Receptor Occupancy on an Ellipsoidal Cell in the Presence of a Point Source of a Chemoattractant

Igor Weber*

Department of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia

Received April 29, 2005

Diffusion of a chemoattractant from a micropipet is routinely used to examine the different aspects of a cell's chemotactic response. To quantify the effect of cell elongation on chemotactic sensitivity in the micropipet assay, the chemoattractant concentration at the cell plasma membrane was determined by solving the equation for diffusion from a point source in the presence of a prolate ellipsoid of varying eccentricity. The results show that cell elongation can significantly increase the difference in receptor occupancy between near and far cell ends and thereby enhance the sensitivity of chemotactic cells to shallow chemoattractant gradients.

INTRODUCTION

In the process of chemotaxis, cells sense the presence of extracellular signaling molecules called chemoattractants and migrate in the direction along shallow gradients of these signals. Chemotaxis leads leukocytes to sites of infection and allows their trafficking in the immune system, directs cells to the proper locations during embryogenesis, guides cells in wound healing, and contributes to pathological states such as allergic inflammation and tumor metastasis. To sense gradients, eukaryotic cells compare differences in receptor occupancy along their length and convert this signal into a localized response. They can respond to differences in chemoattractant concentration that are as small as 1−2% between the front and the back of the cell.[1]

Many key molecular constituents of the chemotactic sensory apparatus of eukaryotic cells have been uncovered in neutrophils and *Dictyostelium*. The primary components of the chemotactic signaling pathway, such as the receptors and heterotrimeric G protein subunits, are distributed uniformly along the cell membrane.[2] Chemoattractant binding activates G proteins and further downstream events, whose initial spatial distribution mirrors receptor occupancy. Asymmetry necessary for directed movement develops further downstream and becomes evident in a sharp localization of the key intermediate in the pathway, phosphatidylinositol 3,4,5-trisphosphate, PI(3,4,5)P_3, at the leading edge of cells exposed to a gradient. In *Dictyostelium*, PI(3,4,5)P_3 localization is regulated through recruitment of phosphoinositide 3-kinase, PI3K, and a phosphatase, PTEN, to membranes at the opposite ends of the cell. This spatial sensing mechanism is functional even when cells are immobilized, and their movement and elongation is inhibited by disruption of the actin cytoskeleton. Mechanisms of amplification and adaptation of an initial chemotactic response based on the activity of PI3K and PTEN have also been proposed recently.[3]

Although a great deal has been learned about signaling networks involved in directional sensing of chemotactic cells, it is still not known how a shallow gradient of chemoattractant can be processed to generate the initial localized response at a specific site along the cell envelope. Ultimately, the initial response has to rely on a difference of the receptor occupancy between the parts of a cell oriented toward and away from the source of chemoattractant. A popular way used to test a cell's chemotactic activity is the micropipet assay.[4] In this assay, cells are exposed to a gradient of chemoattractant diffusing from an orifice of a glass micropipet. Although mostly used to simply test whether cells respond to a chemoattractant or not, a micropipet assay can in principle be used to quantify the response. Theoretical analysis of the assay has been presented for spherical cells in a study of yeast cells' response to mating factor α.[5] In the present study, analysis of the micropipet assay is extended to the general case of cells shaped as prolate ellipsoids, resembling the elongated aggregation-competent *Dictyostelium* cells. Conclusions are drawn about an increase of sensitivity to chemotactic signals of elongated cells, as compared to spherical, in the initial chemotactic response.

THEORETICAL CALCULATIONS

The problem of determining the distribution of occupied receptors on an ellipsoidal cell in the vicinity of a micropipet filled with chemoattractant amounts to solving the Poisson equation for diffusion from a point source in the presence of an impermeable prolate ellipsoid

$$D\nabla^2 C(\vec{r}) = q\delta(\vec{r} - \vec{r}_0) \qquad (1)$$

where $C(\vec{r})$ is the three-dimensional chemoattractant concentration, D is the diffusion coefficient of the chemoattractant, q is the constant release rate of the chemoattractant from the micropipet, whereas the pointlike nature of the source is represented by the delta function. It is appropriate to solve this differential equation by using an orthogonal set of prolate spheroidal coordinates (ξ, η, ϕ).[6] The planar projection presented in Figure 1 fully characterizes the problem, since there is an azimuthal symmetry with respect to the line connecting the center of the ellipsoid and the micropipet tip.

* Corresponding author phone: + 385 1 457 1219; fax + 385 1 456 1177; e-mail: iweber@irb.hr.

10.1021/ci050165x CCC: $30.25
Published on Web 10/14/2005

Figure 1. Two-dimensional presentation of the problem. Source of the chemoattractant is designated by $q(\xi_0)$, distance of the source from the center of the prolate ellipsoid by ξ_0, and the ellipsoid by $\xi = \xi_1$. This figure is not drawn to scale, since in reality ξ_0 is many times larger than ξ_1. Prolate spheroidal coordinates are used throughout.

For a given interfocal distance a, the surface of an ellipsoid is defined by $\xi =$ constant. The concentration on any point of the ellipsoid surface will vary with the meridional coordinate η, which is related to the cosine of the angle between the long axis and the line connecting the center of the ellipsoid to the point on the surface, and not with the azimuthal coordinate ϕ. The method of Green's function expansion is used to solve the Poisson equation. Starting from the general expression for prolate spheroids,[6] utilizing the azimuthal symmetry, and imposing reflecting boundary conditions on the surface, we arrive at the final expression for concentration on the surface of a prolate ellipsoid (a,ξ_1) in the presence of a point source at ξ_0

$$C(\xi_1,\eta) = \frac{2K}{a}\sum_n (2n+1)\frac{Q_n(\xi_0)}{Q'_n(\xi_1)}[P_n(\xi_1)Q'_n(\xi_1) - P'_n(\xi_1)Q_n(\xi_1)]P_n(\eta) \quad (2)$$

where K is a constant quantity $K = (q/4\pi D)\ C_{\text{source}}$, with C_{source} being the concentration of the chemoattractant solution in the pipet, P_n is a Legendre polynomial of order n, Q_n is a Legendre polynomial of the second kind of order n, and P'_n and Q'_n are their respective first derivatives.

To estimate the number of occupied receptors on halves of the ellipsoidal cell facing toward and away from the source of chemoattractant, it was assumed that the number of occupied receptors is determined by $N_{\text{total}}C/(C+K_d)$, where N_{total} is the total number of receptors on the cell surface, C is the chemoattractant concentration on the surface, and K_d is the equilibrium dissociation constant for binding of chemoattractant molecules to the receptor. The calculation was done under the assumption that receptors are homogeneously distributed across the cell surface, an assumption which has a solid experimental foundation.[7] The final expression for the number of occupied receptors on an ellipsoidal cell reads

$$N = \frac{N_{\text{total}}}{\sqrt{\xi_1^2 - 1} + \xi_1^2 \text{Arcsin}\ \xi_1^{-1}} \int \frac{C(\xi_1,\eta)}{C(\xi_1,\eta) + K_d}\sqrt{\xi_1^2 - \eta^2}\,d\eta \quad (3)$$

where $C(\xi_1,\eta)$ is defined by eq 2. Integration over η in the interval [0, 1] will result in the number of bound receptors on the front half ellipsoid, N_f, and integration in the interval [−1, 0] will give the number of bound receptors on the back half ellipsoid, N_b. Numerical integration of the eq 3 was performed in *Mathematica*, Wolfram Research, Inc.

Since we are primarily interested in the dependence of N_f and N_b on cell shape, we will use a shape descriptor defined by $E = \log_2 (a/b)$ to describe cell elongation, where a and b are the major and the minor axes of an ellipsoid, respectively.[8] Integration of eq 3 is then performed for ellipsoids of varying elongation E. As elongation rises from $E = 0$, which corresponds to a spherical cell, the volume-to-area ratio will diminish. We choose to keep the volume constant and calculate the interfocal distance a from it, whereas ξ_1 is determined by E. This decision is substantiated by observations made by scanning electron microscopy that rounded, nearly spherical *Dictyostelium* cells have a rippled, undulated surface, whereas elongated aggregation-competent cells have a smooth surface. A reservoir of cell plasma membrane available for extension has also been discovered in other cell types.[9] For extreme elongation considered in this paper, $E = 4$, the total cell surface area increases by a factor of 2 with respect to a spherical cell, which is biologically plausible.

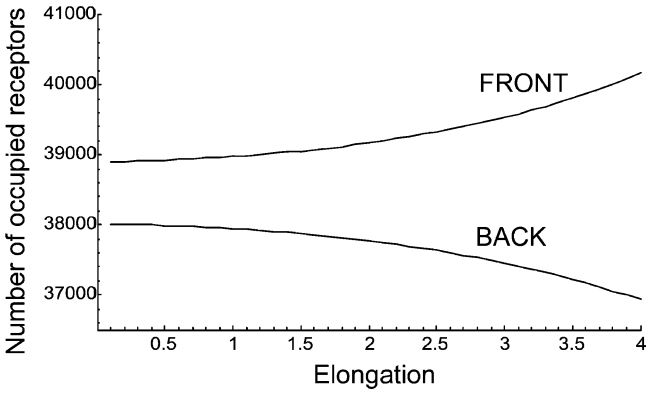

Figure 2. Number of occupied chemoattractant receptors plotted against cell elongation E for the front, N_f, and back, N_b, half ellipsoids. Concentration at a distance from the point source equivalent to the position of the cell midpoint, K_c, was set equal to K_d. Radius of the initial spherical cell was set to $R = 2\ \mu$m, and the distance of the cell center from the source was 40 times larger ($d = 80\ \mu$m). The total number of receptors was set to 100 000.

The average total number of cAMP receptors of the CAR1 type on the surface of an aggregation-competent *Dictyostelium* cell has been estimated to be approximately 10^5, and we will use that estimate in this calculation.[7] Other constants of calculation that determine receptor occupancy on the surface of an ellipsoidal cell, D, C_{source}, K_d, and q, are lumped together in terms of the chemoattractant concentration K_c at a distance from the source equivalent to the position of the middle of the cell, which is varied in the calculation from $2^{-10} \times K_d$ to $2^{10} \times K_d$, an interval that covers 6 orders of magnitude. The sole remaining free parameter of the model is the distance between the point source and the center of the cell, d, which can be compared to the radius of a spherical cell, a cell that has elongation $E = 0$.

RESULTS

The total number of occupied receptors on the surface of an ellipsoidal cell depends only weakly on cell elongation E. As it can be seen from Figure 2, while a number of occupied receptors slowly increases on the front half, it slowly falls off on the back half. This is to be expected, since due to the cell elongation more receptors are brought closer to the source at the front, and more receptors are brought farther from the source at the back. It has to be kept in mind that elongation E is a logarithmic quantity and that a ratio of 16 between lengths of the major and the minor

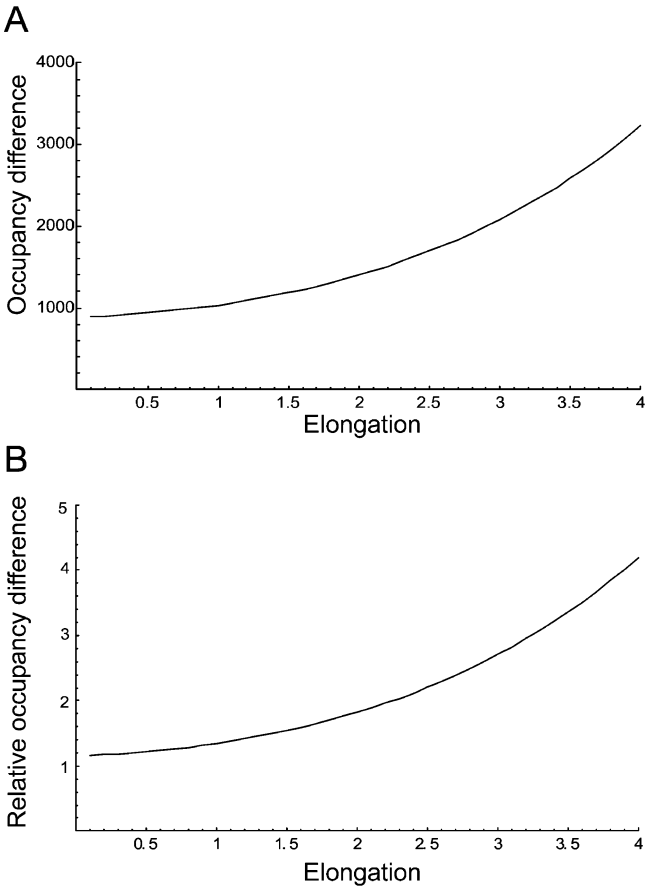

Figure 3. Absolute difference ΔN, (A), and relative difference ΔN_R expressed in percentages, (B), in the number of occupied chemoattractant receptors between the front and the back half of an ellipsoidal cell plotted against cell elongation E. The parameters of calculation are identical to those used in Figure 2. $\Delta N(E=4)/\Delta N(E=0) \approx \Delta N_R(E=4)/\Delta N_R(E=0) \approx 3.6$.

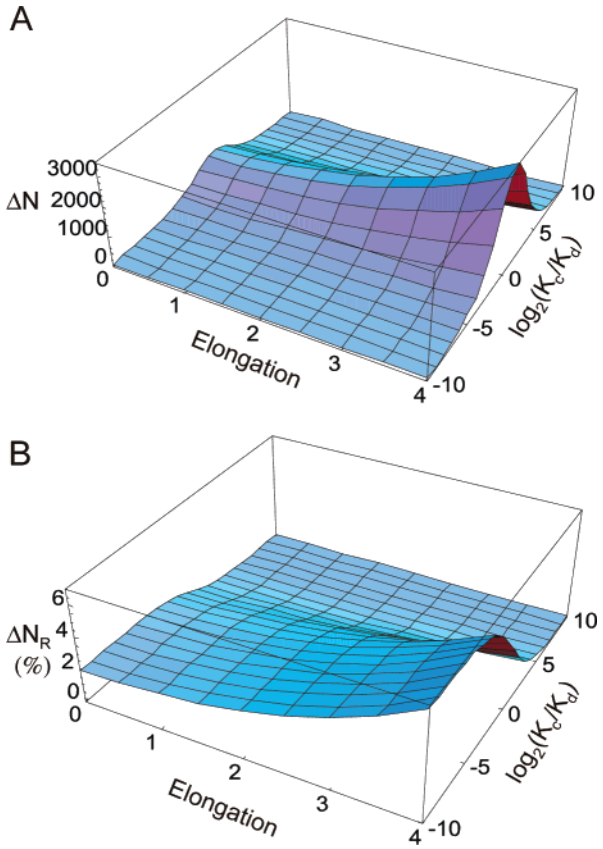

Figure 4. Absolute difference ΔN, (A), and relative difference ΔN_R expressed in percentages, (B), in the number of occupied chemoattractant receptors between the front and the back half of an ellipsoidal cell plotted against cell elongation E and the parameter $\log_2 (K_c/K_d)$, which relates chemoattractant concentration at the cell center, K_c, to the equilibrium dissociation constant of chemotactic receptors, K_d. Note that both parameters are binary logarithms. Other parameters of calculation are identical to those used in Figure 2.

ellipsoid axes, corresponding to $E = 4$, brings about only a difference of about 3% in total receptor occupancy compared to a spherical cell.

Quantities relevant for chemotactic sensitivity of cells are the occupancy difference, $\Delta N = N_f - N_b$, and, in particular, the relative occupancy difference, $\Delta N_R = (N_f - N_b)/(N_f + N_b)$, of chemotactic receptors between the two cell compartments. As usual,[10] we approximated these compartments by two halves of the cell proximal and distal to the point source of chemoattractant, in this case the front and the back half ellipsoids. Figure 3 shows that these important differentials increase by a factor of approximately 3.6 in the course of cell elongation from $E = 0$ to $E = 4$. This increase is relatively insensitive to changes of the absolute chemoattractant concentration in the medium, i.e., to changes of K_c, meaning that a cell will profit from a certain elongation to the same degree, no matter what absolute concentrations it has to measure. This fact can be appreciated at best by representing the two quantities, ΔN and ΔN_R in a three-dimensional plot, with elongation E and a chemoattractant concentration at the center of the cell, K_c, as parameters (Figure 4).

Cells can detect differences in the absolute number of occupied receptors most efficiently at external chemoattractant concentrations near the equilibrium dissociation constant of the receptors. This is corroborated by our calculations for the case of ellipsoidal cells, where ΔN is maximal for $K_c \approx K_d$, and falls off nearly symmetrically for higher and lower chemoattractant concentrations (Figure 4A). We note again that ΔN increases with elongation by the same factor for all values of K_c, which means that the effect of E on ΔN is the same irrespective of the absolute concentration of the chemoattractant. ΔN itself, however, strongly diminishes with increasing or decreasing concentrations, resulting in less than 10 occupied receptors difference between front and back halves for $K_c/K_d = 1000$ or 10^{-3}, a reduction by a factor of more than 150.

Since cells have to resolve relatively small differences in the number of occupied receptors between two spatial compartments against a much larger background of the total number of occupied receptors, the relative difference in receptor occupancy, ΔN_R, is the relevant measure of chemotactic sensitivity. As it can be seen from Figure 4B, ΔN_R increases with a decreasing chemoattractant concentration. It should be noted, however, that at extremely low values of K_c the ability of cells to sense spatial variations will diminish again because of the effects of noise. For instance, even at a relatively high $K_c = K_d/64$, $\Delta N = 36$, whereas $\sqrt{N_f} = 31$. This problem of noise, which invokes the need for temporal integration of chemotactic signal, has been treated extensively in the literature.[11] Let us just note that cells probably can orientate best in chemoattractant concentrations somewhat lower than the K_d of their receptors. Analogous to ΔN, the

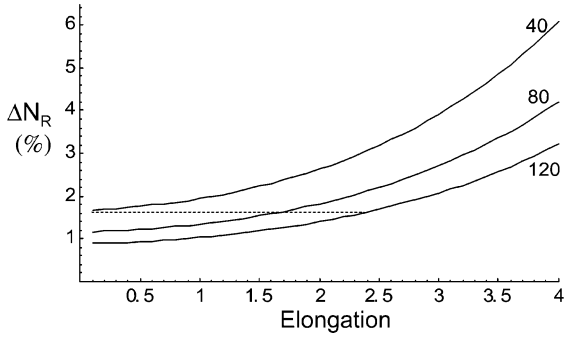

Figure 5. Relative difference ΔN_R in the number of occupied chemoattractant receptors between the front and the back half of an ellipsoidal cell plotted against cell elongation E for three values of distance between the cell center and the chemoattractant source, $d = 40\ \mu$m, $d = 80\ \mu$m, and $d = 120\ \mu$m. The dashed line intersects the $d = 80\ \mu$m and $d = 120\ \mu$m curves at the values of elongation that cells placed at these positions would need to have in order to achieve the same ΔN_R as a spherical cell at $d = 40\ \mu$m. Each of these curves was calculated under the assumption that $K_c = K_d$ at the cell center. Other parameters of calculation are identical to those used in Figure 2.

increase of ΔN_R with E is similar for all values of K_c, and we reiterate that it will be similarly beneficial for a cell to elongate irrespective of the range of concentration it has to measure.

Finally, we tested the effect of cell elongation on ΔN_R at different distances of a cell from a point source of the chemoattractant (Figure 5). The cell size was kept constant, and it was placed at three different distances from the source, 40 μm, 80 μm, and 120 μm. Note that all previous calculations were performed with this distance set to 80 μm. To reach the same ΔN_R as a spherical cell at $d = 40\ \mu$m from the source, a cell at $d = 80\ \mu$m would have to elongate by $E = 1.7$ ($a/b = 3.25$), and a cell at $d = 120\ \mu$m would have to elongate by $E = 2.4$ ($a/b = 5.28$). These values of elongation are typically attained by chemotactic aggregation-competent *Dictyostelium* cells.[12] Loosely speaking, in terms of chemotactic sensitivity it is more efficient for a cell to stretch out than to move many times its length.

DISCUSSION

Chemotactic eukaryotic cells can respond to small differences in chemoattractant concentration between the front and the back of the cell. To sense such weak gradients, cells must compare differences in receptor occupancy along their length and convert this signal into a localized response. Diffusion of a chemoattractant from a micropipet is routinely used to examine cell's chemotactic response. In a quantitative analysis of this response, cells were considered to be spherical bodies,[5] which is not appropriate for elongated cells such as neutrophils and especially for amoeboid *Dictyostelium* cells during aggregation. The aim of this paper was to investigate to what extent cell elongation influences the distribution of occupied chemotactic receptors on a cell placed in a gradient of chemoattractant diffusing from a micropipet.

In the standard micropipet assay for testing chemotaxis of *Dictyostelium* cells,[7,13] the chemoattractant cAMP ($D = 300\ \mu m^2/s$) is diffusing at a typical rate $q = 10^{-11}$ mL/s from a pipet containing a 10^{-4} molar solution of cAMP. The concentration gradient of cAMP, in the absence of cells and other objects, will under these conditions be defined by $C(d) \approx 3 \times 10^{-7}\ d^{-1}$, where C is expressed in moles and the distance between the micropipet tip and the cell center, d, in micrometers. Since equilibrium dissociation constant for binding of cAMP to the high-affinity species of the primary cAMP receptor in *Dictyostelium*, cAR1, is $K_d \approx 30$ nM,[14] the condition used for calculations presented in this paper, $K_c = K_d$, is satisfied at $d = 10\ \mu$m. It follows from these considerations that the standard micropipet assays for *Dictyostelium* chemotaxis, where cells are positioned at distances between 20 and 200 μm away from the pipet, are performed under conditions where K_c is between $K_d/2$ and $K_d/20$, a range of concentrations optimal for cell orientation. It is most likely that, while establishing the assay, experimenters arrived at these optimal conditions by varying release rate and cAMP concentration in the pipet. This is also a range of concentrations in which cell elongation is the only way to increase cell sensitivity during the initial chemotactic response (see below).

The micropipet assay has recently been turned into a quantitative tool by which *Dictyostelium* cells can be exposed to precisely controlled chemoattractant gradients.[3,15] To simplify quantitative analysis of a number of chemotactic responses, cells were immobilized and rounded in these experiments by treatment with an actin-depolymerizing substance. Results of theoretical analysis presented in the present paper could be used to investigate and quantitatively interpret chemotactic responses of elongated cells.

Apart from being relevant for quantitative interpretation of the micropipet assay, this investigation also has more general implications for research on chemotaxis. It follows from the presented results that elongation is an efficient way for a cell to increase its ability to orientate in gradients of chemoattractant concentration. The relevant measure of this ability, ΔN_R, approximately doubles at elongation $E = 2.5$ and triples at elongation $E = 3.5$, values that can be readily attained by *Dictyostelium* cells in the phase of their highest chemotactic activity during aggregation.[12] A cell has a rather limited repertoire of measures at its disposal to increase its chemotactic sensitivity. Agonist-induced phosphorylation of G-protein-coupled cAMP receptors has been shown to facilitate the desensitization processes by lowering ligand affinity of these receptors in *Dictyostelium*.[16] Such desensitization at the level of chemotactic receptors serves as an efficient way of adaptation that enables a cell to keep K_d of its receptors in the range optimal for measuring concentration present in medium.

The agonist-induced phosphorylation of chemoattractant receptors which lowers their affinity for the ligand may be useful in maintaining the cell's sensitivity at high cAMP levels. Equilibrium dissociation constant of the phosphorylated cAR1 receptor is approximately 2 orders of magnitude lower than the K_d of unmodified receptor.[14] It would be of interest to extend the present model to include the phosphorylation step. This would amount to sum occupancy of two receptor populations over a range of the phosphorylated receptor fractions, using eq 3 with the two respective dissociation constants. Such a calculation would obviously result in an increase of sensitivity in the range of cAMP concentrations corresponding to the K_d of phosphorylated receptors, proportionally to the fraction of phosphorylated receptors. An exact approach, however, would have to

include the full kinetics of the receptor phosphorylation and dephosphorylation.

Aggregation-competent *Dictyostelium* cells secrete a highly specific cAMP phosphodiesterase into an extracellular medium which decreases the cAMP levels in the cell vicinity by degrading it. Although the importance of this enzyme has been demonstrated for aggregation and late development in *Dictyostelium*,[17] no information is available about its possible role in adaptation during the chemotactic response on the level of single cells. Since the activity of the cAMP phosphodiesterase is triggered by cAMP, it is possible that this enzyme is involved, by a feedback mechanism, in adjusting the external cAMP concentration to the optimal affinity interval of cell's cAMP receptors.

Receptor desensitization, i.e., the lowering of their affinity for the ligand, will obviously be the most efficient way for a cell to adapt and measure concentrations higher than the K_d of unexposed receptors. For concentrations lower that the receptor's K_d, however, cell elongation is the only way to increase a cell's ability to orientate in shallow gradients. The main advantages of cell elongation is that it can be effected instantaneously since it does not depend on any complex biochemical feedback mechanism and that it is equally efficient under all conditions of external chemoattractant concentration. Cell elongation can be of decisive importance in augmenting the initial chemotactic response, when the cell is exposed to a weak signal gradient for the first time. It is therefore plausible to propose that the elongated shape of aggregation-competent *Dictyostelium* cells has evolved, at least in part, for the reasons of improved directional sensing.

ACKNOWLEDGMENT

The author thanks Jeffrey E. Segall for his support in the early phase of this research.

REFERENCES AND NOTES

(1) Van Haastert, P. J.; Devreotes, P. N. Chemotaxis: signaling the way forward. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 626−634.

(2) Jin, T.; Zhang, N.; Long, Y.; Parent, C. A.; Devreotes, P. N. Localization of the G protein $\beta\gamma$ complex in living cells during chemotaxis. *Science* **2000**, *287*, 1034−1036.

(3) Janetopoulos, C.; Ma, L.; Devreotes, P. N.; Iglesias, P. A. Chemoattractant-induced phosphatidylinositol 3,4,5-trisphosphate accumulation is spatially amplified and adapts, independent of the actin cytoskeleton. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8951−8956.

(4) Gerisch, G.; Keller, H. U. Chemotactic reorientation of granulocytes stimulated with micropipets containing fMet-Leu-Phe. *J. Cell Sci.* **1981**, *52*, 1−10.

(5) Segall, J. E. Polarization of yeast cells in spatial gradients of α mating factor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8332−8336.

(6) Morse, P. M.; Feshbach, H. *Methods of Theoretical Physics*; McGraw-Hill Book Company, Inc.: New York, 1953; pp 1284−1292.

(7) Xiao, Z.; Zhang, N.; Murphy, D. B.; Devreotes, P. N. Dynamic distribution of chemoattractant receptors in living cells during chemotaxis and persistent stimulation. *J. Cell Biol.* **1997**, *139*, 365−374.

(8) Dunn, G. A.; Brown, A. F. *Quantifying cellular shape using moment invariants;* Springer series: Lecture Notes In Biomathematics, Vol. 89: Biological Motion; Alt, W., Hoffmann, G., Eds.; Springer-Verlag: Berlin, 1990; pp 254−268.

(9) Raucher, D.; Sheetz, M. P. Characteristics of a membrane reservoir buffering membrane tension. *Biophys. J.* **1999**, *77*, 1992−2002.

(10) Lauffenburger, D. A. Influence of external concentration fluctuations on leukocyte chemotactic orientation. *Cell Biophys.* **1982**, *4*, 177−209.

(11) Moghe, P. V.; Tranquillo, R. T. Stochastic model of chemoattractant receptor dynamics in leukocyte chemosensory movement. *Bull. Math. Biol.* **1994**, *56*, 1041−1093.

(12) Weber, I. Computer-assisted morphometry of cell-substratum contacts. *Croatian Med. J.* **1999**, *40*, 334−339.

(13) Weber, I.; Wallraff, E.; Albrecht, R.; Gerisch, G. Motility and substratum adhesion of *Dictyostelium* wild-type and cytoskeletal mutant cells: a study by RICM/bright-field double-view image analysis. *J. Cell* Sci. **1995**, *108*, 1519−1530.

(14) Dormann, D.; Kim, J. Y.; Devreotes, P. N.; Weijer, C. J. cAMP receptor affinity controls wave dynamics, geometry and morphogenesis in *Dictyostelium*. *J. Cell Sci.* **2001**, *114*, 2513−2523.

(15) Xu, X.; Meier-Schellersheim, M.; Jiao, X.; Nelson, L. E.; Jin, T. Quantitative imaging of single live cells reveals spatiotemporal dynamics of multistep signaling events of chemoattractant gradient sensing in *Dictyostelium*. *Mol. Biol. Cell* **2005**, *16*, 676−688.

(16) Caterina, M. J.; Devreotes, P. N.; Borleis, J.; Hereld, D. Agonist-induced loss of ligand binding is correlated with phosphorylation of cAR1, a G protein-coupled chemoattractant receptor from *Dictyostelium*. *J. Biol. Chem.* **1995**, *270*, 8667−8672.

(17) Wessels, D. J.; Zhang, H.; Reynolds, J.; Daniels, K.; Heid, P.; Lu, S.; Kuspa, A.; Shaulsky, G.; Loomis, W. F.; Soll, D. R. The internal phosphodiesterase RegA is essential for the suppression of lateral pseudopods during *Dictyostelium* chemotaxis. *Mol. Biol. Cell* **2000**, *11*, 2803−2820.

CI050165X