higher adhesion to the substrate[4]. Similar transitions also take place during cell spreading over a substrate[55].

How cells switch from one type of protrusion to another is unclear; cortical contractility and/or the strength of substrate adhesions might be key determinants. Because bleb formation depends on myosin contractility, lower myosin activity might favour the formation of lamellipodia rather than blebs. Indeed, myosin II-null *D. discoideum* cells form lamellipodia under conditions in which wild-type cells would use blebbing motility[7]. Conversely, factors that facilitate membrane detachment from the cortex should favour bleb formation, perhaps explaining why blebbing dominates during *D. discoideum* migration in low-osmolarity media[7]. However, strong adhesion to the substrate seems to promote lamellipodia formation[9,30]. Adhesions might promote actin polymerization, reduce cortical contractility, stabilize the actin cortex, or cause all three simultaneously. Studies on cells that can switch between modes of motility will be necessary to understand what regulates the type of protrusion that is formed.

Finally, given the scarcity of data available, we can only speculate as to the respective advantages of blebbing and lamellipodial motilities. Bleb expansion is faster than lamellipodial protrusion and can occur in any direction, and because blebs have no cortex they can easily adapt to the shape of the extracellular environment. Blebs might therefore be useful in complex 3D environments, such as ECM gels or living tissues (FIG. 4a,b,e), in which lamellipodia have no obvious surface to follow[9,10,16]. However, lamellipodia might allow for a precise and controlled sensing of the substrate. Focal adhesion formation can be activated by tension[56], which, when coupled to myosin contractions

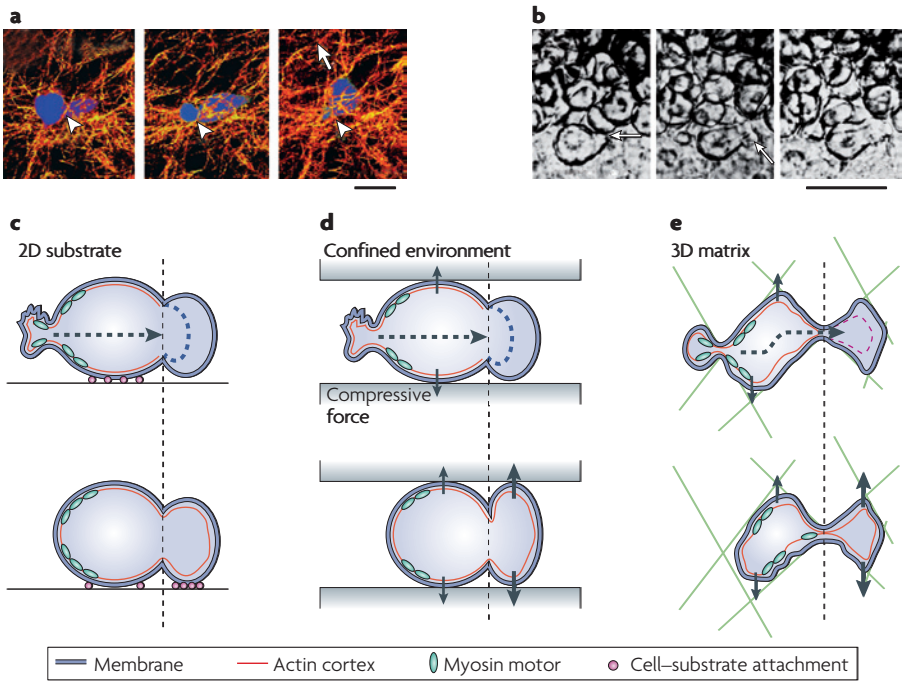

Figure 4 | **From blebbing to movement. a** | A tumour cell (blue) migrating through a collagen matrix (orange). Contraction of the uropod (arrowhead in all images) moves the cell body through the collagen mesh and, subsequently, a new protrusion is created (in the direction of the arrow, right image). Time between images ~7 minutes. Scale bar, 20 µm. Reproduced, with permission, from REF. 25 © (2003) Rockefeller University Press. **b** | Bleb migration of a deep cell in a mid-blastula fish embryo. The cells in the top part of the image are part of the periblast. A bleb can clearly be distinguished at the leading edge of the lowest cell (arrow, left image). This bleb broadens and possibly adheres to the periblast (middle image) before elongating into a lobopodium (right image). Time between images is 4 seconds. Scale bar, 50 µm. Reproduced, with permission, from REF. 13 © (1977) Blackwell Scientific Publications. **c** | In two-dimensional (2D) cultures, in order to translate polarized blebbing into movement, the cell must adhere to the substrate. When a new bleb is formed and comes in contact with the substrate, new cell–substrate adhesions are formed and the cell mass can stream forward. The pink dots indicate cell–substrate attachment points. **d** | When the cell is in a confined environment (for example, between two glass coverslips or in a thin microfluidic channel), it can move in the absence of cell–substrate adhesions. Instead, the cell exerts forces perpendicularly to the substrate and can squeeze itself forward; this mechanism is known as chimneying[53]. **e** | When the cell is migrating in an extracellular matrix (ECM) gel (three-dimensional (3D) matrix), it can move by a combination of the mechanisms described. The fluid nature of growing blebs enables the cell to squeeze through the ECM network mesh. The dashed line indicates the position of the leading edge before bleb nucleation, arrows indicate the forces that are exerted by the cells on the extracellular environment and dashed arrows indicate the streaming of cytoplasm.

Table 1 | **Proteins that have roles in cell blebbing**

Protein	Possible role	Observed in blebbing motility*	References
Bleb expansion			
RhoA	Triggers actin nucleation? Activates myosin contractility during retraction	–	10,43
ROCK1	Activates contractility	Yes	10,36,60
RhoE	Downregulates ROCK1	Yes	10
KIAA0861	Activates RhoA?	–	43
Ankyrin	Might form part of an erythrocytic cytoskeleton under the growing bleb membrane	–	43
Protein 4.1	Might form part of an erythrocytic cytoskeleton under the growing bleb membrane	–	43
Myosin I	Unknown	–	43
Src	Controls RhoA?	–	38
Cortex regrowth			
Ezrin	Tethers the actin cortex to the membrane	Yes	6,43,46
Moesin	Tethers the actin cortex to the membrane	–	43
Actin	Main component of the cortical network	Yes	5,19,33
α-Actinin	Bundles actin	Yes	29,43
Coronin	Bundles actin	–	43
Fimbrin	Bundles actin	–	43
Cortactin	Bundles actin	–	69
Tropomyosin	Controls cortical contractility?	–	43
Tropomodulin	Caps actin-filament pointed ends?	–	43
Myosin II	Contracts the cortex during bleb retraction	Yes	5,34,43,46
Anillin	Coordinates myosin II and actin contractility?	–	43
Myosin light chain kinase	Activates myosin contractility	Yes	5,59

*–, not investigated; ROCK1, Rho-kinase-1.

at the base of the lamellipodium, can mediate sensing of the underlying substrate rigidity[57], allowing cells to selectively migrate towards stiffer regions of the substrate (durotaxis)[58].

Blebbing motility might represent a simpler mode of locomotion than lamellipodial motility. Indeed, myosin-driven cortical contractility alone can lead to bleb nucleation and expansion[32,47]. By contrast, lamellipodial growth might require precise coordination of actin nucleation, polarized growth of a dendritic actin network, adhesion to ECM and, in some cases, matrix proteolysis[2,9]. Moreover, data on cell spreading suggest that bleb growth requires less energy than lamellipodium formation[55]. Taken together, these observations suggest that blebs (the formation of which only requires the presence of a contractile cortex), could be a more ancestral protrusion than the finely tuned and energetically costly lamellipodium.

Conclusions and future perspectives

Blebs are evolutionarily conserved cell protrusions that are commonly observed during cell spreading, cell division and apoptosis. A wealth of data from the 'pre-molecular' era and a growing number of recent observations demonstrate that blebs are a common alternative to lamellipodia during migration, from amoebae to vertebrates. Many embryonic cells use bleb-based migration during development and some cancer cells use blebbing motility as an escape migration mechanism in response to treatments that block matrix proteolysis. As tailoring anti-cancer treatments becomes the norm, understanding blebbing motility of tumour cells will be important to ensure successful chemotherapy.

Surprisingly, considering its widespread use, blebbing migration has been mostly ignored, perhaps owing to the frequent interpretation of blebs as a pathological sign. Moreover, blebbing motility usually occurs in 3D matrices or living tissues, making its study technically challenging. Along with the wider availability of the appropriate tools for high-resolution imaging in 3D environments, a number of cell lines have been identified that use blebbing for motility[5,6,9,30], providing model systems for molecular and physical studies of this migration mode.

Many key issues still need to be addressed. Even basic questions, such as how cells generate polarized blebbing and translate blebbing into movement, are not fully understood. More complex questions, such as the relative energetic efficiencies of blebbing rather than lamellipodial motility, why and how cells switch from one mode to the other, or whether and how Rho GTPases interact to give rise to polarized blebbing, merit further attention. Given its physiological and pathological importance, blebbing motility represents an exciting new avenue of research for the cell motility field that should no longer be overshadowed by its lamellipodial counterpart.

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DATABASES

UniProtKB: http://ca.expasy.org/sprot
diaphanous | ezrin | MLCK | moesin | myosin II | radixin | RhoA | ROCK | SDF1

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