Dynamic instability of the intracellular pressure drives bleb-based motility

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Summary

We have demonstrated that the two- and three-dimensional motility of the human pathogenic parasite Entamoeba histolytica (Eh) depends on sustained instability of the intracellular hydrostatic pressure. This instability drives the cyclic generation and healing of membrane blebs, with typical protrusion velocities of 10–20 μm/second over a few hundred milliseconds and healing times of 10 seconds. The use of a novel micro-electroporation method to control the intracellular pressure enabled us to develop a qualitative model with three parameters: the rate of the myosin-driven internal pressure increase; the critical disjunction stress of membrane–cytoskeleton bonds; and the turnover time of the F-actin cortex. Although blebs occur randomly in space and irregularly in time, they can be forced to occur with a defined periodicity in confined geometries, thus confirming our model. Given the highly efficient bleb-based motility of Eh in vitro and in vivo, Eh cells represent a unique model for studying the physical and biological aspects of amoeboid versus mesenchymal motility in two- and three-dimensional environments.

Key words: Blebs, Cytoskeleton, Motility

Introduction

On the basis of extensive investigations in different cell types and in various contexts, two distinct modes of cell motility (mesenchymal and amoeboid motility) have been proposed (Friedl and Wolf, 2003; Sahai, 2007). These modes differ in many respects: cell morphology, the organization and dynamics of cell–substrate adhesion, the localization and activity of the actomyosin contractile machinery, and the distribution of the forces exerted on two-dimensional (2D) and three-dimensional (3D) substrates (Bray, 2001; Mierke et al., 2008). It has also been reported that some cells can undergo a transition between these modes (Friedl and Wolf, 2003; Friedl, 2004), and that the motility mode strongly influences migration efficiency (Carragher et al., 2006). It is therefore likely that cells optimize their motility by selecting distinct strategies, probably as a function of the state of their molecular ‘machinery’ and of their environment. This optimization could be relevant for metastatic invasion, leukocyte migration in lymph nodes or target tissues, and cell movements during embryonic development, where cells successively encounter different 3D contexts (Hugues et al., 2004; Raz and Reichman-Fried, 2006). For instance, during immune surveillance and the inflammatory response, leukocytes move through environments such as blood, mucus, epithelium and the lymphatic circulatory system (Hugues et al., 2004). We studied Entamoeba histolytica (Eh) cells, the causative agent of amoebiasis (dysentery) (Stanley, 2003). This pathogen passes through various environments (intestinal tissue layers, the portal vein and the liver microcirculation) as it invades and ultimately destroys the host intestine and produces liver abscesses (Stanley, 2001). Given this context, the objective of the present work was to elucidate the physical basis of Eh cell motility and elaborate a model of amoeboid behavior, with special focus on the role of the internal hydrostatic pressure.

In previous work using in vivo two-photon imaging, we observed that Eh migration in the liver during the infectious process is accompanied by very intense, cyclic production of spherical protrusions of the plasma membrane (Coudrier et al., 2005). In vitro, these protrusions can either retract or become stable and thus sustain the exploration of the local environment by the cell over several hours (Coudrier et al., 2005). In the absence of externally guided cell motility, there is no correlation between the directions of the protrusions, and efficient, random exploration of the substrate is observed. However, appropriate chemotactic gradients do orient and stabilize protrusions in particular directions (Blazquez et al., 2006; Zaki et al., 2006). It is known that myosin II is essential for Eh motility, both in vitro (Arhets et al., 1998) and in vivo (Coudrier et al., 2005). In the present work, we demonstrated that the Eh cell protrusions are blebs (Keller et al., 2002; Fackler and Grosse, 2008; Charras and Paluch, 2008). By studying the mechanism underlying their production, we further showed that the blebs directly actuate cell motility in a physiological context. Our observations suggest that Eh cells could serve as a valuable prototype for studying amoeboid motility in general.

Results

Spherical protrusions produced in 3D, 2D and liquid environments

Infection by Eh requires efficient motility through environments that vary in terms of their geometrical, mechanical and biochemical properties. In an initial report (Coudrier et al., 2005), we observed a strong correlation between the efficiency of the infectious process (as measured experimentally in animal models), in vivo cell motility and the active generation of cell protrusions. Here, we show that these protrusions are spherical and thus very distinct from the
lamellipodia and filopodia involved in mesenchymal migration. Protrusion generation is similarly active in both 2D and 3D, as shown in the 3D liver parenchyma (supplementary material Movie 1) and on bare glass (supplementary material Movie 2). This suggests that the microenvironment in contact with the cell has a limited effect on protrusive activity. To further assess the role of cell–substrate contacts, amoebae were loaded at the interface between two fluids of differing density (a Percoll solution and culture medium). In this fluid environment, cells continue to actively produce round protrusions (supplementary material Movie 3). Similarly, when cells simply sediment through culture medium, active protrusions are still observed and persist once contact with the substrate is made (supplementary material Movie 4). These results clearly indicate that the protrusive activity is intrinsic to amoeba cells and is not induced by cell–substrate adhesion.

Relationship between motility and asymmetric and dynamic contacts
Because Eh cells do not have specialized organelles for moving in liquids (e.g. flagella or cilia), they can only achieve net motion relative to their environment by interacting with a solid substrate. Although no net motion is observed in floating or sedimenting cells (supplementary material Movies 3 and 4), the latter start to move as soon as they contact with the substrate, which suggests an ability to transfer momentum.

To further investigate cell–substrate contact and its role in protrusive activity, we combined conventional phase-contrast imaging with the observation of adhesion patterns using reflection interference contrast microscopy (RICM) (Fig. 1; supplementary material Movie 5 and Fig. S3). Strikingly, the shape of Eh cells is quite distinct from their contacting surface; this is in contrast to fibroblasts and most adherent cells, for which adhesion strongly dictates the cell profile. Despite extended regions of tight contact (revealed by continuous zones of dark RICM signal), the production of protrusions leads to discontinuous contact zones away from the tight contact area (Fig. 1B). This observation indicates that protrusions are produced as elevated structures that can contact the substrate at a remote site. This is in agreement with observations of 3D protrusions in a liquid environment (supplementary material Movie 3) and with previous electron microscopy pictures (Gonzalez-Robles and Martinez-Palomo, 1983) showing that protrusions are triggered all around the cell surface (which has a typical size of 20 μm in all three dimensions).

The initial, discontinuous contacts then either nucleate and grow into larger surfaces (which eventually bridge with the main zone (supplementary material Fig. S3) or disappear if the protrusions retracted. Hence, protrusion dynamics is coupled to dynamic spreading of adhesive contacts. The cell boundary seen in phase-contrast microscopy always extends beyond the dark RICM zone, indicating an ‘overhang’ type of geometry (sketched in Fig. 1D). Interestingly, this overhang is much greater in protruding regions than in retracting regions. This clear asymmetry can be interpreted as a contact angle difference, which is probably due to the plasma membrane and the associated cortex being peeled away in retracting zones.

For further investigation of the protrusion mechanism of Eh cells, it is important to note that cell–glass adhesion forces (which could in principle lead to an extensive contact area and a rather flat morphology) are dominated by the contraction-induced and elastic forces inside the cortex. These forces probably drive cell deformation in 2D and in 3D, as mentioned above. Nevertheless, the adhesion forces are strong enough to produce effective contacts and the momentum transfer required for the observed motility. In principle, the adhesive interactions are not specific on the molecular level, despite the surface expression of specific adhesion receptors (Coudrier et al., 2005). Indeed, similar motile behaviors have been observed on substrates with greater or lower hydrophobicity than bare glass (supplementary material Figs S1 and S2). These observations indicate that excessive adhesion could inhibit the production of Eh cell protrusions, as reported elsewhere (Friedl et al., 2001).

Plasma membrane abruptly detaches from the cytoskeleton
We next focused on the physical mechanism underlying protrusive activity by Eh cells moving on a glass surface. The results presented in the preceding section indicated that adhesion does not directly interfere with the intracellular processes that lead to protrusion production, which can be considered as operating independently of contact with the substrate. Phase-contrast video microscopy observations revealed deformations of the cell boundary on two distinct time scales: slow (10–30 seconds) overall changes (supplementary material Movie 6) and fast (0.1–5 seconds) local changes (supplementary material Movie 7). The slow, overall deformations exhibit a constant phase-contrast pattern, suggesting a stationary cell cortex structure. By contrast, fast deformations are protrusions that correspond locally to major changes in the phase-contrast signal; this indicates that the cortical structure is strongly modified. Interestingly, myosin inhibition produces conditions under which fast deformations are blocked and slow deformations...
velocities measured here. Furthermore, the observed velocities are similar to other ‘explosive’ processes that are not powered by polymerization, such as the Thysone acrosomal process (Tilney and Inoue, 1985). Our observations strongly suggest that Eh cell protrusions are bona fide blebs, as indicated by their sphericity, the absence of submembrane structure and the hyaline-like appearance of the inner face. Protrusion production via membrane disjunction is analogous to the formation of apoptotic blebs by proteolytic cleavage of cytoskeleton–membrane links (Mills et al., 1998; Mills et al., 1999). Unlike necrotic blebs, Eh blebs are highly dynamic in nature; rapid expansion is followed by cytosol invasion and gradual association between the membrane and a new cortex. These events occur in a cyclic fashion.

On the molecular level, the actin cortex (seen in parallel time-lapse videos of phase contrast and F-actin fluorescence; Fig. 3) shows fast disjunction, with no change in the fluorescence of the initial structure. However, 4 seconds later, the fluorescence decays and the rim of the bleb becomes fluorescent (indicating the accumulation of F-actin).

The time courses of F-actin dynamics along the initial cortex and along the bleb rim (Fig. 4A) are shown as a series of kymographs (Fig. 4B,C) and are integrated over time (Fig. 4D,E). These semiquantitative data indicate that the typical times for depolymerization and distal repolymerization are similar (on the order of 5–10 seconds). This is in agreement with the notion that the F-actin cortex turns over constantly, with the balance between depolymerization and distal repolymerization are similar (on the order of 5–10 seconds).

The preceding observations are schematically summarized in Fig. 5A. Blebs drive the net motion of the cells via three schematic transitions occurring on different time-scales. From the initial state (a), the plasma membrane detaches from the cytoskeleton (typically within 0.1 seconds). From the second state (b), the cytoskeleton gradually depolymerizes and repolymerizes under the bleb membrane (c). Within 5–10 seconds, the ‘old’ cortex has vanished.
and the new cortex has fully matured (d). In this situation, contraction forces can deform the cell and eventually lead to a new disjunction.

Analysis of forces and proposal of a cyclic model
Most investigations of cell motility have focused on situations in which actin polymerization has a dominant role. In these situations the membrane has an ancillary role, which mainly consists of templating actin polymerization while remaining attached to the cortex. Here, given the disjunction, one must reconsider the forces exerted on the linkers by the membrane and the cytoskeleton (Fig. 5B) and their relationship with contractile activity.

The plasma membrane is subjected (Fig. 5B, blue arrows) to hydrostatic pressure from the outside fluid ($\pi_{\text{ext}}$) and the internal medium ($\pi_{\text{int}}$). It is also subjected to forces exerted by individual links, the surface density of which yields an effective pressure ($\pi_{\text{links}}$). Given the mechanical equilibrium of the links (i.e. as long as they hold firm), this pressure also equals the contractile pressure exerted upon the links by the cortex ($\pi_c$). The latter is produced by cortical contractile tension ($\gamma_c$) and curvature.

After membrane disjunction (Fig. 5B'), a different set of forces must be considered. The linker pressure ($\pi_{\text{links}}$) on the membrane and its opposing cytoskeletal counterpart disappear. This change corresponds to effective outward pressure of the same magnitude on the membrane, together with effective inward pressure on the cytoskeleton. We also consider that the outward hydrodynamic flux through the cortex leads to viscous resistance (corresponding to outward pressure on the cortex, $\pi_{\text{hydro}}$). Membrane disjunction implies the local disruption of a large number of non-covalent bonds (a few square micrometers), characterized at the individual level by finite on/off rates. When stressed, the off rate increases exponentially with the stress (Bell, 1978) and an ‘avalanche effect’ is expected above a critical pressure $\pi^*_{\text{links}}$ which will directly depend on the density and binding energy of the bond.

As discussed below, variations in the osmotic pressure on the membrane have been neglected because no water influx is observed during protrusion production and resorption. In principle, two additional forces should be considered. First, the membrane is subjected to surface tension that could (along with curvature) slow down or stop bleb expansion. This was suggested by our observation that blebs often exhibit larger front velocities in concave regions (data not shown). This aspect has been considered in a theoretical publication (Brugues et al., 2010) but cannot be easily taken into account in the experiments presented herein. Second, cell–substrate contact generates surface forces at the membrane and primarily prevents local bleb formation. These forces do not impede blebbing in contact-free zones.

On the basis of the above mechanical analysis, we propose a cyclic model (Fig. 5C) in which cortical contraction builds up the inside pressure difference across the plasma membrane to the point where it exceeds the critical disjunction pressure ($\pi^*_{\text{links}}$) that the linker distribution can withstand. When the membrane detaches

![Image](image-url)
Concomitantly, the excess internal pressure \( \Delta \pi \) is hydrostatic, and the effective inward pressure is the hydrostatic outside expansion. (a) The distribution of forces exerted on the cytoskeleton, the plasma membrane and molecular linkers. These forces are mainly: the hydrostatic outside \( \pi_{\text{ext}} \) and inside \( \pi_{\text{int}} \) pressures, the membrane tension \( \gamma_{m} \), the cortical tension \( \gamma_{c} \) resulting in pressure \( \pi_{c} \), and the forces exerted on and transmitted by individual linkers, resulting in pressure \( \pi_{\text{link}} \). Arrows indicate the direction in which the forces act. (B) Forces exerted during membrane disjunction. The linker pressure \( \pi_{\text{link}} \) on the membrane and its opposing cytoskeletal counterpart disappear. Dashed blue arrow indicates effective outward pressure and dashed red arrow the effective inward pressure. \( \Delta \pi_{\text{hydro}} \) is the outward pressure on the cortex. (C) The cyclic change in the inside–outside pressure difference over time. Letters a–e correspond to the states shown in A.

**Respective roles of pressure effects and contraction**

We first determined to what extent bleb formation was sensitive to external perturbations of the inside–outside pressure difference. Using micropipette aspiration (supplementary material Movie 9), we observed that the membrane region exposed to low external pressure (~500 Pa) exhibited active blebbing, whereas the opposite end of the cell stopped making blebs. This strongly suggests that the inside–outside pressure difference is a key parameter in triggering membrane disjunction. In agreement with several previous reports (Arhets et al., 1998; Paluch et al., 2005; Charras et al., 2005), the inhibition of actomyosin contraction with ML-7 and Y27632 was found to block bleb formation (supplementary material Fig. S4B,D). To elucidate the respective roles of contraction and the pressure increase (both of which appear to be required for blebbing), we designed an experiment in which the inside–outside pressure difference was cancelled while the contractile machinery remained intact.

Electroporation is known to transiently permeate the cell membrane and can therefore be used in conjunction with a micropipette to cancel the hydrostatic pressure difference at will on the subcellular scale and with good temporal resolution. Eth cells were held in tight contact with the micropipette tip. The electrical resistance was in the order of 25–30 MΩ. In this situation, the cells produced blebs that immediately disappeared at the onset of electrical pulse trains (see supplementary material Movie 10). As long as pulses were maintained, the cells shrank (Fig. 6 and supplementary material Fig. S5). As soon as the pulses ceased, blebbing resumed within a few seconds. In addition, electroporation-induced shrinkage was absent when either of two actomyosin inhibitors (ML-7 and Y27632) were added to the medium (see supplementary material Movie 11). Taken together, these results indicate that the contraction machinery is not affected by electroporation but cannot alone induce blebs when the hydrostatic pressure difference is cancelled. Meanwhile, the fact that a normally membrane-impermeant dye (propidium iodide) penetrated into the cell suggests that pores are formed. Interestingly, dye influx systematically occurred through a single permeation point (see supplementary material Movie 12) located outside of the micropipette. Indeed, the effect of electroporation, as revealed by propidium iodide experiments (see supplementary material Movie 12), is to create pores at a single point, through which endoplasmic material immediately leaks out and through which the dye enters a few seconds later, before it equilibrates throughout the cell (supplementary material Fig. 12). The effect of pores is to cancel, at least locally, the contraction-induced pressure difference. The fact that the electroporation-induced local pressure relaxation is immediately followed by a global effect on bleb inhibition strongly suggests that membrane disruption and blebbing result from a direct pressure on the membrane and from linker rupture, and also that the local pressure drop immediately propagates throughout the cell.

**Blebbing can be forced into a periodic mode**

Assuming that the rate of contraction and linker strength and density are constant and uniform, our model predicts that blebs
will be produced periodically. In cell-on-glass, blebs occurred one by one. There was neither time overlap (supplementary material Fig. S6) nor a well-defined period. Indeed, the blebs were heterogeneous in terms of duration and size (supplementary material Fig. S7) and the fluctuations over time were so large that no statistically meaningful period could be distinguished. According to our model, this temporal irregularity suggests that the control parameters are not constant over time and/or are not uniform in space. The simplest explanation is that the local pressure on the linkers (πc) depends on the local membrane curvature, i.e. πc≈2R k/R (Fig. 5B). Curvature is indeed strongly non-uniform in Eh cells (Fig. 2). Interestingly, when the cell is forced to adopt a more regular shape in micropipette aspiration experiments, a clear periodicity appears (Fig. 7). The period (8±2 seconds, n=46 blebs) matches that of the polymerization/depolymerization measured above. This clearly shows that blebbing frequency fluctuations are dominated by geometric effects and that the control parameters are constant. Our micropipette experiments have been extensively described in a detailed theoretical analysis of various dynamic modes for cortical instability (Brugue et al., 2010).

Discussion

Bleb formation is observed in apoptotic cells (Mills et al., 1998; Sebbagh et al., 2005; Leverrier and Ridley, 2001; Sebbagh et al., 2001), migrating cells (Blaser et al., 2006; Yoshida and Soldati, 2006), non-migrating cells (Keller and Eggl, 1998; Gutjahr et al., 2005; Charras et al., 2005; Paluch et al., 2005) and in cells before they fully adhere to a flat substrate (Norman et al., 2010). Nevertheless, the only previously reported situation in which motility is entirely driven by blebs is primordial germ cell (PGC) migration in zebrafish (Blaser et al., 2006; Raz and Reichman-Fried, 2006). The key message of the present work is that Eh cell migration (as with PGCs) is solely driven by blebbing, at least under the circumstances investigated here. However, unlike zebrafish PGCs (which are immobile in vitro), Eh cells exhibit bleb-based motility both in vitro and in vivo and therefore appear to constitute a unique model for investigating the mechanism of bleb formation and its relationship with motility in a relatively normal physiological context. We took advantage of this unique feature and used RICM to qualitatively elucidate the relationship between bleb formation and retraction, cell-substrate contact dynamics and net cell motion.

The present data and our previous observations on in vivo infection-related Eh cell motility (Coudrier et al., 2005) together strongly suggest that blebbing-based cell motion is efficient compared to the well-known mesenchymal migration mode. We observed extremely high bleb front velocities: up to two orders of magnitude higher than the average speed for mesenchymal cells (Bray, 2001). Nevertheless, these velocities are transient and do not directly reflect average motility speed. Given that the Eh cell blebs are produce isotropically and lead to random motion (supplementary material Movie 13), the efficiency of motility is better reflected by the mean square displacement (supplementary material Fig. S8), which is typically in the order of 0.1–10 μm²/second. One of the most remarkable features of Eh bleb-based motility is the very high blebbing frequency, which makes it possible for the cell to change direction several times per minute.

Comments on the blebbing model

In the initial state in the cycle (Fig. 5Aa), the cell is in a static situation in which the increasing stress does not translate into significant deformation. The internal pressure is uniform and one would expect to have a uniform distribution of the disjunction probability if the linker distribution and curvature are uniform. However, it should be borne in mind that linkers at the membrane cytoskeleton interface can be very dynamic (Coscoy et al., 2002); hence, density fluctuations can trigger local changes in the disruption pressure πiink. In a few reports, collapse of the cortex is seen as the first event. However, this situation has only been seen
in non-physiological situations (Keller and Eggli, 1998; Paluch et al., 2005).

In our present model, we decided to neglect the possible role of osmotic forces in membrane disruption and swelling. Osmotic forces have indeed been proposed to account for membrane swelling (Oster and Perelson, 1987) due to an outside–inside water flux. In the present situation, no such inward flux is seen. Instead, the swelling is accompanied throughout the whole cell by a small but detectable flow that is directed towards and then fills the bleb (supplementary material Fig. S9). Incidentally, this observation provides a strong indication that the local hydrostatic pressure drop induced at the time of membrane disjunction propagates immediately through the whole cell. This propagation uniformly relieves the pressure on the links and inhibits the formation of new blebs elsewhere. This hypothesis is in agreement with our observation (supplementary material Fig. S6) that (in general) new blebs only appear once the previous one has ‘matured’ back to the starting point. This mutual bleb exclusion [which has also been seen in zebrafish PGCs (Blaser et al., 2006)] might account for the relatively high observed diffusion coefficient.

Our data strongly suggest that contractility exerts overall control over the internal hydrostatic pressure and that this pressure remains uniform. As a consequence, a local increase in contractile activity alone is not likely to generate a sustained local pressure increase and thus trigger local bleb formation. This contradicts the interpretation of experiments in which bleb formation could be suppressed (Charras et al., 2005) by the local inhibition of contractility. These observations prompted Charras and coworkers to hypothesize that the cytoskeleton can display poroelastic behavior in which the hydraulic resistance and elasticity of the cytoskeleton results in relatively long propagation times for local pressure variations (10 seconds across the cell). This is obviously not the case with free cells, because local relaxation of the inside–outside pressure difference immediately cancelled blebbing throughout the cell, which suggests that a local hydrostatic pressure jump was relieved almost instantaneously. Furthermore, we observed that the cortex clearly moves inward upon blebbing (supplementary material Fig. S10). This recoil can be accounted for by the interplay of two antagonist pressures occurring during the disjunction: the loss of the pressure \( p_{\text{link}} \) exerted on the cytoskeleton, and the outward-directed pressure \( p_{\text{hydro}} \) due to the hydrodynamic resistance caused by the outward flow. Hence, the recoil of the cortex indicates that the linker pressure exceeds the viscous resistance and that poroelastic effects at the cytoskeleton–membrane junction can therefore be neglected. In addition, the observation that blebbing is immediately followed by a small but detectable granular flow throughout the whole cell (supplementary material Fig. S9) is also a strong indication that the local hydrostatic pressure drop induced by blebbing propagates almost instantaneously through the whole cell.

Our finding that myosin activity drives bleb formation in a non-local way via the overall internal hydrostatic pressure should also be considered in light of a report showing that blebs in zebrafish PGCs colocalize with hot spots of a calcium ionophore protein and thus coincide with local calcium increases and the accompanying contraction (Blaser et al., 2006). In these experiments, contraction was necessary to produce blebs but did not necessarily dictate their location. These blebs might result from a local reduction in linker density triggered by either calcium pulses or contraction-induced cortex heterogeneities. These previous observations are consistent with our hypothesis of a uniform hydrostatic pressure, but more detailed experiments are needed to distinguish between the possible mechanisms controlling bleb location.

### Biochemical control of the dynamic instability

Our model is founded on three key parameters: (1) the rate at which pressure (due to actomyosin contraction and curvature) builds up, (2) the critical disjunction pressure (resisted by membrane–cytoskeleton linkers) and (3) the turnover rate of the actin cortex. The rate of pressure build-up depends on both the level of mechano-enzymatic activity and the cortex density. Therefore, in the presence of a structurally stable cortex, the time needed to reach the disjunction pressure is expected to vary inversely with the reciprocal of the contraction rate. However, the actin cortex is far from being a static structure (Pantaloni et al., 2001; Pollard and Borisy, 2003) and its turnover will certainly interfere with the pressure build-up. Indeed, each time a microfilament is lost through turnover, the accumulated stress disappears and is subtracted from the network stress. One therefore expects the tensile stress to reach a steady-state level that is modulated by the contraction rate and the actin turnover rate. This steady state will not be reached if disjunction occurs faster than stress saturation. In this context, the kinetic competition between contraction and polymerization rates (along with the critical disjunction pressure) should lead to different dynamic modes, with or without disjunction (supplementary material Fig. S11). Although a quantitative, theoretical investigation of these dynamic modes has been carried out (Brugues et al., 2010), the qualitative model developed here should help us to understand how blebbing is biochemically controlled.

### Efficiency of motility and external mechanical control

Our RICM experiments showed that \( Eh \) cells even adhere to a bare glass slide, probably due to the relatively nonspecific nature of the molecular adhesive machinery of the parasite. If blebs are to produce motion in a viscoelastic environment, the sum of the net momentum successively transmitted to the substrate (first during bleb emission and then during the maturation/contraction steps) must be non-zero. During bleb emission, the opposing forces exerted on the bleb arise from either viscous drag in the liquid phase or from friction between the membrane and the substrate. Both types of forces are effectively counteracted by adhesion of the rest of the cell to the substrate, as suggested by the fact that the centre of mass does move forward during that phase. As the bleb matures, cortex polymerization and the formation of links with adhesion receptors result in a rather uniform solid-like friction. Upon further contraction, the centre of mass is therefore not expected to move. Qualitatively, the liquid-like structure of the bleb breaks the symmetry of the friction forces in space and time and thus powers net motion.

In addition to chemical signals, external mechanical factors could also direct bleb-based motion by restricting bleb emission in directions where blebs are not limited by contact pressure, such as the holes in the extracellular microenvironment. In principle, any factor that interferes with contraction-induced strain (such as adhesion to a rigid substrate) could limit the internal pressure build-up and thus inhibit bleb-based motility.

We hereby propose that the use of bleb-driven motility depends on the balance between contractile activity and the elastic compliance of the microenvironment (providing cell adhesion is strong enough to couple these forces). In addition to intracellular controls on motility (Lammermann and Sixt, 2009), we suggest that cells can be prompted to switch between bleb-driven and mesenchymal motility modes by changes in the surrounding milieu (namely the adhesiveness, geometry and elastic compliance).
is an attractive concept that should help us to understand how cells optimize migration strategies in various 'soft' and 'stiff' microenvironments and enhance our knowledge of cellular behavior during embryogenesis, parasite infection, tumorigenesis and immune cell migration (Hugues et al., 2004; Coudrier et al., 2005; Raz and Reichman-Fried, 2006; Beadle et al., 2008).

Materials and Methods

Preparation of Entamoeba histolytica cells and drug treatments

E. histolytica HM1-IMSS (Diamond, 1961) wild-type strain was grown and resuspended to 5 × 10⁶ cells/ml. Attached trophozoites of pathogenic Entamoeba histolytica were used for checking the membrane integrity during experiments, Vincent Semetey together with Pia Streicher for helping the LifeAct construct, Sylvie Syan for the parasite culture, Isabel Llanco and Jeremie Barral for their help during electroporation experiments, Vincent Semetey together with Pia Streicher for helping with surface treatments, and Gil Toombes for critical reading of the manuscript. B.M. and F.A. are members of GdR CellTiss of the CNRS. B.M. was supported by a doctoral fellowship from Region Ile-de-France. This work was supported by grants from the ANR (to N.G. and F.A., to P.N. and P.S.), the Institut Curie and the Region Ile-de-France.

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