Inversely Correlated Cycles in Speed and Turning in an Ameba: An Oscillatory Model of Cell Locomotion

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ABSTRACT Previous biophysical models of ameboid crawling have described cell movement in terms of a persistent random walk. Speed and orientation were treated in the latter model as independent and temporally homogeneous stochastic processes. We show here that, at least in the case of *Dictyostelium discoideum*, both speed control and reorientation processes involve a deterministic, periodic component. We also show that the processes are synchronized and negatively correlated, as was suggested by earlier findings. That is, increased turning correlates with periods of slow movement. Therefore, previous models are inconsistent with the behaivor of cells. Using a heuristic approach, we have developed a mathematical model that describes the statistical properties of the cell's velocity and movement of its centroid. Our observations and the model are consistent with the phenomenological description of ameboid motility as a cyclic process of pseudopod extension and retraction.

INTRODUCTION

Many motile eukaryotic cells move by a crawling process. A fast-moving cell like a neutrophil or *Dictyostelium discoideum* goes through the following sequence of steps: pseudopod extension, cytoplasmic flow into the pseudopod, and finally contraction, which includes tail detachment and culminates in a "cringe" stage when the cell momentarily ceases translocation (Satoh et al., 1985; Murray et al., 1992; Wessels et al., 1994). Translocation of a cell may be characterized by speed and direction. At short observation times cell movement can be described with one parameter, speed. This is in the directed (or persistent) mode of movement. At longer observation times the cell no longer moves in the original direction. If the cell's environment is uniform, the travel direction eventually becomes totally randomized. The time it takes a cell to randomize the travel direction is termed "directional persistence time," or "persistence time" for short. At observation times longer than the persistence time, deterministic description of cell movement is no longer possible; instead of predictions regarding the actual position of the cell, one can only estimate the probability of finding a cell in a certain area. Therefore, at these times motility is in its stochastic (or diffusive) mode.

Fundamental work by Doob (1942) has served as a basis for mathematical models (Gail and Boone, 1970; Schienbein and Gruler, 1993) of cell locomotion that adequately describe the transition from deterministic to stochastic behavior. In the last paper, speed and direction of cell movement were treated as two independent stochastic functions of time, and both processes were assumed to be temporally homogeneous. The model predictions were surprisingly accurate for time-averaged measures of cell locomotion, even for galvanotaxis or chemotaxis. The dose-response curve predicted by this model for cell orientation in a polar field was in good agreement with experimental ones obtained with various cell types and in different kinds of fields (Gruler and Franke, 1990). Nevertheless, the predictions of this model for time-dependent measures of cell movement were not adequate. For example, it failed to explain observations made by the same group that cell reorientation after change in polarity of an applied electric field was delayed (Franke and Gruler, 1990). More importantly, the model predictions for mean square displacement of cell centroid (centroid MSD) in the absence of a field did not always hold. If one used parameter estimates obtained from the initial part of the MSD curve to predict the behavior of the curve at larger observation times, experimental MSDs often declined below those predicted by the theory (see, for example, figure. 3 in Stokes et al., 1991).

If predictions of a mathematical model are at odds with experimental results, the problem usually originates from the assumptions that the model is based on. Let us first consider the assumption of temporal homogeneity. This feature of a system (with regard to a certain external perturbation) means that the reaction of the system to a perturbation is always the same, no matter when the perturbation occurs. The original work by Doob (and even earlier analyses by Uhlenbeck and Ornstein, 1930) considered the diffusion of gas molecules at low pressures. In such a system, nothing but collisions among moving particles could alter their velocities. Apparently, each molecule of an inert gas is a temporally homogeneous system with regard to the random kicks it receives from the other molecules. Unlike the gas molecules, cells have a locomotory behavior of their own. In particular, turns of gas molecules as a result of collisions depend only on relative velocities and mass ratios, regardless of the time of a collision, whereas a cell turns into a new pseudopod only after this pseudopod has

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been created and chosen to form a new leading edge. This cannot happen at any moment regardless of the stage of the locomotory cycle the cell is at (Sylwester et al., 1995; Murray et al., 1992; Wessels et al., 1994). In fact, the fraction of the cycle period when a cell is turning-competent may be rather small. Therefore, the assumption of temporal homogeneity does not seem to hold for a motile cell. One would expect statistical properties of cell dispersal to differ most dramatically from those of Ornstein-Uhlenbeck diffusion if the directional persistence time is close to the cycle time, that is, if it takes a cell only a few motility cycles to randomize the direction of movement.

Another important assumption is the one of statistical independence of speed and orientation. Whereas it is obviously true for gas molecules, cells are again quite different. On one hand, agents are known that alter speed without noticeably affecting persistence time (Gruler, 1987). One can also suppress the chemotactic movement of *D. discoideum* cells, by clamping Ca^{2+}, without impairing the cell's ability to extend pseudopods and elongate preferentially toward the source of chemoattractant (Van Duijn and Van Haastert, 1992). These authors interpreted their observations as evidence for independent control of locomotion and orientation. On the other hand, as we mentioned above, big turns are synchronized with the motility cycle, and so are major speed changes (Nossal and Zigmond, 1976; Wessels et al., 1994; Sylwester et al., 1995; Murray et al., 1992). To resolve the issue of dependence or independence of the two parameters, one has to experimentally characterize their cross-correlation. No data on this could be found in the literature.

Yet another concern about the applicability of a theory based on Ornstein-Uhlenbeck processes to cell locomotion comes from the non-Markov behavior of partial autocorrelation functions of cell speed and the cosine of its travel angle. If the locomotion process were purely random, the partial autocorrelation functions must have been found to contain only one nonzero value, because any purely random process is a Markov chain and events of a Markov chain are correlated only with a step lag of 1, but not any other step lag. Experimental observations of significant correlations for other step lags (Hartman et al., 1994) suggest a deterministic component in the speed and orientation control processes. Likewise, if cell reorientation were driven by a purely random process, the fraction of cells that retain initial direction of their movement would decay as a single-exponential function of time (Gruler and Bultmann, 1984). The experimentally determined dependencies are always bimodal, with a large drop after a certain time lag and relatively slow decay after that. It is important to mention here that both studies were done with fast-moving human neutrophils; thoroughly analyzing the slow crawling of chick heart fibroblasts, Dunn and Brown (1987) found no evidence for non-Markov behavior. Evidence for a nonstochastic component has led the authors (Gruler and Franke, 1990; Hartman et al., 1994) to suggest that a deterministic, perhaps cyclic process was involved in both orientation and speed controls. It has also led to the concepts of an internal clock and an internal program governing cell locomotion.

A variety of motile cell types demonstrate oscillatory, quasiperiodic behavior (Satoh et al., 1985, and references therein; Killich et al., 1993; Mandeville et al., 1995; Wessels et al., 1994; Murray et al., 1992). The cyclic character of migration results in a certain degree of periodicity. Shown in Fig. 1 is a cell centroid track illustrating this kind of quasiperiodicity. The stretches of persistent movement are interrupted with brief stops in centroid translocation, corresponding either to completion of pseudopod extension or to the end of contraction. It is during these brief stops (pauses) that the cell reorients itself, or turns (Mandeville et al., 1995; Wessels et al., 1994; Murray et al., 1992). The only mathematical model in the literature we are aware of that deals with the locomotory cycle as a periodic process (DiMilla et al., 1991) provides a detailed one-dimensional description of influence of cell-substratum adhesion and cell mechanics on translocation speed. It does not consider reorientation and therefore cannot predict centroid MSDs, because MSD behavior depends on reorientation as well as on speed.

The purpose of the present work was to find experimentally a set of physically justifiable principles for constructing a model of cell locomotion that would account for its cyclicity and give predictions about the traditional measure of motility, the centroid MSD. We also developed a simple mathematical description of the cell dynamics that describes the cycles as well as the peculiarities of centroid MSDs at longer observation times.

MATERIALS AND METHODS

We used wild-type *D. discoideum* cells, strain Ax3, at 4–6 h into the development cycle plated on alcohol-washed glass. The cells were observed in bright field with 10× objective (final scale 1 μm/pix) and video recorded for 1–2 h for further processing. The following equipment was used: Olympus BH-2 microscope (Olympus Optical Co., Tokyo, Japan), Hamamatsu C2400 videocamera and Argus-10 videoprocessor (Hamamatsu Photonics K.K., Hamamatsu City, Japan), and model PV-98A PC-VCR (NEC Corporation, Tokyo, Japan). Images were subsequently digitized and stored on a real-time disk every 0.6 s. The cell centroid tracking was performed with the use of a routine described elsewhere (Gelles et al., 1988). The resulting data table was fed to a MATLAB software package (MathWorks, Natick, MA) as an input for a dedicated routine for generating an arranged data table after excluding the data on the cells that have been lost during the tracking. Only tracks longer than 20 min

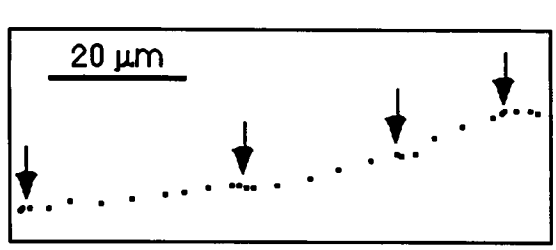

FIGURE 1 Typical cell centroid track. Time lag between the points is 12 s. Arrows indicate points of near-stopping.

were used. Further processing, including filtering (final high-frequency cutoff at 0.208 Hz), as well as fitting procedures, was also done with use of the MATLAB package. For calculating mean square displacements of cell centroids (centroid MSD; see below), the displacements were taken over nonoverlapping time intervals to avoid artifacts due to mutual correlation of displacements (see Dickinson and Tranquillo, 1993).

RESULTS

Periodicity of turning

When the cells were followed for up to 2 h with the video microscope, they moved continuously with no obvious change in speed or frequency of turning due to photodamage or anoxia. A turning rate was defined as illustrated in Fig. 2 *A*. Our preliminary experiments showed that the choice of the time lag between points defining a turn was improrant. When the lags were too short, the turns were obscured by noise, whereas when the lags were too long, the turns tended to be smoothed out.

We calculated autocorrelation functions (ACFs; see definition in the legend to Fig. 2) of turn rate. Typical results for an individual cell are shown in Fig. 2 *B*. Several secondary peaks are observed, separated by lags of 1–3 min, indicating turning cycles with this period. This is a representative trace of 14 analyzed tracks, and all of those showed quasiperiodicity with minimum cycle times of 1–3 min.

Periodicity of speed control

The speed ACF for an individual cell is shown in Fig. 2 *C*. Again, we observed strong secondary peaks at intervals of a few minutes, indicating that cell speed was also quasiperiodical (in all analyzed cases). This is the same cell as in Fig. 2 *B* and shows the same periodicity for speed as was observed for turning.

Negative correlation between turning and speed

Because the same periodicity of turning and speed was commonly observed, we analyzed the phases of the two parameters to determine if they were correlated. Plotting the speed and turn rate of an individual cell versus time, one immediately sees that they are out of phase (Fig. 3 *A*). To analyze data from a larger pool of cells, we grouped points of 14 tracks according to the instantaneous speed at a given point (0 . . . 2, 2 . . . 4, and 4 . . . 6 μm/min) and calculated the conditional angular ACFs for each group separately. The term "conditional" means that a condition was applied for selecting data points before computing the ACFs. The ACFs differed from each other so that the slowest moving corresponded to the fastest randomization of direction (Fig. 3 *B*). Scatter plots of speed versus turn rate (Fig. 3 *C*) give negative correlation coefficients and appear to consist of two distinct zones: turns and stretches of persistent movement. This corre-

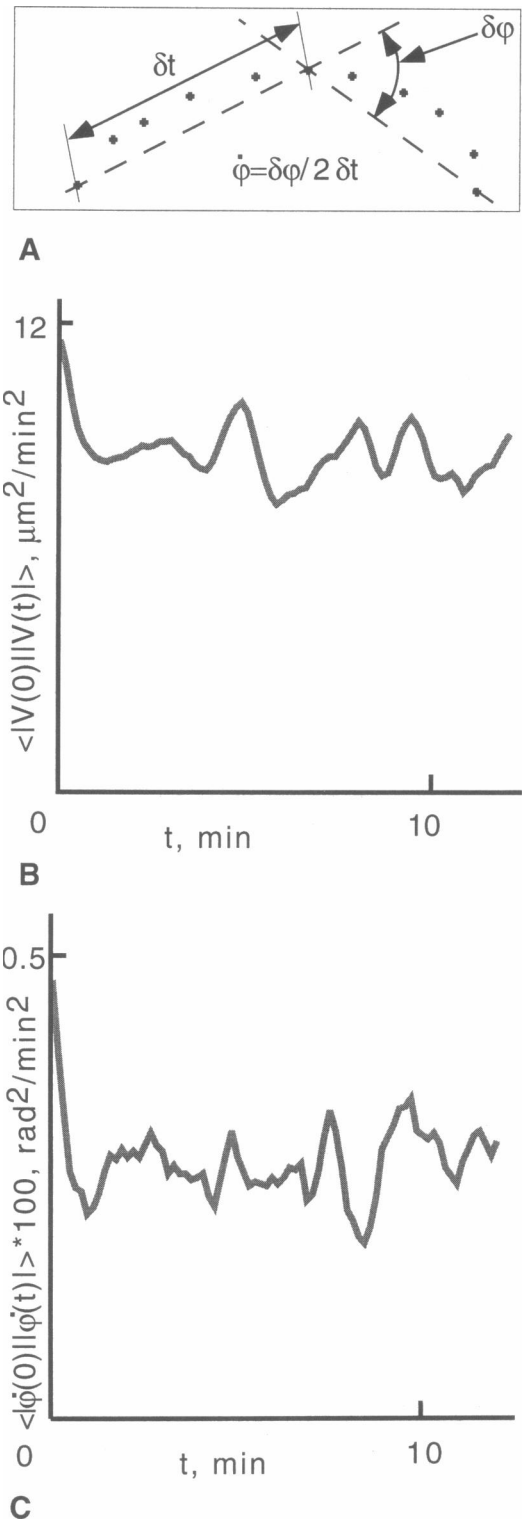

FIGURE 2 (*A*) Definition of the turn rate. To calculate the turn rate at a point of the track, two more points of the track were used, 38.4 s before and 38.4 s after the point in consideration. The absolute value of the angle between the two vectors of total centroid translocations was divided by total time to yield the turn rate. (*B*, *C*) Autocorrelation functions of (*B*) turn rate and (*C*) velocity for an individual cell track. The autocorrelation function was calculated as $\mathrm{ACF}(f,\tau) = \langle f(t)f(t+\tau)\rangle$, where $\langle f\rangle$ denotes an average of the function f over the period of observation.

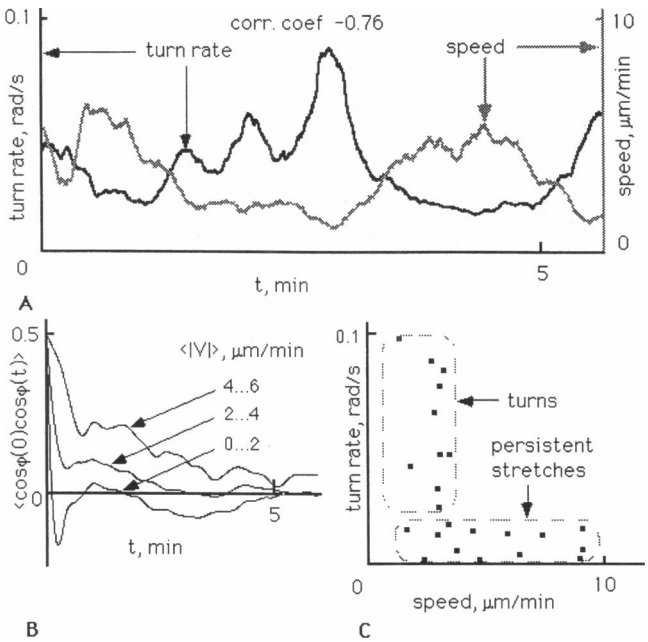

FIGURE 3 (*A*) "Instantaneous" velocity and turn rate versus time. Notice the opposite phases of the two processes. (*B*) Conditional ACFs for cosine of the travel angle, with respect to the initial travel direction, for different initial velocities. The term "conditional" denotes that before calculating the ACFs, a condition was imposed on the initial velocity of a cell. The initial velocities were chosen in the intervals 0 . . . 2, 2 . . . 4, and 4 . . . 6 μm/s. (*C*) Velocity-turn rate scatter plot. Notice the clear separation of the points into two groups.

lation exhibits itself in trajectory plots (Fig. 4) that consist of stretches of relatively persistent movement, separated by brief stops used by the cell for reorientation.

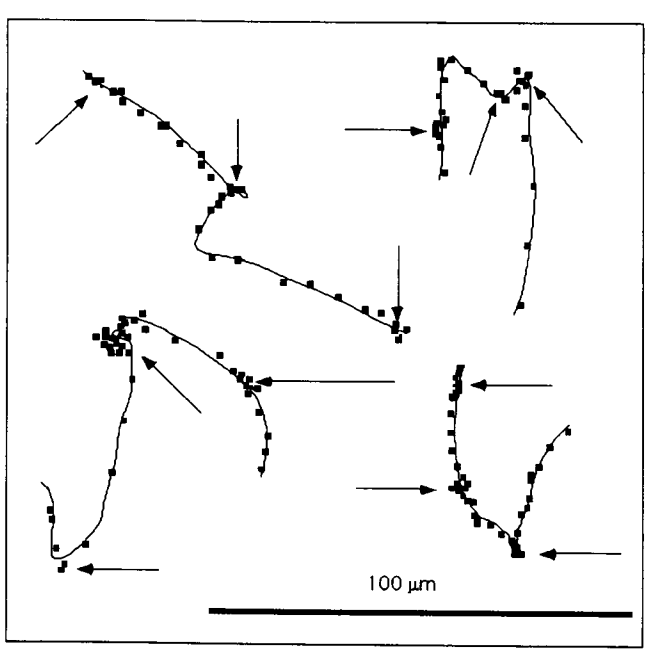

FIGURE 4 Experimental cell centroid tracks. Time lag between the points is 12 s. Scale bar, 100 μm.

Oscillatory model for cell dispersal

The previous models of cell migration over two-dimensional surfaces have been based on the Langevin equation for velocity. The velocity in such a theory is a two-component vector $[V_x, V_y]$. For clarity, we will explicitly write out only one out of each pair of identical equations for the two components. The Langevin equation for the component of cell velocity along a selected axis x is

$$\dot{V}_x + \gamma V_x = G_x, \tag{1}$$

where G_x is the x component of a random force acting on the cell centroid:

$$\langle G_x \rangle = 0, \quad \langle G_x(t_1) G_x(t_2) \rangle = q\delta(t_1 - t_2), \tag{2}$$

and all averages and correlation functions that can be measured experimentally were calculated from the solution of this equation. To introduce periodicity into the process, we added an extra term to the equation:

$$\dot{V}_x(t) + \gamma V_x(t) + \beta \int_{-\infty}^{t} V_x(\tau) e^{-\alpha(t-\tau)}\, d\tau = G_x(t), \tag{3}$$

which, after Fourier transform, gives

$$\tilde{V}_x = \tilde{G}_x \frac{i\omega + \alpha}{(i\omega + \gamma)(i\omega + \alpha) + \beta/\sqrt{2\pi}}. \tag{4}$$

For the roots of the denominator one obtains

$$\omega_{1,2} = i\frac{\alpha + \gamma}{2} \pm i\sqrt{\left(\frac{\alpha - \gamma}{2}\right)^2 - \beta/\sqrt{2\pi}}, \tag{5}$$

and the motility process is oscillatory if the roots have a nonzero real part (Fig. 5). This, in particular, means that, depending on combination of values of the parameters, cells may or may not exhibit oscillatory behavior, or may exhibit

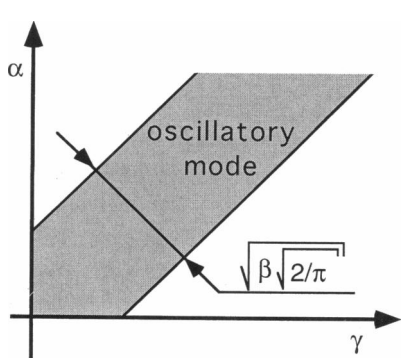

FIGURE 5 Parameter-space mode chart for Eq. 3. This plot shows that cells for which the parameters α and γ are within certain limits have oscillatory behavior, whereas other cells do not. The width of the oscillatory mode zone depends on β. Because values of the three parameters vary among the cells, some cells in the population may be in the oscillatory mode, and others in the nonoscillatory mode.

it to various degrees. The solution of Eq. 3 reads

$$V_x = M_1 \int_{-\infty}^{t} d\tau G_x(\tau) e^{i\omega_1(t-\tau)} + M_2 \int_{-\infty}^{t} d\tau G_x(\tau) e^{i\omega_2(t-\tau)}, \tag{6}$$

$$M_{1,2} = \frac{1}{\sqrt{2\pi}}\left(1 \pm \left[1 - \frac{4\beta}{\sqrt{2\pi}(\alpha-\gamma)^2}\right]^{-1/2}\right),$$

from where the velocity autocorrelation function is

$$\frac{1}{2\pi q}\langle V_x(t_1)V_x(t_2)\rangle = M_3 e^{i\omega_1|t_1-t_2|} + M_4 e^{i\omega_2|t_1-t_2|}, \tag{7}$$

$$M_{3,4} = M_{1,2}^2/2i\omega_{1,2} + M_1M_2/(i\omega_1 + i\omega_2),$$

and for the centroid MSD one obtains

$$\frac{1}{2\pi q}\langle (X(t) - X(0))^2\rangle = \frac{M_3}{i\omega_1}\left(t + \frac{1 - e^{i\omega_1 t}}{i\omega_1}\right) + \frac{M_4}{i\omega_2}\left(t + \frac{1 - e^{i\omega_2 t}}{i\omega_2}\right). \tag{8}$$

Assuming Gaussian distribution for the noise, we obtain the two-dimensional Maxwell distribution of speed (absolute value of the velocity):

$$\varphi(|V|) = \frac{|V|}{\sigma^2}\exp\left(-\frac{|V|^2}{2\sigma^2}\right)$$
$$\sigma = q(M_3 + M_4), \tag{9}$$

which gives a very good approximation of the experimental distribution (Fig. 6). Denoting $\langle V_x(t_1)V_x(t_2)\rangle/\sigma^2$ as $\rho(|t1 - t_2|)$ and $V_x/|V|$ as cos φ, we obtain

$$\langle |V(t_1)||V(t_2)|\rangle \cong \frac{2\sigma^2}{\pi}\left(1 + \rho^2(|t_1 - t_2|)\left(\frac{4}{\pi} - 1\right)\right) \tag{10}$$

$$\langle \cos\varphi(t_1)\cos\varphi(t_2)\rangle \cong \rho(|t_1 - t_2|)/2, \tag{11}$$

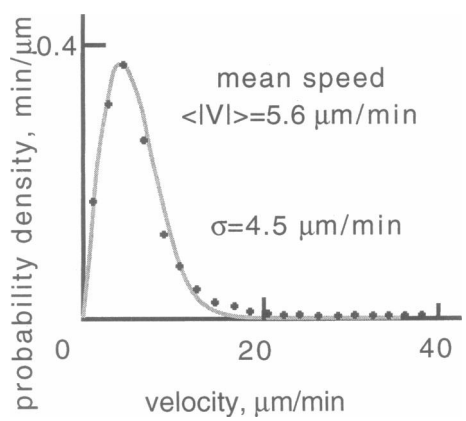

FIGURE 6 Speed distribution of an individual cell and its fit with Maxwell distribution (Eq. 9).

which offers a way to check theoretical predictions against experimental data (Fig. 7 *A*): this plot shows experimental speed ACF and predictions for it from Eq. 10, where $\rho(|t_1 - t_2|)$ was determined from the experimental angular ACF according to Eq. 11, whereas σ was calculated by nonlinear fit of Eq. 9 to experimental speed distribution. The conformity of the two plots is remarkably good. Notice that the time dependencies of the two ACFs are independently derived from those of speed and travel angle, respectively; therefore, these data also support the mutual dependence of cell speed and reorientation. The validity of the model is also supported by the good fits it yields for other experimental curves (Fig. 7 *B*, centroid MSD for a fast-moving cell with pronounced periodicity), as well as its accurate predictions for control values. For example, a prediction is that

$$\frac{\langle |V(0)||V(\infty)|\rangle}{\langle |V(0)||V(0)|\rangle} = \frac{\pi}{4} \cong 0.785.$$

Our measurements gave 0.73 ± 0.04 (mean ± SD), and Schienbein and Gruler (1993) have found this value to be 0.82 for human granulocytes. Similarly, another prediction is

$$\frac{\langle |V|\rangle}{\sqrt{\langle |V|^2\rangle - \langle |V|\rangle^2}} = \sqrt{\frac{\pi}{4 - \pi}} \cong 1.91.$$

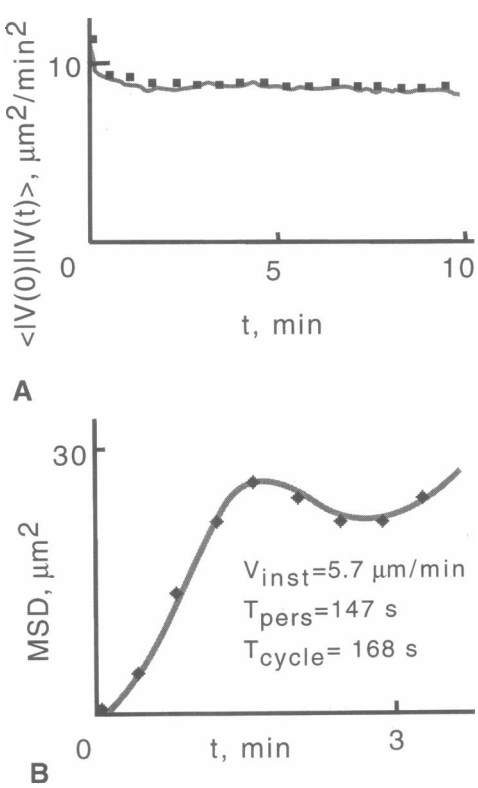

FIGURE 7 (*A*) Comparison of an experimental speed ACF with one derived from angular ACF for the same cell according to Eqs. 10 and 11. Parameter σ for the calculations was obtained by nonlinear fit of the velocities' distribution (Eq. 9, Fig. 6). (*B*) Fit of an experimental centroid MSD curve with Eq. 8. The fitting parameters were $V_{inst} = \sigma$; $T_{pers} = 1/\text{Real}(\omega_{1,2})$; and $T_{cycle} = 2\pi/\text{Imag}(\omega_{1,2})$.

We found this value to be 1.68 ± 0.15 (mean ± SD), and Schienbein and Gruler (1993) used it as a control parameter (they discarded data for a preparation of cells if this parameter did not exceed 1.5).

DISCUSSION

Locomotory cycle and stochastic models of cell motility

Our observations quantitatively describe the cyclic sequence of movement and turning of ameboid cells (E. L. Elson, personal communication; Murray et al., 1992; Sylwester et al., 1995; Wessels et al., 1994). The process of movement includes pseudopod extension, cytoplasmic flow into the pseudopod, and cell contraction with concomitant tail detachment. Changes in travel direction are associated with a certain stage of this cycle, namely pseudopod extension after cell rounding, which is necessarily correlated with slow movement. The cell speed, defined as the amplitude of the velocity of the cell centroid, also changes as the cell progresses through the cycle. Basically, both speed control and reorientation processes involved a deterministic, periodic component, consistent with the cyclic character of locomotion. We also showed that the processes are synchronized and negatively correlated.

On the other hand, previous biophysical models of ameboid crawling (Gail and Boone, 1970; Schienbein and Gruler, 1993) have treated speed and orientation control as two independent and temporally homogeneous stochastic processes. Analyzing our experimental tracks of crawling amoeba *D. discoideum* to test the assumptions of those theories, we found no evidence either for mutual independence or for temporal homogeneity of the two processes. Thus, only at observation times not exceeding the cycle periods do such models give a good representation of the centroid MSD data. At times on the order of the cycle period or longer, the MSD data are not modeled by the previous theory.

Features of the improved model

We have developed a heuristic approach to derive a stochastic equation of motion to fit the observations detailed here. Unlike the rigorous approach, the heuristic method of modeling allows one to predict the behavior of a system, within the framework of available phenomenological information, to a desired level of detail (Hartman et al., 1994; Risken, 1984). It is possible, then, to make corrections as more experimental information becomes available or to incorporate hypotheses about the influence of various factors on the behavior of the system (see examples of this in Lauffenburger, 1991).

Compared to the previous theories (Schienbein and Gruler, 1993; Gail and Boone, 1970), we made three major modifications. First, we treated velocity as a complex value rather than separating it into amplitude (speed) and direction for separate analyses. Therefore, consistent with our own observations, we described velocity control and reorientation as coordinated processes. As an important consequence of that, we no longer needed any assumptions about a "preferred speed" as a separate entity stored in the cell's memory (Gruler and Franke, 1990; Schienbein and Gruler, 1993). The RMS speed in our model is a combination of properties of the cell: power of the cell motor and parameters characterizing the dynamics of the locomotory machinery. Second, we derived speed distribution in the form of Maxwell distribution for two dimensions (Doob, 1942) rather than use its approximation by a Gaussian distribution (Schienbein and Gruler, 1993). This is important for an experimentalist who measures the absolute value of speed and needs a theoretical prediction for that value; a Gaussian is not a good approximation for this, because it gives nonzero probabilities for negative values of a quantity that is never negative. Finally, we introduced an extra term to the equation to explain the observed cyclic behavior.

Our model provides for the first time a mathematical description of the behavior displayed by many centroid MSDs. Cell populations are not uniform with respect to behaviors of individual cell MSDs. Initial portions of centroid MSD plots give reasonable fits to the Ornstein-Uhlenbeck model, but for times comparable to the duration of the motility cycle, the plots often show an inflection point or bounded behavior. Because damaged or injured cells will potentially give the bounded behavior, the cells showing slow movement and bounded behavior are usually excluded from MSD data. Even with cells that give good initial fits to the Ornstein-Uhlenbeck model, for the remainder of the curve the MSDs decline below the theoretical curve (for example, see figure 3 in Stokes et al., 1991). Our theory gives a very good fit of both the MSD plots at the intermediate as well as larger observation times.

Fast ameboid crawling is not a purely random process, as has been shown previously (Hartman et al., 1994; Gruler and Bultmann, 1984). Rather, an internal clock and an internal program govern cell motility. Cyclic behavior, consistent with these concepts and with the phenomenological description of ameboid crawling as cycles of pseudopod extension and cell contraction, is a characteristic feature of the present model.

On the interpretation of the oscillatory model

Because our model describes experimental data well, it may reflect the actual mechanism of crawling. One possible interpretation for Eq. 3 would be that it is in fact a force balance equation, similar to the Langevin equation (Eq. 1), but including an extra term in a "creep function" (Fung, 1993) describing viscoelastic properties of the moving cell. However, biomechanical data from studies on granulocytes suggest that inertia plays a very minor role in the mechanical balance of a crawling cell (R. M. Hochmuth, personal communication). Mechanical oscillations in such a system

would be overdamped, which indeed has been observed (Oster and Odell, 1984). Calculations show that Eq. 3, if understood as a force balance equation, would contradict these observations. At the same time, oscillatory modes of gelation-solation reactions in cytoplasm have been observed in both computational (Alt, 1985) and actual (Pohl, 1989) experiments. We therefore favor the idea that the origin of the observed dynamics, well described by Eq. 3, is physicochemical rather than mechanical. Let us consider, for example, a tripartite chemical system:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C.$$

Kinetics in this system are determined by the system of equations

$$\begin{cases} \dot{b} = G + k_1(B_0 - B - b - C - c) \\ \qquad - (k_{-1} + k_2)(B + b) + k_{-2}(C + c) \\ \dot{B} = k_1(B_0 - B - C) - (k_{-1} + k_2)B + k_{-2}C \\ \dot{c} = k_2(B + b) - k_{-2}(C + c) \\ \dot{C} = k_2B - k_{-2}C \end{cases}$$

where B is the equilibrium and b the nonequilibrium fractions of the concentration of the component B, C and c are the same for the component C, and G is noise in the system leading to concentration fluctuations. B_0 is the total concentration of the three interconvertible species. For the nonequilibrium fractions one obtains

$$\begin{cases} \dot{b} + \gamma b + \beta \int b(\tau) e^{-\alpha(t-\tau)} d\tau = G \\ c = k_2 \int b(\tau) e^{-\alpha(t-\tau)} d\tau, \end{cases}$$

where

$$\alpha = k_{-2},$$

$$\beta = k_2(k_1 - k_{-2}),$$

$$\gamma = k_1 + k_{-1} + k_2.$$

Assuming that A is G-actin, B is a labile pool of F-actin, and C is a stable pool of F-actin, we have the equation for the nonequilibrium fraction of the labile F-actin concentration, which is exactly like that for velocity. It is reasonable to assume that cell velocity is actually proportional to the gradient of labile F-actin concentration or some other component in a similar chemical process.

It is worth mentioning here that nonrandom, cyclic behavior of complex chemical systems (like, for example, a Belousov-Zhabotinsky reaction) exhibits itself in a "far from equilibrium" situation. In the above example of a tripartite system, the nonequilibrium fraction of the intermediate species has the oscillatory dynamics characteristic of the cell velocity. It is intuitively clear that in neutrophils or slime molds the cell cortex is much further from equilibrium than in slow crawling cells like fibroblasts. Therefore, finding non-Markov behavior in fast crawlers (Gruler and Franke, 1990; Hartman et al., 1994) and failure to find it in slow ones (Dunn and Brown, 1987) are both consistent with the idea of cell cortex dynamics governing the cellular internal clock.

CONCLUDING REMARKS

The tripartite model is far from complete, as it does not take into account many important features of the actin cytoskeleton (diffusibility of actin, for example). Therefore, it should be considered an example of a system yielding the dynamic law identical to Eq. 3 rather than the "true" mechanism of cell locomotion. It also serves to illustrate how a mechanistic model may yield predictions testable on experimental tracking data. In particular, the tripartite model permits the calculation of correlations among changes in parameters of Eq. 3 as functions of the kinetic constants of the system. Such correlations may then be experimentally tested, even when the true values of any of the constants are not known.

The oscillatory model by itself can be used by experimentalists to quantify the motile behavior of cells in a more successive and comprehensive manner than has been achieved before (see, for example, Dunn and Brown, 1987). It may also be useful for correlating immune disorders with motile characteristics of white blood cells (see, for example, Sylwester et al., 1995).

In summary, we found that in the slime mold *D. discoideum*, variations in speed and orientation of movement are correlated and quasiperiodical. Thus speed and direction are not controlled independently of each other in the crawling amoeba, which contradicts the assumptions of previous biophysical theories of ameboid movement (Schienbein and Gruler, 1993). We suggest a simple theory that describes the quasiperiodical character of crawling. The theory correctly predicts deviations from the "classical" persistent random-walk behavior observed in MSD plots at intermediate observation times. It also correctly describes the shape of speed distribution for an individual cell and correctly predicts the relationship among this distribution, cell centroid MSD, and autocorrelation functions for speed and cosine of the travel angle. Thus, for the first time, we can start to model cell locomotion on surfaces as a cyclical process.

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