The cell ratchet: Interplay between efficient protrusions and adhesion determines cell motion

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Many physiological and pathological processes involve directed cell motion. In general, migrating cells are represented with a polarized morphology with extending and retracting protrusions at the leading edge. However, cell motion is a more complex phenomenon. Cells show heterogeneous morphologies and high protrusive dynamics is not always related to cell shape. This prevents the quantitative prediction of cell motion and the identification of cellular mechanisms setting directionality. Here we discuss the importance of protrusion fluctuations in directed cell motion. We show how their spatiotemporal distribution and dynamics determine the fluctuations and directions of cell motion for NIH3T3 fibroblasts plated on micro-patterned adhesive ratchets.¹ We introduce efficient protrusions and direction index which capture short-term cell motility over hours: these new read-outs allow the prediction of parameters characteristic for the long-term motion of cells over days. The results may have important implications for the study of biological phenomena where directed cell migration is involved, in morphogenesis and in cancer.

Introduction

Cell migration is involved in several physiological and pathological processes, including tissue development or cancer metastasis.² ³ ⁴ Directed cell migration is typically described by the presence of chemoattractants, both in vivo and in vitro, associated to the activation or inhibition of sets of genes, and leading to the cytoskeleton reorganization and polarization.⁵ This implies a functional asymmetry between the front and back sides generating preferential locations for cellular protrusion activity (lamellipodia and filopodia) to set the future direction of migration.⁶ Some works have identified lamellipodia and filopodia as key players in the machinery of cell motion.⁷ ⁸ These protrusions are precursors for the establishment of focal contacts eventually acting as mechanosensors for cell motion.⁹ On uniformly coated in vitro surfaces, protrusions stochastically fluctuate around the cell edge exploring cell microenvironment seeking for a docking site where to adhere, stabilize and apply forces. In order to migrate, these traction forces are unbalanced and eventually lead to cell polarization. This change in cell shape is also related to the switch from random to directional motility. In fact, we recently demonstrated in vitro that the typical random-like motion of cells on 2-dimensional (2D) surfaces could be transformed (rectified) into directional motion by means of micro-patterned asymmetric adhesive patches (ratchet).¹ The interplay between protrusions dynamics and adhesion regions determined the final direction of cell motion, while taking into account stochasticity in directions.

In this Commentary, we discuss the important role of protrusion fluctuations in the physico-chemical mechanism of directed cell motion. We introduce the new tools and concepts developed to describe cell motion, such as efficient...
protrusions and the associated direction index $D_{\text{dir}}$. We highlight the key role of asymmetric protrusion fluctuations in guiding cell motion and how perturbation of these fluctuations using inhibitors of the Rho/Rac pathways and microtubule polymerization affects cell migration. Within this framework, we developed a theoretical description based on the persistent random walk model and found a clear correlation between short- and long-term cell motion. This demonstrates that efficient protrusions quantification allows to predict parameters characteristic of long-term cell motion.

Protrusions fluctuations as regulators of directionality

Cells plated on uniform 2D microenvironments are characterized by a random distribution of protrusions which grow and shrink stochastically around the cell periphery (see Movie 1). When symmetry is broken, cells start to migrate. Typically, these cells are depicted as triangular (polarized) cells moving straight with a wide front edge and a thin tail at the back (see Fig. 1A). In this context, cell polarity generates preferential locations at the front for protrusions fluctuations and force. Generation in a tug-of-war mechanism, setting the future direction of cell motion $(D_{\text{cell}})$. The lifetime of this ‘unbalance’ sets the duration of the migration path, typically 1–2 cell unit sizes before cells switch direction and re-start this cycle.

However, this simplified vision of the phenomena holds true only in some cases. In others, cells show complex morphologies (see Fig. 1B and Movie 1), and the direction of migration is unpredictable using these standard rules. Cells probing in one side and moving in unexpected directions, compared to the one expected by the initial morphological polarity $(D_{\text{cell0}}, \text{dir0})$, can be frequently observed (see Fig. 1C). Therefore, spatial distribution of protrusions is not sufficient for predicting all parameters characteristic of cell migration, such as directionality, persistence length $L_p$ and time $T_p$ (length and time during which a cell migrates directionally) and associated speed $v$ (see Fig. 1B). Spatio-temporal distribution and dynamics of protrusions must be also investigated. These observations suggest that: (i) morphological polarity cannot be considered as the unique indicator of directionality, but other mechanisms must be also taken into consideration; (ii) protrusion fluctuations are one of the main regulators in directing cell motion; and (iii) the classical concept of protrusion needs to be extended to efficient protrusion, a cellular structure with probing and adhering capabilities leading to local force applications through focal contacts.

In his famous ratchet and pawl chapter, Richard Feynman showed that random motion driven by fluctuations could be transformed into directed motion by breaking the spatial symmetry of the system (see Fig. 2A). If protrusions are the main sources of fluctuations during cell motility, one should be able to bias the direction of motion by modulating a spatially asymmetric protrusions; this will eventually promote directed cell motion (see Fig. 2B). With this purpose, we designed a “ratchet”-based set-up consisting of a sequence of micro-patterned fibronectin (FN) adhesive patches spaced by gaps (see Fig. 2B and Fig. 3B). Non-patterned areas were passivated with a cell repellent compound – PLL-g-PEG – to prevent cell adhesion away from the ratchet. We plated NIH3T3 cells at low density (to minimize cell-cell interaction) on the adhesive patches; cells adhered on patterns adopting the geometry set by the FN motifs. In comparison to 2D scenario, where protrusions extended (and retracted) stochastically around the cell edge (see Fig. 3A), cells on ratchets mainly extended (fluctuating) protrusions toward the neighboring motifs, named for clarity + and − directions (see Fig. 2B ii and Fig. 3). In this scenario, protrusions could only adhere on the adhesive patches - efficient - whereas protrusions extending on the passivated regions could not adhere (non efficient). The area of the motifs was adjusted to the mean ‘resting’ area of cells plated on 2D surfaces covered with the same FN concentration.

![Figure 1. NIH3T3 fibroblast moving on a uniform 2D surface. (A) Scheme showing the traditional view of a triangular cell moving on a 2D surface. (B) Time-lapse projection showing a NIH3T3 fibroblast migrating in a sequence of straight displacements on a homogenously-coated fibronectin surface (10 µg ml⁻¹) for about 40 h (see Ref.1 for details in materials and methods). The cell shows complex morphologies during migration. $L_p$, $T_p$, and $v$ represent the persistence length, time and cell speed on the linear migration path ($v_i$ = 1, 2 in this example). (C) Images showing 2 different time points (start and end) of a cell migrating (upper) parallel $D_{\text{Polarity}}$ and (lower) perpendicular $(D_{\text{Polarity}} \perp D_{\text{Cell}})$ to the direction expected by its initial morphological polarity; cells are outlined for clarity. Scale bar: 50 µm.](image-url)
This ratchet-like configuration resulted in a clear unbalance in protrusion dynamics and spatio-temporal distribution, and as a consequence, in a tug-of-war force distribution of protrusions. Protrusions extended a similar distance ($d_p$) toward the C and – direction ($d_{pC}$ and $d_{p-}$). Taken together with the asymmetric morphology of the adhesive FN patches, the available adhesive area was therefore different in both directions (see Fig. 3B). This asymmetry induced cell migration in majority, and strikingly, toward the C direction (first-step motion) (see Fig. 2B iii and Fig. 3B). It is worth noting that this direction is the opposite to the expected direction set by the cell shape polarity.

We next identified the mechanism involved in this biased migration. Dynamics of efficient protrusions were characterized by 2 measurable parameters: the frequency of probing $v$, and stabilization time $\tau$. They are defined as the number (per unit of time) and the adhesive lifetime of efficient protrusions, respectively, on both sides (– and +) of the ratchet-like patches. We found that even though $v_+ > v_-$, $\tau_- < \tau_+$, and cells, as mentioned above, migrated mostly toward the + direction (see Fig. 3B, lower). This suggests that the combinatory effect of both parameters was strongly correlated with the direction of migration. In order to prove this hypothesis, both parameters were incorporated in a dimensionless parameter defined as the direction index $I_{dir}$ (see Eq. 1) which encodes the asymmetry of efficient protrusions dynamics. Note that $-1 < I_{dir} < 1$, and the sign of the resulting value indicates the direction of an elementary step motion ($-$ or $+$).

$$I_{dir} = \frac{v_+ \tau_+ - v_- \tau_-}{v_+ \tau_+ + v_- \tau_-}$$

$I_{dir}$ describes the probability $p_\pm$ of...
migration toward the + or − direction, respectively. This probability is set by $I_{dir} = p_+ - p_-$ with the normalization factor $p_+ + p_- = 1$. The predicted probability is therefore:

$$p_\pm = \frac{1 \pm I_{dir}}{2} \quad (2)$$

Experimental and predicted values of the bias (defined as the % of cells with a resulting net motion toward the + direction over the cells migrating to the − direction) given by $p_{\pm}$ matched accurately. This result highlighted the predictive power of $I_{dir}$ and confirmed the correlation between $\nu$ and $\tau$ with the actual direction of migration.

In order to give further evidence for the predictive power of $I_{dir}$, the geometry of the neighboring motifs was modified by symmetric rectangles (Rectangle-Triangle-Rectangle, R-T-R), resulting in a perturbation of the efficient protrusions activity ($\nu$ and $\tau$) (see Fig. 3C). Cells had more adhesive area available, in particular on the − side, and cells migrated mainly toward this direction, as predicted by $I_{dir}$. Note finally that if spatial and temporal symmetry of the system was not broken (Rectangle-Spot-Rectangle, R-S-R), efficient protrusion fluctuations were similar on both sides. Cells had equal chances to migrate either + or − (see Fig. 3D and movies S1–S3 in ref.1) (again, confirmed by $I_{dir}$) showing that an asymmetric protrusion landscape is necessary to induce biased migration. Altogether, we show that the direction of an elementary step is described by the asymmetry on the dynamics of efficient protrusions and its probability is quantified in $I_{dir}$. In this study, we used the values of $\nu$ and $\tau$ to quantify force asymmetry of efficient protrusions but we did not actually measure it. Even though we cannot establish in which cases force is established, we assumed that a force was applied whenever a protrusion is stabilized based on the fact that the focal contacts mediate a traction force. Future improvements to this work will require the use of traction force microscopy experiments in order to confirm this assumption.

**Perturbing $\nu$ and $\tau$ spectrum: inhibition of the Rho-GTPases**

A variation in the geometry of the neighboring motifs resulted in perturbations of efficient protrusions activity and, consequently, this change had an impact in cell migration. We next studied the effect of directly perturbing protrusions activity in cells using drugs, while keeping the same micro-pattern ratchet geometry.

The Rho-family of GTPases (Rho-GTPases) plays an important role in multiple aspects of cell behavior, in particular cell adhesion and migration. They participate in cell directionality by regulating acto-myosin dynamics. Among Rho-GTPases, Rho and Rac have been shown to have crucial roles in cellular processes, including acto-myosin dynamics, actin polymerization, and protrusion formation, polarization, motility or adhesion. Schematically, Rac is established to be involved in the regulation of lamellipodia and Rho regulates stress fibers dynamics and the formation (and maturation) of focal adhesions. Altogether, this suggests that, if cell directionality is dictated by protrusion dynamics, which are regulated by the Rho GTPases, perturbing their activity could have an impact on cell motion. In fact, inhibition of the Rho and Rac pathways (named here Rho$^-$ and Rac$^-$) on NIH3T3 cells deposited on uniformly-coated 2D fibronectin surfaces, caused cells to move more directionally, in particular for Rac$^{-13}$. When Rho$^-$ and Rac$^-$ cells were transferred on the ratchet, we measured $\nu_- < \nu_+$ in both cases and, most surprisingly, obtained larger values than for WT cells. This increase in dynamics might be related to the presence of feedback loops between both pathways. Some evidence have suggested a crosstalk between them, where one can regulate the other (e.g. Rac can inhibit Rho). The stabilization time $\tau$ was also perturbed even though it showed a trend similar to...
WT cells, *i.e.* \( \tau_{\text{m}} < \tau_{\text{k}} \). This perturbation in \( \nu \) and \( \tau \) suggested also a variation on \( I_{\text{dir}} \). However, Rac \(^-\) and Rho \(^-\) led to similar \( I_{\text{dir}} (0) \) values and cells mostly migrated toward the \( + \) direction. The probability predicted by \( I_{\text{dir}} \) matched again the experimental measurements in both conditions (confirming \( I_{\text{dir}} \) as a reliable read-out). These results suggested that the adhesive patches imposed cell behavior for the first step (short-term motion) and that Rho \(^-\) and Rac \(^-\) showed their effect on cell motility after this first step (long-term motion), even though efficient protrusion activity was perturbed. Finally, depolymerization of microtubules prevented motion, suggesting a key role for microtubules in allowing efficient probing (Movie 4).

**Long-term cell motion and cell bias**

Results for a single step motion led also to the hypothesis that long-term cell motion on a ratchet sequence will show a similar trend by repeating the mechanism outlined for the first steps. Cells on the ratchet protruded and moved to the neighboring motif with a probability dictated by \( I_{\text{dir}} \); then, we hypothesized the cycle to start again. The results showed a different scenario. Prior to the first step, the shape of the cell within an individual triangular patch was constrained protruding (asymmetrically) toward the \( + \) and \( - \) directions (Fig. 3B).Cell polarity was stabilized as shown by the distribution of polarity markers (focal contacts and centrosome). Then, the cell moved (e.g., toward the \( + \) direction) with a less constrained shape (Fig. 4).

As expected for a ratchet where stochasticity is essential, cells showed different behaviors: with or without back-and-forth motions, cells migrated to the \( + \) direction, to the \( - \) direction, or fluctuated in both directions with a no resulting net motion (Fig. 4A–C). However, long-term cell motion was mainly higher in percentage toward the \( + \) direction (Fig. 4A).\(^1\) This was a consequence of 2 main factors: (i) a spatio-temporal asymmetry in efficient protrusion (dictated by \( \nu \) and \( \tau \)), and (ii) a stability in cell polarity; cells maintained their direction of motion if polarity was stabilized. This result also suggested an increase in the velocity of cell motion if polarity was stable, as confirmed by the measurement of the average instantaneous velocity \( v_{\text{inst}} \) (Fig. 4D–F); cell motility increased after \( 10-20 \, \text{h} \) and reached a plateau at about \( 30 \, \text{h} \). Two distinct distributions of cell velocities were identified, before and after \( t = 10 \, \text{h} \) (Figs. 4D–F, insets). Note also that the motion of cells migrating could also show high fluctuations in \( v_{\text{inst}} \) mainly at large time scales (Fig. 4E). Finally, a small decrease in \( v_{\text{inst}} \) could also be observed at large time scales in fluctuating cells (Fig. 4F). This rich dynamics will be further probed in the future.

We next evaluated the long-term effect of perturbing protrusion fluctuations in long-term cell motion. Rho \(^-\) and Rac \(^-\) cells showed favorable motion mainly toward the \( + \) direction (Fig. 4G–H and Movie 2 and 3). However, they did not show a significant variation in the percentage of cells migrating \(+\) or \(-\) (+/- ratio) compared to WT. Other parameters characteristic of directed (i.e., persistent) cell motion showed a clear perturbation though. In particular, Rac \(^-\) showed a significant variation in \( I_{\text{p}} \) and \( T_{\text{p}} \) compared to WT. \( I_{\text{p}} \) (\( T_{\text{p}} \)) showed an increase (decrease, resp.); whereas for Rho \(^-\) a decrease (increase, resp) was obtained.\(^1\)

We proposed that Rac \(^-\) cells are more polarized and less sensitive to the adhesive ratchet, whereas Rho \(^-\) cells are more sensitive to the ratchets: both effects compensate in the ‘long-term’.

We also measured the instantaneous velocity for cells migrating toward the \( + \) direction for Rho \(^-\) and Rac \(^-\) conditions. Similar to WT condition, we observed an increase in velocity for Rac \(^-\) starting at around \( t = 10 \, \text{h} \) and reaching a plateau at about \( 30 \, \text{h} \) (Fig. 4K). In contrast, no increase in cell velocity was measured for Rho \(^-\) condition (Fig. 4J). These long term behaviors suggest an adaptive response from cells on adhesive ratchets, and our results favor a key role for the Rho pathway regulation through feedback loops.

In addition to the actin cytoskeleton, microtubules (\( \mu \)T) have been shown to contribute to the establishment of polarity in directed cell motion.\(^20\) Taking this into account, we next studied the role of microtubules inhibition (\( \mu \)T\(^-\)) on long-term cell motion using 10 \( \mu \)M nocodazole (Fig. 4I). Even though a high protrusive activity was observed in \( \mu \)T\(^-\) (see Movie 4), and contrary to the effect observed upon the inhibition of Rho and Rac, NIH3T3 cells (>95 %) were unable to hop toward the neighboring patches. Microtubule disassembly has been shown to activate Rho which in turn, induces acto-myosin contractility.\(^21\) This might perturb protrusion activity and impede cell migration, potentially by preventing efficient protrusions to reach the neighboring motifs. Altogether, these results confirmed the key role of microtubules in allowing cell locomotion.

**Impact of micro-pattern design for cell motion**

To understand the effect of the micro-pattern shapes in rectification, different geometries and morphologies were tested but keeping the adhesive area always constant (Fig. 5). We observed that \( I_{\text{p}}, T_{\text{p}}, \nu \) and direction depended on the selected configurations, as expected.\(^1\) We first studied cell behavior in a completely symmetrical system: a sequence of circular FN patches (Fig. 5A). In this configuration, \( \nu_{\text{x}} \approx \nu_{\text{y}} \) and \( \tau_{\text{m}} \approx \tau_{\text{k}} \); cells fluctuated between \(+\) and \(-\) directions with equal probability with low \( L_{\text{p}} \) and \( T_{\text{p}} \). We compared cell migration on circular and triangles patches based on the capability of cells to migrate and maintain directionality, as indicated by the measurement of the number of cell turns per unit of time (\( N_{\text{t}}^{-1} \)) (Fig. 4C). We found \( N_{\text{t}}^{-1} \) values of 0.17 (± 0.10) h\(^{-1}\) and 0.11 (± 0.02) h\(^{-1}\), respectively. On a FN self-assembled monolayer (SAM) though, cells switched directions more frequently as expected, with an \( N_{\text{t}}^{-1} \) value 3-fold larger than the triangle scenario (0.29 (± 0.16) h\(^{-1}\)).

We next studied the impact of triangle sharpness \( s \) in cell motility, keeping an asymmetric protrusion landscape, using 2 types of triangles defined as \( s^{++} \) (very sharp; the one mainly described in this work) and \( s^+ \) (sharp; wider front edge) (Fig. 5B–C). We found that cells deposited on \( s^+ \) triangles showed similar behaviors observed on \( s^{++} \) triangles (see Fig. 4 and Fig. 5B) and, in both cases, a clear bias toward the \( + \) direction was obtained (+/- = 2.2 and 2.3, respectively).
Figure 4. Long-term cell motion in a ratchet. Time sequence of an NIH3T3 cell on a micro-patterned ratchet migrating directionally toward the (A) + direction, (B) − direction and (C) fluctuating + and − (see movie S4 in ref.1). Above, the percentages for the different behaviors. (D–F) $v_{\text{av}}$ plot for cells migrating +, −, or fluctuating, respectively. Inset in each panel, the $v_{\text{av}}$ histograms. Data in orange and blue correspond to $v_{\text{av}}$ in the range of 0h–10h and 10h–48h, respectively. (G–H) NIH3T3 cell migrating toward the + direction upon the inhibition of Rho (80 nM C3) (see Movie 2) and Rac (100 µM NSC23766) (see Movie 3). (I) Fluctuating cell unable to hop toward the neighboring patches upon inhibition of microtubules (10 µM nocodazole) (see Movie 4). (J–K) $v_{\text{av}}$ plots for Rho− (j) and Rac− (k) cells migrating +. Inset in each panel, the corresponding velocity histograms. Error bars: SE. Time in hh:mm. Scale bars: 50 µm.
However, cells moved in average slower on $s^+$ triangles (in both directions), most probably because cells were more spread and less elongated, suggesting a potential larger friction with adhesive motifs (see Fig. 5C). Interestingly, even though the actual area is the same in both cases, the acto-myosin cytoskeleton sets focal contacts locations differently, and thereby may affect force balance.

To give further evidence for the interplay between protrusion fluctuations and adhesion, we finally tested what would be the effect if a single adhesive patch would be flipped – we called it a stopper – (see Fig. 5C and Movie 5). We observed that a cell migrating in the + direction paused and reversed its motion in the 'stopper' case. This suggests that protrusions dynamics was perturbed (reversed + → –) causing the cell to switch direction. Note finally that the switching direction time was low and similar to cells reversing their motion on normal – ratchet – configuration.

**Conclusions and Perspectives**

In this Commentary, we have discussed about the key role of protrusions fluctuations and adhesion in directing cell motion. Their asymmetric distribution guide cells on micro-patterned adhesive ratchets. Only two parameters, $v$ and $\tau$, which are encoded in $L_p$, robustly predict cell migration. This framework could be easily extended to other cell types.
Similarly, $I_{d}$ determination allows to successfully predict cell behavior in all experimental conditions. Finally, a theoretical model was developed showing a clear correlation between short- and long-term cell motion, highlighting that the motion of the cell can be thoroughly captured only with 2 read-outs, $v$ and $\tau$.

Our results are consistent with other studies on cell guiding assays. Cells were shown to migrate directionally in systems where the spatial symmetry was broken and in the absence of any environmental cue. 22-24 Cells deposited on ratchet-like microchannels or under tilted micropillars were shown to migrate by mechanically interacting with the environment. Clearly, symmetry can be broken by many means. And this suggests that migration in vivo may not be always a consequence of external chemical cues. But mechanical interactions and in particular, asymmetries in the spatial micro-environment of cells, may be a key determinant for guiding cell directions. Future studies using our framework will allow to evaluate the relevance of these mechanisms in vivo, in particular in the numerous cases where no chemical gradients are reported.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher’s website.

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