Fast-crawling cell types migrate to avoid the direction of periodic substratum stretching

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ABSTRACT

To investigate the relationship between mechanical stimuli from substrata and related cell functions, one of the most useful techniques is the application of mechanical stimuli via periodic stretching of elastic substrata. In response to this stimulus, \textit{Dictyostelium discoideum} cells migrate in a direction perpendicular to the stretching direction. The origins of directional migration, higher migration velocity in the direction perpendicular to the stretching direction or the higher probability of a switch of migration direction to perpendicular to the stretching direction, however, remain unknown. In this study, we applied periodic stretching stimuli to neutrophil-like differentiated HL-60 cells, which migrate perpendicular to the direction of stretch. Detailed analysis of the trajectories of HL-60 cells and \textit{Dictyostelium} cells obtained in a previous study revealed that the higher probability of a switch of migration direction to that perpendicular to the direction of stretching was the main cause of such directional migration. This directional migration appears to be a strategy adopted by fast-crawling cells in which they do not migrate faster in the direction they want to go, but migrate to avoid a direction they do not want to go.

Introduction

Living cells are constantly subjected to a range of mechanical stimuli, such as shear flow, osmotic pressure, and changes in hardness of substratum. To permit effective cell function, they need to sense the mechanical characteristics of their environment and respond appropriately. It appears that, under physiological conditions, cells adhering to substrata receive mechanical stimuli mainly from the substrata: these stimuli then decide their shape and/or migration properties.\textsuperscript{1,2} Crawling cells obviously cannot migrate without adhering to the substratum, indicating that one of the most fundamental mechanisms may be polarity generation for migration utilizing the force exerted by the substratum. To investigate the relationship between a mechanical signal from substrata and related cell functions, one of the most effective techniques for applying mechanical stimuli artificially is to stretch the elastic substratum to which the cells are adhered.\textsuperscript{3-6} In response to periodic stretching of the elastic substratum, intracellular stress fibers in fibroblasts, and endothelial, osteosarcoma, and smooth muscle cells rearrange themselves perpendicular to the direction of stretching, with the result that the shape of the cells extends in that direction.\textsuperscript{7-13}

It is also an interesting question as to whether or not the direction of cell crawling is regulated by periodic stretching of the substratum. We found recently that \textit{Dictyostelium} cells, which are fast-crawling cells that have no stress fibers, migrate perpendicular to the direction of periodic stretching.\textsuperscript{14,15} This finding raises 2 major questions: (I) Is this directional migration of \textit{Dictyostelium} cells in response to the force exerted by the substratum limited to \textit{Dictyostelium} cells, or is it common in other cell types? and (II) How do the cells realize directional migration perpendicular to the stretching direction? There are 2 possible origins of directional migration. One is that the probability of a switch of migration direction to perpendicular to the stretching direction is higher than that of a switch to other directions. The other is that the migration velocity in the direction perpendicular to the stretching direction is higher than that in any other direction.

To answer these 2 questions (I) and (II), we dispersed neutrophil-like differentiated HL-60 cells on the elastic sheets, made from polydimethylsiloxane (PDMS), and applied periodic stretching stimuli to them. They migrated perpendicular to the direction of stretching, as was seen in the \textit{Dictyostelium} cells. We then clarified the origin of
directional migration of both the HL-60 cells and the Dictyostelium cells that was perpendicular to that of stretching by carrying out a detailed analysis of their trajectories. Trajectory analysis is a powerful tool for revealing the behavioral strategy that crawling cells adopt for survival. For example, the amoeboid motion of freely crawling cells, such as Dictyostelium and microglia, is not a simple random walk but a zigzag motion in which they make a turn in the opposite direction to their previous turn. This behavior seems to improve their chances of finding a target over that of performing a random walk.

Our results suggest that directional migration perpendicular to that of stretching is common in fast-crawling cells, such as HL-60 and Dictyostelium cells, and that its chief cause is the high probability of a switch of direction of migration to perpendicular to the stretching direction.

Results

Directional migration of HL-60 cells in response to periodic stretching of elastic substratum at various time cycles

The differentiated HL-60 cells were transferred to the PDMS elastic sheets, whose surface was coated with fibronectin and applied periodic stretching stimuli with various stretching conditions. When the sheet was stretched, perpendicular shrinkage was suppressed at $\leq 1/4$ of the stretch as was the case of previous study with Dictyostelium cells.

First, periodic stretching stimuli of a fixed stretch ratio (115%) and different time cycles (30, 15 and 7.5 s) were applied to HL-60 cells by repeated stretching and relaxation of the substratum. In changing the time cycles of periodic stretching, the duty ratio of stretch and shrinkage was kept at 1:1, and the velocities of stretching and relaxation also constant. The trajectories of cell migration under each time cycle and that under zero stretching stimulus are shown in Figure 1A-D. The probability of each direction of migration ($\theta$) (Fig. 2A-D) was individually calculated from Figure 1A-D. The cells tended to migrate perpendicularly to the direction of stretching (90° or 270°) when subjected to periodic stretching (Figs. 1B-D, 2B-D and Movie S1), whereas they migrated isotropically and uniformly in its absence (Figs. 1A, 2A and Movie S2). The $|\sin \theta|$ values calculated from Figure 2A-D are shown in Figure 3A. (See Materials and Methods for full details.) The values rose with shortening time cycle, indicating that HL-60 cells can sense periodic stretching stimuli applied through the substratum and migrate perpendicular to the stretching direction. These results suggest that HL-60 cells prefer a non-deformed direction.

Effect of stretch ratio of periodic stretching on directional migration of HL-60 cells

Next, we examined the relationship between stretch ratio and degree of directionality of migration of HL-60 cells. Cell migration was analyzed under the periodic stretching stimuli of a fixed time cycle (15 s) and different stretch ratios (zero stretching, 107.5, 115 and 130%). Based on the trajectories of cell migration (Fig. 1A, C, E and F), frequencies of migration directions under each stretch ratio (Fig. 2A, C, E and F) and the $|\sin \theta|$ values (Fig. 3B) were calculated. The $|\sin \theta|$ value rose with increasing stretch ratio, indicating the degree of directional migration to be positively correlated with the stretch ratio, as was time-cycle dependency (Fig. 3A).

Difference in migration velocity perpendicular to the stretch direction and that parallel to the stretching is not the main cause of directional migration

To test whether the migration velocity perpendicular to the stretch direction is higher than that parallel to it, we calculated them under each set of stretch conditions of HL-60 cells (Fig. 4). (See Materials and Methods for full details.) When the stretch ratio was fixed at 115%, there was no significant difference in migration velocity between the perpendicular direction (⊥) and the parallel direction (∥) at a time cycle of 15 s or more (Fig. 4A-C). Only at the 7.5-s time cycle was velocity in the perpendicular direction significantly higher than that in the parallel direction (‘ in Fig. 4D). When the time cycle was fixed at 15 s, only at the 130% stretch ratio was the velocity in the perpendicular direction significantly higher than that in the parallel direction (Fig. 4A, C, E and F).

In a previous paper, we obtained the trajectory data of migrating cells of the Dictyostelium discoideum cAR1/cAR3 [receptors of chemoattractant, cyclic adenosine 3’,5’-monophosphate (cAMP)] double-mutant cell line R19 under the periodic stretching stimuli with various stretching conditions. To test whether or not this situation is common in Dictyostelium cells, we calculated the migration velocities of R19 Dictyostelium cells perpendicular and parallel to the stretch direction under each set of stretch conditions (Fig. 5). In the case of Dictyostelium cells too, only at strong stretching stimuli of 130% stretch ratio and 10-s time cycle was the velocity in the perpendicular direction (⊥) significantly higher than that in the parallel direction (∥), with only a small difference seen (‘ in Fig. 5F). These results indicate that periodic stretching of the substratum causes little difference in the migration velocity of fast-crawling cells between the perpendicular and the parallel directions.
A switch of migration direction from parallel to perpendicular to the stretch direction, the main cause of directional migration

To test whether the probability of a switch of migration direction from parallel to perpendicular is higher than that from perpendicular to parallel, we analyzed the trajectories of HL-60 cells under a range of stretch conditions (Fig. 1) and calculated the probability of a switch of migration direction from perpendicular to parallel (Fig. 6A and the red columns in C and D) and that from parallel to perpendicular (Fig. 6B and the blue columns in C and D) under each stretch condition. (See Materials and Methods for full details.) When the stretch ratio was fixed at 115%, the probability of a switch from perpendicular to parallel did not change at a time cycle of 15 s or more (red columns in Fig. 6C). Only at a 7.5-s time cycle did the probability decrease (* in Fig. 6C). On the other hand, the probability of a switch of migration direction from parallel to perpendicular increased on shortening the time cycle (blue columns in Fig. 6C). When the time cycle was fixed at 15 s, the probability of a switch from perpendicular to parallel did not change at stretch ratios of 115% or below (red columns in Fig. 6D). Only at a 130% stretch ratio did the probability decrease (* in Fig. 6D). However, the probability of a switch from parallel to perpendicular increased with increasing stretch ratio (blue columns in Fig. 6D).

The probability of a switch of migration direction was also calculated from the trajectories of Dictyostelium cells (Fig. 6E and F). The resultant graphs show the same characteristics as those of HL-60 cells (Fig. 6C and D). At a 120% stretch ratio, the probability of a switch from perpendicular to parallel (Fig. 6A) did not change when the time cycle was 10 s or above (red columns in Fig. 6E). Only at a 5-s time cycle did

Figure 1. Trajectories of migration of differentiated HL-60 cells on an elastic substratum with or without periodic stretching for 30 min. (A) Without periodic stretching (n = 102 from 4 experiments). (B) Periodic stretching at 115% stretch ratio and 30-s time cycle (n = 101 from 4 experiments). (C) 115% and 15 s (n = 103 from 4 experiments). (D) 115% and 7.5 s (n = 101 from 4 experiments). (E) 107.5% and 15 s (n = 101 from 5 experiments). (F) 130% and 15 s (n = 101 from 4 experiments). Periodic stretching stimuli were applied in the direction parallel to the x-axis in B-E. Stretch conditions are shown as green double-headed arrows. Length and thickness of the arrows represent stretching ratio and frequency, respectively.
the probability decrease (* in Fig. 6E). As with HL-60 cells, the probability of a switch of migration direction from parallel to perpendicular (Fig. 6B) increased with shortening time cycle (blue columns in Fig. 6E). When the time cycle was fixed at 10 s, the probability of a switch from perpendicular to parallel did not change at stretch ratios of 120% or below (red columns in Fig. 6F). Only at a 130% stretch ratio did the probability decrease (* in Fig. 6F). However, the probability of a switch from parallel to perpendicular increased with increasing stretch ratio (blue columns in Fig. 6F). These results indicate that periodic stretching of the substratum can turn the migration direction of fast-crawling cells from parallel to the stretch direction to perpendicular to it.

**Discussion**

Fast-crawling cells migrate toward chemoattractants such as N-formyl-methionine-leucine-phenylalanine (fMLP) for neutrophils and neutrophil-like HL-60 cells, and cAMP for *Dictyostelium* cells. Interestingly, even in media containing a uniform concentration of fMLP, HL-60 cells localize F-actin to a portion of the cell and myosin IIA or RhoA to the opposite side. In *Dictyostelium* cells, localization of phosphatidylinositol 3,4,5-trisphosphate (PIP3) plays a role in the generation of migrating polarity, not only in the presence of extracellular cAMP, but also in its absence. The distribution of PIP3 is regulated by PI3K and PTEN. In spite of these facts, a sextuple mutant lacking all 5 PI3K genes and PTEN can migrate without localization of PIP3, albeit at low velocities. These observations indicate that fast-crawling cells do not necessarily require upstream signals for polarity generation to induce migration. Polarity generation for migration utilizing the force from the substratum may be a fundamental mechanism, with chemotaxis being a highly sophisticated version.

This study shows that application of periodic stretching stimuli to neutrophil-like differentiated HL-60 cells induces migration perpendicular to the direction of stretching, in the same way as seen with *Dictyostelium*
Our results showed the probability of a switch to perpendicular migration increased on decreasing the time cycle or on increasing the stretch ratio (blue columns in Fig. 6C-F); although the velocity in the perpendicular direction was significantly higher than that in the parallel direction only when subjected to strong periodic stretching stimuli (Figs. 4D, F and 5F). Such strong stimuli may cause the edges of the cells, which are perpendicular to the stretching direction, to peel off the substratum, decreasing the velocity in the parallel direction, and reducing the likelihood of a switch of direction to parallel migration (‘s in Fig. 6). Thus, the main trigger for perpendicular migration appears to be not a difference in migration velocity between the perpendicular and parallel directions but a rise in the probability of a switch to perpendicular migration. This directional migration appears to be a strategy adopted by fast-crawling cells in which they do not migrate faster in the direction they want to go, but migrate to avoid a direction they do not want to go.

Freely crawling cells, such as Dictyostelium and microglia, travel in a zigzag motion, in which they make a turn in the line-symmetrical direction to their previous turn as the axis of long-range migration direction. However, it has not been clarified how the cells realize this zigzag motion. Dictyostelium cells exert a traction force on the substratum at their extending pseudopods. At the same time, the cells experience a reaction force from the substratum, which has the same amplitude but acts in the direction opposite to the traction force. This study, however, shows that fast-crawling cells such as Dictyostelium and HL-60 cells migrate to avoid the directions in which they receive forces from the substratum. The above facts and the results of this study suggest that the mechanosensing reaction between the cells and the substratum may induce the zigzag crawling movement as follows. (I) A crawling cell extends a pseudopod in one direction while exerting a traction force onto the substratum at the pseudopod. (II) The cell experiences the reaction force from the substratum at the pseudopod and stops extending the pseudopod. (III) It then extends another pseudopod in the line-symmetrical direction. Repeating these 3 steps results in zigzag movement.

The aim of this study was to elucidate the common action principle of fast-crawling cell types by means of trajectory analysis. Thus, we did not deal with the signaling cascade that causes migration. We previously revealed that stretching of the substratum induced myosin II localization equally at the edges of the cells, which are perpendicular to the stretching direction. The mechano-sensor molecules which respond to the force from the substratum and the subsequent signaling cascade are unknown. Deformation of actin filaments is a possible candidate for inducing myosin II localization, since an actin meshwork is required for myosin II localization. The detailed mechanism of how myosin II localizes in response to stretching of the substratum is a potentially interesting topic for future study.

In this paper, artificial force was applied to crawling cells via the substratum. Even in the absence of such external forces, the cells can generate polarity for migration. In the situation without application of any external force, it appears that the forces that the adherent cells experience from the substratum are reaction forces from the...
substratum caused by their own traction to it. Neutrophils exert larger traction forces on the rigid substratum than on the soft one. The traction/reaction forces are likely to depend on the rigidity of the substratum. The sensing of such reaction forces has been proposed as “active touch.” Fibroblasts sense rigidity in the substratum and move toward rigid areas (a process called durotaxis). Migration of fast-crawling cells on a substratum with anisotropic rigidity is a potentially interesting avenue for future investigation.

**Materials and methods**

**HL-60 cell line**

The neutrophil-like HL-60 cell line, a model of human promyelocytic leukemia, was obtained from Riken Cell Bank (Cell No. RCB0041; Tsukuba, Japan) and grown in RPMI 1640 medium (18902145; Wako, Osaka, Japan) supplemented with 10% FBS (Nichirei, Tokyo, Japan), 100 U/ml streptomycin, 100 U/ml penicillin G and 0.25 μg/ml amphotericin B (09366-44; Nacalai tesque, Kyoto, Japan). Cell differentiation was induced by transfer into culture medium containing 1.3% DMSO. After 3 d in 5% CO₂ at 37 °C, cells showed neutrophil-like migration.

**Periodic stretching of the substratum**

Elastic sheets, 22 mm × 40 mm, were made from PDMS (Sylgard 184, Dow Corning Toray, Tokyo, Japan) according to previously-described methods. These are same substratum used for previous experiment of Dictyostelium cells. The surface of the sheet was coated

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**Figure 4.** Migration velocity of differentiated HL-60 cells on elastic substratum with periodic stretching. (A) Under zero periodic stretching. (B-D) Migration velocities under periodic stretching at a 115% stretch ratio and different time cycles (B: 30 s, C: 15 s, D: 7.5 s). (E and F) Migration velocities during periodic stretching with a 15-s time cycle and different stretch ratios (E: 107.5%, F: 130%). The left- and right-hand columns in each panel respectively represent velocities parallel (∥) and perpendicular (⊥) to the stretching direction (n = 60 switches × 102, 101, 103, 101, 101 and 101 cells from (A-F), mean ± SEM; * p < 0.05, Welch’s t-test).
with fibronectin (354008, BD Japan, Tokyo, Japan). The differentiated cells in the culture chamber were transferred directly to the elastic sheets and given one hour to adhere to the sheets. The culture medium containing 1.3% DMSO was then replaced with that containing no DMSO. After allowing about 30 min for cell recovery, stretching of the substratum was performed according to the previously-described methods. Using elastic sheets, it is difficult to exclude the Poisson’s effect completely. When the sheet was stretched, perpendicular shrinkage was suppressed at ≤ 1/4 of the stretch as was the case of previous study with Dictyostelium cells. In changing the frequency of stretching, the duty ratio of stretch and shrinkage was kept at 1:1, and the velocities of stretching and relaxation also constant. The periods of being stretched to a maximum and shrunk to a minimum were adjusted to change the frequency.

**Statistical analysis of cell migration**

The analysis was performed according to previously-described methods. Migrating cells subjected to periodic stretching were observed under phase contrast microscopy using an inverted microscope (TS100; Nikon, Tokyo, Japan) with a 20× objective lens (LWD ADL 20×F; Nikon). Images taken with a CMOS camera (DMK41BUC02; Argo, Osaka, Japan) were transferred to a PC at 30-s intervals for 30 min. The data were then analyzed using ImageJ with 2 plug-ins: Manual Tracking, and the Chemotaxis and

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**Figure 5.** Migration velocity of RI9 Dictyostelium cells on elastic substratum with periodic stretching. (A) Under zero periodic stretching. (B-D) Migration velocities under periodic stretching at a 120% stretch ratio and different time cycles (B: 20 s, C: 10 s, D: 5 s). (E and F) Migration velocities during periodic stretching with a 10-s time cycle and different stretch ratios (E: 110%, F: 130%). The left and right columns in each panel represent velocities parallel (∥) and perpendicular (⊥) to the stretching direction (n = 60 switches × 55, 54, 61, 85, 89 and 50 cells from (A-F), mean ± SEM; *p < 0.05, Welch’s t-test). (A) and (C-F) were calculated from the trajectory data, Figs. 2F, 3C, G, A and E, in our previous study, respectively. (B) was calculated from the data newly obtained in this study.
Migration Tool. In this analysis, we measured the angle \( \theta \) (in Fig. 7A) made by the x-axis which was parallel to stretching direction and the vector from the initial location of a cell \( (t = 0 \text{ min}) \) to the final one during observation \( (t = 30 \text{ min}) \) (thick arrow in Fig. 7A) was measured. The absolute value of the sine of the angle, \(|\sin \theta|\), was calculated to provide an index of directional migration. If all cells migrate parallel to the direction of stretching \( (0^\circ \text{ or } 180^\circ \text{; hereinafter ‘parallel (migration)’}) \), all the values of \(|\sin \theta|\) should be 0. On the other hand, if all cells migrate perpendicularly \( (90^\circ \text{ or } 270^\circ \text{; hereinafter ‘perpendicular (migration)’}) \), the value should be 1. If they show isotropic migration, the value should be \( 2/\pi = 0.64 \). In preparing histograms for Fig. 3, the interior angle of each datum was set at 66.0°. This means that, for example, the value at 90° includes the number of cells that migrated in directions from 57° to 123°.

Migration velocities in the parallel and perpendicular directions, and the probabilities of a switch from parallel to perpendicular and vice versa, were estimated as follows. A vector from the initial location of a cell \( (t = T) \) to that in the next time frame \( (t = T + 30 \text{ s}) \) was defined (thick arrow in Fig. 7B). When the angle \( \phi \) between the x-axis \((x\text{ in Fig. 7B}) \) and the vector was between 315 \( (-45^\circ) \) and 45° or between 135 and 225° (gray region in Fig. 7B), the migration direction at \( t = T \) was labeled as “parallel” to the stretching. On the other hand, when it was 45 – 135° or 225 – 315°, it was labeled as “perpendicular.” Average parallel and perpendicular velocities were individually calculated throughout the migration.
period of 30 min. The probability of a switch of migration direction from parallel to perpendicular was obtained by dividing the number of label changes from "parallel" to "perpendicular" by the total number of vectors throughout the 30-min migration period. The probability from perpendicular to parallel was obtained by dividing the number of label changes from "perpendicular" to "parallel" by the total number of vectors during the 30-min migration period. Each transition to the same direction was regarded as a discrete event.

**Abbreviations**

- cAMP: cyclic adenosine 3’5’-monophosphate
- fMLP: N-formyl-methionine-leucine-phenylalanine
- PDMS: polydimethylsiloxane
- PIP3: phosphatidylinositol 3,4,5-trisphosphate

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**References**

[1] Giannone G, Sheetz MP. Substrate rigidity and force define form through tyrosine phosphatase and kinase pathways. Trends Cell Biol 2006; 16:213-23; PMID:16529933; http://dx.doi.org/10.1016/j.tcb.2006.02.005
[2] Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. Nat Rev Mol Cell Biol 2006; 7:265-75; PMID:16607289; http://dx.doi.org/10.1038/nrm1890
[3] Naruse K, Yamada T, Sai XR, Hamaguchi M, Sokabe M. Pp125FAK is required for stretch dependent morphological response of endothelial cells. Oncogene 1998; 17:455-63; PMID:9696039; http://dx.doi.org/10.1038/sj.onc.1201950
[4] Naruse K, Yamada T, Sokabe M. Involvement of SA channels in orienting response of cultured endothelial cells to cyclic stretch. Am J Physiol Heart Circ Physiol 1998; 274:H1532-8
[5] Crosby LM, Luellen C, Zhang Z, Tague LL, Sinclair SE, Waters CM. Balance of life and death in alveolar epithelial
type II cells: proliferation, apoptosis, and the effects of cyclic stretch on wound healing. Am J Physiol Lung Cell Mol Physiol 2011; 301:L536–46; PMID:21724858; http://dx.doi.org/10.1152/japplphysiol.00371.2010

[6] Desai LP, White SR, Waters CM. Cyclic mechanical stretch decreases cell migration by inhibiting phosphatidylinositol 3-kinase- and focal adhesion kinase-mediated JNK1 activation. J Biol Chem 2010; 285:4511–9; PMID:20018857; http://dx.doi.org/10.1074/jbc.M109.084335

[7] Birukov KG, Jacobson JR, Flores AA, Ye SQ, Birukova AA, Verin AD, Garcia JGN. Magnitude-dependent regulation of pulmonary endothelial cell barrier function by cyclic stretch. Am J Physiol Lung Cell Mol Physiol 2003; 285:L785–97; PMID:12639843; http://dx.doi.org/10.1152/japplphysiol.00336.2002

[8] Kaunas R, Nguyen P, Usami S, Chien S. Cooperative effects of Rho and cyclic stretch on stress fiber organization. Proc Natl Acad Sci USA 2005; 102:15895–900; PMID:16247009; http://dx.doi.org/10.1073/pnas.0506041102

[9] Tondon A, Hsu H-J, Kaunas R. Dependence of cyclic stretch-induced stress fiber reorientation on stretch waveform. J Biomech 2011; 45:728–35; PMID:22206828; http://dx.doi.org/10.1016/j.jbiomech.2011.11.028

[10] Lee C-F, Haase C, Deguchi S, Kaunas R. Cyclic stretch-induced stress fiber dynamics – dependence on strain rate, Rho-kinase and MLCK. Biochem Biophys Res Commun 2010; 401:344–9; PMID:20849825; http://dx.doi.org/10.1016/j.bbrc.2010.09.046

[11] Zhao L, Sang C, Yang C, Zhuang F. Effects of stress fiber contractility on uniaxial stretch guiding mitosis orientation and stress fiber alignment. J Biomech 2011; 44:2388–94; PMID:21767844; http://dx.doi.org/10.1016/j.jbiomech.2011.06.033

[12] Sato K, Adachi T, Matsuo M, Tomita Y. Quantitative evaluation of threshold fiber strain that induces reorganization of cytoskeletal actin fiber structure in osteoblastic cells. J Biomech 2005; 38:1895–901; PMID:16023478; http://dx.doi.org/10.1016/j.jbiomech.2004.08.012

[13] Morioka M, Parameswaran H, Naruse K, Kondo M, Sokabe M, Hasegawa Y, Suki B, Ito S. Microtubule dynamics regulate cyclic stretch-induced cell alignment in human airway smooth muscle cells. PLoS ONE 2011; 6:e26384; PMID:22022610; http://dx.doi.org/10.1371/journal.pone.0026384

[14] Iwadate Y, Yumura S. Cyclic stretch of the substratum using a shape-memory alloy induces directional migration in Dictyostelium cells. BioTechniques 2009; 47:757–67; PMID:19852761; http://dx.doi.org/10.2144/00113217

[15] Iwadate Y, Okimura C, Sato K, Nakashima Y, Tsujikoa M, Minami K. Myosin-II-mediated directional migration of Dictyostelium cells in response to cyclic stretching of substratum. Biophys J 2013; 104:748–58; PMID:23442953; http://dx.doi.org/10.1016/j.bpj.2013.01.005

[16] Li L, Norrelykke SF, Cox EC. Persistent cell motion in the absence of external signals: a search strategy for eukaryotic cells. PLoS ONE 2008; 3:e2093; http://dx.doi.org/10.1371/journal.pone.0002093

[17] Yang TD, Park J-S, Choi Y, Choi W, Ko T-W, Lee KJ. Zigzag turning preference of freely crawling cells. PLoS ONE 2011; 6:e20255; PMID:21687729; http://dx.doi.org/10.1371/journal.pone.0020255

[18] Insall RH, Soede RD, Schaap P, Devreotes PN. Two cAMP receptors activate common signaling pathways in Dictyostelium. Mol Biol Cell 1994; 5:703–11; PMID:7949426; http://dx.doi.org/10.1091/mbc.5.6.703

[19] Wang M-J, Artemenko Y, Cai W-J, Iglesias PA, Devreotes PN. The directional response of chemotactic cells depends on a balance between cytoskeletal architecture and the external gradient. Cell Rep 2014; 9:1110–21; PMID:25437564; http://dx.doi.org/10.1016/j.celrep.2014.09.047

[20] Wu J, Pipathsouk A, Keizer-Gunnink A, Fusetti F, Alkema W, Liu S, Altschuler S, Wu L, Kortholt A, Weiner OD. Homer3 regulates the establishment of neutrophil polarity. Mol Biol Cell 2015; 26:1629–39; PMID:25739453; http://dx.doi.org/10.1091/mbc.E14-07-1197

[21] Nichols JM, Veltman D, Kay RR. Chemotaxis of a model organism: progress with Dictyostelium. Curr Opin Cell Biol 2015; 36:7–12; PMID:26183444; http://dx.doi.org/10.1016/j.jceb.2015.06.005

[22] Shin ME, He Y, Li D, Na S, Chowdhury F, Poh Y-C, Collin O, Su P, de Lanerolle P, Schwartz MA, et al. Spatiotemporal organization, regulation, and functions of tractions during neutrophil chemotaxis. Blood 2010; 116:3297–310; PMID:20616216; http://dx.doi.org/10.1182/blood-2009-12-260851

[23] Xu J, Wang F, Van Keymeulen A, Herzmark P, Straight A, Kelly K, Takuwa Y, Sugimoto N, Mitchison T, Bourne HR. Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. Cell 2003; 114:201–14; PMID:12887922; http://dx.doi.org/10.1016/S0092-8674(03)00555-5

[24] Van Haastert PJM, Devreotes PN. Chemotaxis: signalling the way forward. Nat Rev Mol Cell Biol 2004; 5:626–34; PMID:15366706; http://dx.doi.org/10.1038/nrm1435

[25] Kölsch V, Charest PG, Firtel RA. The regulation of cell motility and chemotaxis by phospholipid signaling. J Cell Sci 2008; 121:551–9; PMID:18287584; http://dx.doi.org/10.1242/jcs.023333

[26] Arau Y, Shibata T, Matsuoka S, Sato MJ, Yanagida T, Ueda M. Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. Proc Natl Acad Sci USA 2010; 107:12399–404; PMID:20562345; http://dx.doi.org/10.1073/pnas.0908278107

[27] Sasaki AT, Janetopoulos C, Lee S, Charest PG, Takeda K, Sundheimer LW, Meil R, Devreotes PN, Firtel RA. G protein-independent Ras/Pi3K/F-actin circuit regulates basic cell motility. J Cell Biol 2007; 178:185–91; PMID:17635933; http://dx.doi.org/10.1083/jcb.200611138

[28] Shibata T, Nishikawa M, Matsuoka S, Ueda M. Intracellular encoding of spatiotemporal guidance cues in a self-organizing signaling system for chemotaxis in Dictyostelium cells. Biophys J 2013; 105:2199–209; PMID:24209866; http://dx.doi.org/10.1016/j.bpj.2013.09.024

[29] Nishikawa M, Hörning M, Ueda M, Shibata T. Excitable signal transduction induces both spontaneous and directional cell asymmetries in the phosphatidylinositol lipid signaling system for eukaryotic chemotaxis. Biophys J 2014; 106:723–34; PMID:24507613; http://dx.doi.org/10.1016/j.bpj.2013.12.023

[30] Iijima M, Devreotes P. Tumor suppressor PTEN mediates sensing of chemotactant gradients. Cell 2002; 109:599–610; PMID:12062103; http://dx.doi.org/10.1016/S0092-8674(02)00745-6
Comer FI, Parent CA. PI 3-kinases and PTEN: how opposites chemoattract. Cell 2002; 109:541-4; PMID:12062096; http://dx.doi.org/10.1016/S0092-8674(02)00765-1

Matsuoka S, Shibata T, Ueda M. Asymmetric PTEN distribution regulated by spatial heterogeneity in membrane-binding state transitions. PLoS Comput Biol 2013; 9:e1002862; PMID:23326224; http://dx.doi.org/10.1371/journal.pcbi.1002862

Hoeller O, Kay RR. Chemotaxis in the absence of PtdIns (3,4,5)P3 gradients. Curr Biol 2007; 17:813-7; PMID:17462897; http://dx.doi.org/10.1016/j.cub.2007.04.004

Iwadate Y, Yumura S. Actin-based propulsive forces and myosin-II-based contractile forces in migrating Dictyostelium cells. J Cell Sci 2008; 121:1314-24; PMID:18388319; http://dx.doi.org/10.1242/jcs.021576

Bosgraaf L, van Haastert PJM. The regulation of myosin II in Dictyostelium. Eur J Cell Biol 2006; 85:969-79; PMID:16814425; http://dx.doi.org/10.1016/j.ejcb.2006.04.004

Uyeda TQP, Iwadate Y, Umeki N, Nagasaki A, Yumura S. Stretching actin filaments within cells enhances their affinity for the myosin II motor domain. PLoS One 2011; 6:e26200; PMID:22022566; http://dx.doi.org/10.1371/journal.pone.0026200

Kobayashi T, Sokabe M. Sensing substrate rigidity by mechanosensitive ion channels with stress fibers and focal adhesions. Curr Opin Cell Biol 2010; 22:669-76; PMID:20850289; http://dx.doi.org/10.1016/j.cel.2010.08.023

Lazopoulos KA, Stamenović D. Durotaxis as an elastic stability phenomenon. J Biomech 2008; 41:1289-94; PMID:18308324; http://dx.doi.org/10.1016/j.jbiomech.2008.01.008

Jiang G, Huang AH, Cai Y, Tanase M, Sheetz MP. Rigidity sensing at the leading edge through alphav-beta3 integrins and RPTPalpha. Biophys J 2006; 90:1804-9; PMID:16339875; http://dx.doi.org/10.1529/biophysj.105.072462

Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. Biophys J 2000; 79:144-52; PMID:10866943; http://dx.doi.org/10.1016/S0006-3495(00)76279-5