Introduction

Eukaryotic cells move by extending pseudopodia, which are actin-filled protrusions of the cell surface [1]. Pseudopod formation by Dictyostelium cells, like many other moving cells, shows a typical pseudopod cycle: upon their initiation, pseudopodia grow at a constant rate during their first ~15 s and then stop. The next pseudopod is typically formed a few seconds later, but sometimes commences while the present pseudopod is still growing, giving rise to a cell with two pseudopodia. The fate of the pseudopod after its initial growth phase determines its role in cell movement: the pseudopod is either retracted, or is maintained by flow of the cytoplasm into the pseudopod thereby moving the cell body. The frequency, position and directions of the maintained pseudopodia form the basis of cell movement, because they determine the speed and trajectory of the cell. An important aspect of cell motility is the ability of cells to respond to directional cues with oriented movement. Gradients of chemicals give rise to chemotaxis [2]. Other directional cues that can induce oriented movement are temperature gradients (thermotaxis) or electric fields (electrotaxis) [3,4]. These signals somehow modulate basal pseudopod extension such that, on average, cells move in the direction of the positional cues. In this respect, studies on cell movement are critical for understanding directional movement.

Cells in the absence of external cues do not move in random directions but exhibit a so-called correlated random walk [5–9]. This tendency to move in the same direction is called persistence, and the duration of the correlation is the persistence time. Cells with strong persistence make fewer turns, move for prolonged periods of time in the same direction, and thereby effectively penetrate into the surrounding space. Other search strategies for efficient exploration are local diffusive search and Levi walks [8,10]. Can we understand the cell trajectory by analyzing how cells extend pseudopodia?

To obtain large data sets of extending pseudopodia we developed a computer algorithm that identifies the cell contour and its protrusions. The extending pseudopod is characterized by a vector that connects the x,y,t coordinates of the pseudopod at the beginning and end of the growth phase, respectively [11]. A picture of ordered cell movement has emerged from the analysis of ~6000 pseudopodia that are extended by wild type and mutant cells in buffer [12]. Dictyostelium cells, as many other eukaryotic cells, may extend two types of pseudopodia: de novo at regions devoid of recent pseudopod activity, or by splitting of an existing pseudopod [12,13]. Pseudopod splitting occurs very frequently alternating to the right and left at a relatively small angle of ~55 degrees. Therefore, pseudopod splitting may lead to a persistent zig-zag trajectory [14]. In contrast, de novo pseudopodia are extended in all directions and do not exhibit a right/left bias, suggesting that de novo pseudopodia induce a random turn of the cells. We observed strong persistence for cells that extend many pseudopodia. The aim is to define the descriptive persistence time or average turn angle with primary experimentally-derived pseudopod properties. First we obtained detailed quantitative data on the probability frequency distributions of the size and direction of pseudopod activity. We then formulated a model that consists of five components: pseudopod size, fraction of splitting...
Author Summary

Even in the absence of external information, many organisms do not move in purely random directions. Usually, the current direction is correlated with the direction of prior movement. This persistent random walk is the typical way that simple cells or complex organisms move. Cells with poor persistence exhibit Brownian motion with little displacement. In contrast, cells with strong persistence explore much larger areas. We have explored the principle of the persistent random walk by analyzing how Dictyostelium cells extend protrusions called pseudopodia. These cells can extend a new pseudopod in a random direction. However, usually cells use the current pseudopod for alternating right/left splittings, by which they move in a persistent zig-zag trajectory. A stochastic model was designed for the persistent random walk, which is based on the observed angular frequencies of pseudopod extensions. Critical elements for persistent movement are the ratio of de novo and splitting pseudopodia, and, unexpectedly, the shape of the cell. A relatively round cell moves with much more persistence than a cell with an irregular shape. These predictions of the model were confirmed by experiments that record the movement of mutant cells that are specifically defective in pseudopod splitting or have a very irregular shape.

Methods

Dictyostelium strains and cell recordings

The strains used are wild type AX3, p3k-null strain GMP1 with a deletion of p3k1 and p3k2 genes [15], pla2-null with a deletion of the pla2 gene [16], sgc/gca-null cells (abbreviated as gc-null cells) with a deletion of gea and sgc genes [17], sgc/pla2-null cells with a deletion of sgc and pla2 genes [18], and dha2-null cells lacking the forH gene encoding the Dictyostelium homologue of formin [19]. Cells were grown in HG5 medium (contains per liter: 14.3 g oxoid peptone, 7.15 g bacto yeast extract, 1.36 g Na2HPO4, 0.49 g KH2PO4, 10.0 g glucose), harvested in PB (10 mM KH2PO4/Na2HPO4, pH 6.5), and allowed to develop in 1 ml PB in a well of a 6-wells plate (Nunc). Movies were recorded at a rate of 1 frame per second for at least 15 minutes with an inverted light microscope (Olympus Type CK40 with 20 XI objective) and images were captured with a JVC CCD camera. Cell trajectories were recorded as the movement of the centroid of the cell as described [20].

Pseudopod analysis

Images were analyzed with the fully automatic pseudopod-tracking algorithm Quimp3, which is described in detail [11]. Briefly, the program uses an active contour analysis to represent the outline of the cell using ~150 nodes [21]. Extending pseudopodia that satisfied the user-defined minimum number of adjacent convex nodes and the minimum area change were identified. The direction of each extending pseudopod was identified by the x,y and time coordinates of the central convex node of the convex area at the start and end of growth, respectively. The tangent to the surface at the node where the pseudopod started was calculated using the position of the adjacent nodes. The automated algorithm annotates each pseudopod as de novo versus splitting using the criterion that the convex area of the new pseudopod exhibits overlap with the convex area of the current pseudopod or is within a user-defined distance. The output files containing the x,y-coordinates of the start and end position of the pseudopod, the tangent of the surface and the annotation of the pseudopod were imported in Excel to calculate pseudopod size, interval, direction to gradient, direction to tangent, etc for de novo and splitting pseudopodia (see Fig. 1), as well as fraction of pseudopod splitting and alternating Right/Left bias a/RL/total splitting; Table 1).

The cell shape parameter \( \psi \) was determined as follows: Using the outline of the cell with ~150 nodes, two ellipsoids were constructed, the largest ellipse inside the cell outline and the smallest ellipse outside the cell outline. Then an intermediate ellipse was constructed by interpolation of the inner and outer ellipse. This intermediate ellipse makes several intersections with the cell outline, thereby forming areas of the cell that are outside the intermediate ellipse (with total surface area \( O \)), and areas of the intermediate ellipse that do not belong to the cell (with total

Figure 1. Pseudopod analysis. Movies at a rate of 1 frame per second were recorded for Dictyostelium cells moving on a solid support in buffer. The program Quimp3 represents the cell outline using a polygon of ~150 nodes, and then identifies extending convex protrusions as pseudopodia, described by the x,y,t coordinates of the start and end of their growing phase. The program also calculates the tangent to the surface at the position where the pseudopod started. These data were used to calculate for each pseudopod the size \( \lambda \), the angle \( \alpha \) relative to a specific point in space, the angle \( \beta \) relative to the tangent, and the angle \( \phi \) relative to the previous pseudopod. doi:10.1371/journal.pcbi.1000874.g001
Table 1. Observed and deduced parameters of wild type and mutant cells.

| Property                        | Symbol | Units | WT 1h | WT 3h | WT 5h | WT 7h | gc-null | pla2-null | sgc/pla2-null | dlia2-null |
|---------------------------------|--------|-------|-------|-------|-------|-------|---------|-----------|---------------|------------|
| Observed pseudopod (n cells/pseudopodia) |        |       | 7/215 | 8/256 | 28/835 | 7/294 | 7/312   | 8/208     | 8/219         | 8/164      |
| Pseudopod size                  | \( \lambda_p \) | \( \mu \) m | 5.0±0.2 | 5.3±0.2 | 5.2±0.2 | 4.7±0.2 | 4.6±0.4 | 7.7±0.5 | 5.3±0.7 | 5.6±0.4 |
| Splitting angle                 | \( \varphi \) | degrees | 62    | 58    | 55    | 55    | 54      | 50        | 54           | 54         |
| SD splitting angle              | \( \sigma_\varphi \) |           | 26.1  | 29.7  | 27.8  | 27.5  | 26.9    | 28.5      | 27.5         | 46.5       |
| Alternating angle               | \( a \) | -      | 0.74±0.02 | 0.74±0.06 | 0.77±0.04 | 0.82±0.06 | 0.67±0.05 | 0.68±0.05 | 0.75±0.08 | 0.75±0.03 |
| Fraction splitting              | \( s \) | -      | 0.55±0.07 | 0.60±0.05 | 0.86±0.06 | 0.89±0.05 | 0.71±0.06 | 0.67±0.10 | 0.41±0.07 | 0.82±0.09 |
| Correlation factor              | \( \gamma_{\text{obs}} \) | -      | 0.46±0.11 | 0.52±0.07 | 0.74±0.09 | 0.81±0.10 | 0.58±0.11 | 0.55±0.11 | 0.35±0.08 | 0.53±0.07 |
| Turn angle                      | \( \theta \) | degrees | 63    | 59    | 42    | 36    | 55      | 57        | 70           | 58         |

The WCD is given by

\[
fwCD(\varphi, \rho) = \frac{1 - \rho^2}{2\pi(1 + \rho^2 - 2\rho \cos(\varphi))}, \quad 0 < \rho < 1. \tag{3}
\]

Monte Carlo simulations

Pseudopod extension is an ordered stochastic event [12]. The position of the tip of the extended pseudopodia depends on pseudopod size \( \lambda_p \), splitting fraction \( s \), left/right alternating ratio \( a \), angle between split pseudopodia \( \varphi \) and variance of this angle \( \sigma_\varphi \).

A Monte Carlo simulation starts with a random angle \( \varphi(1) \) of the first pseudopod. For the next and all subsequent pseudopodia the simulation uses four uniformly distributed random numbers \( R_{ia} \) \((i = 1, \ldots, 4)\) to calculate \( \varphi(n) \), the angle of the \( n \)th pseudopod: \( \varphi(n) \in [0,1]\) with the decision to split if \( R_{ia} < \frac{1}{2} ; \varphi(n) \in [0,1]\) with the decision for alternating splitting if \( R_{ia} < \frac{1}{2} ; \varphi(n) \in [0,1]\) for direction of split after de novo with decision right if \( R_{ia} < \frac{1}{2} ; \varphi(n) \in [-180,180]\) for the direction of the de novo pseudopod. These probabilities result in a projected angle of extension in degrees. Finally, the actual pseudopod direction is drawn from a wrapped von Mises distribution with this projected angle as mean and \( \sigma_\varphi \) as variance (\( \kappa = 1/\sigma_\varphi^2 \); variance converted to radians). The obtained \( \varphi(n) \) and the pseudopod size \( \lambda_p \) are used to calculate the \( x,y \) coordinates of the tip of the pseudopod, followed by a next round of four random numbers to calculate \( \varphi(n+1) \).

Please note that in the simulations the direction of the simulated de novo pseudopodia is random; consequently, a small fraction of de novo pseudopodia are in the same direction of the previous pseudopod, which would be recognized in experiments as splitting pseudopodia. Conversely, a small fraction of the simulated splitting pseudopodia have angles
much larger than 55 degrees and would be recognized in experiments as de novo pseudopodia. From the geometry of the cell, we estimate that the number of simulated de novo in the current pseudopod and the number of splitting pseudopodia outside the current pseudopod are approximately the same, suggesting that the simulations represent the observed ratio of splitting and de novo pseudopodia.

Results

Pseudopod extensions

The angles between pseudopodia were analyzed in detail and the results are presented in Fig. 2. For splitting pseudopodia, the angle between the current and next pseudopod ($\theta_{1,2}$) has a clear bimodal distribution (Fig. 2A). A probability density function (PDF) of angles belongs to the family of circular or wrapped distributions. The data reported in this study were all fitted well by a von Mises distribution (vMD), which is the circular analog of the normal distribution. The wrapped Cauchy distribution has fatter tails and provided a poorer fit of the data (data not shown). The bimodal vMD presented in Fig. 2A is symmetric, yielding two means ($\theta_{1,2} = 7/55$) that have the same variance $\sigma = 0.6 \sigma x$; $\sigma_{1,2} = 28$ degrees). Figure 2B shows the PDF of the angle between the current and next pseudopod ($\theta_{1,3}$), which is best described by a single vMD with a mean of $\theta_{1,3} = 2$ degrees and $\sigma_{1,3} = 42$ degrees. Figure 2C reveals that there is no significant correlation between the magnitude of angles between first/second pseudopod and the magnitude of the angles between second/third pseudopod (thus e.g. splitting at a larger angle is not followed by a split at a smaller angle). The extension of splitting pseudopodia is summarized in Fig. 2D, and is based on the previous observation that a pseudopod split to the right is frequently followed by a split to the left and visa versa [12]. Thus the next pseudopod is extended at an angle of $\sim 55$ degrees to the right or left relative to the current pseudopod, and the next-next pseudopod is extended in roughly the same direction as the current pseudopod.

The angle between a de novo pseudopod and the previous pseudopod shows a very broad distribution (Fig. 2E). Nearly all angles between $-180$ and $+180$ are well represented with a somewhat lower abundance of angles around 0 degrees. This suggests that a de novo pseudopod can be extended in any direction, but with slightly lower probability of the direction of the current pseudopod.

Trajectories of wild type and mutant cells

To investigate the consequence of the observed ordered extension of pseudopodia for cell movement on a coarse time scale for many pseudopodia we recorded the movement of Dictyostelium cells during 15 minutes; in this period about 30 pseudopodia are extended. Previously we have presented the cell trajectories for several strains and developmental stages [12] (see also Fig. S1). The mean square displacement as a function time, $\langle D^2(t) \rangle$, exhibits a slow approach to a linear function (Fig. 3A), which is typical for a transition of a correlated random walk at short times to a Brownian random walk after longer times [6,23]. Previously, the often used equation for a correlated random walk were fit to the data points to estimate persistence time and speed of the cells [12]. The aim of the present study is to analyze the mechanism of cell movement from the perspective of the extending pseudopodia, which have a specific length and direction. A correlated random walk in two dimensions can also be described with steps and turns [24,25]. With the replacement of the number of steps (n) in Eq. 7 in reference [25] for $n = F t$ we obtain

$$\langle D^2(t) \rangle = \lambda^2 \left[ F t \left( 1 + \gamma - 2 \gamma \frac{1 - \gamma F t}{1 - \gamma F t} \right) \right],$$

where $\lambda$ is the step size in $\mu m$, $F$ is the step frequency, and $\gamma$ is the correlation factor of dispersion ($0 < \gamma < 1$), defined as the arithmetic mean of the cosine of the turn angle $\theta$ between steps

$$\gamma = \langle \cos(\theta) \rangle.$$  

With three variables ($F, \lambda, \gamma$) the estimates of the parameters become uncertain. Fortunately, the step size can be deduced accurately from experimental data. As will be shown below in Eq. 10, the step size is given by $\lambda = \lambda_0 \cos(\phi/2)$, where measurements for $\lambda_0$ and $\phi$ are presented in Table 1. Using this value for $\lambda$, the dispersion data were fitted to obtain the observed correlation factor of dispersion ($\gamma_{obs}$) with the corresponding turn angle $\theta$. In cells starved for 1 or 3 hours the correlation factor is only $\sim 0.5$ with turn angle of $\sim 60$ degrees. At 5 and 7 hours of starvation, cells move with much stronger persistence (correlation factor of 0.74 and 0.81 and a small turn angle of 42 and 36 degrees). Deletion of PLA2 or guanylyl cyclases prevents this increase of correlation factor, persistence is very low and cells disperse poorly.

How is pseudopod extension related to the observed correlation factor of dispersion $\gamma_{obs}$? As previously stated (see Fig. 2), Dictyostelium cells may extend either de novo pseudopodia in nearly random directions, or splitting pseudopodia in a direction similar to the previous direction. Therefore, cells that extend exclusively de novo pseudopodia are expected to exhibit a random walk with $\gamma_{obs} = 0$ (turn angle $\theta = 90$ degrees), whereas cells extending exclusively splitting pseudopodia will exhibit strong persistence with large $\gamma$ and small turn angle $\theta$. As a consequence, $\gamma_{obs}$ is expected to depend on the ratio $s$ of splitting/de novo pseudopodia. Fig. 3B demonstrates that within experimental error this relationship is approximately linear; this holds true for the mutants as well as for wild type cells at different stages of development. The linear regression of all data yields $\gamma_{obs} = 0.921s - 0.044$. Thus, when all pseudopodia are de novo ($s = 0$) the correlation factor is small ($\gamma_{obs} = -0.044$) giving a turn angle $\theta_{obs} = 93$ degrees, close to the expected value of 90 degrees for random turns. In contrast, when all pseudopodia are the result of splitting ($s = 1$) the correlation factor is large ($\gamma_{obs} = 0.88$) yielding a small turn angle ($\theta = 29$ degrees). The implication of small turn angles for splitting pseudopodia will be discussed later.

Model for persistent movement

The alternating right/left extension of splitting pseudopodia can be used to simplify a description of the movement of Dictyostelium cells over longer distances. In this approach, the simplification may be valid for movement on a longer time scale only, as we study here, but may not be appropriate over shorter time scales of a few pseudopodia. Because pseudopodia are frequently extended alternating right/left, we consider movement by pairs of two pseudopodia.

Figure 4 shows four possibilities of pairs of splitting pseudopodia, which are the RL, LR, RR and LL, each with corresponding probabilities and angles as indicated. In addition to these splitting pairs, three combinations with de novo pseudopodia are possible: split-de novo, de novo-split, and de novo-de novo. The correlation factor of dispersion yields for the seven pairs:
De novo pseudopodia are extended in a random direction, i.e. $\langle \cos \theta_{ab} \rangle$, $\langle \cos \theta_{ad} \rangle$ and $\langle \cos \theta_{bd} \rangle$ equal zero. The turn angles of the four splitting pairs are $0$, $\varphi$ and $2\varphi$, as indicated in Fig. 4A, and the variance is approximately $2\sigma_{\varphi}^2$ (see Fig. 2B). Consequently Eq. 6 reduces to:

$$\gamma_{pair} = s^2a^2\langle \cos \theta_{RL} \rangle + a(1-a)\langle \cos \theta_{LR} \rangle + a(1-a)\langle \cos \theta_{RR} \rangle + (1-a)^2\langle \cos \theta_{LL} \rangle + s(1-s)\langle \cos \theta_{ab} \rangle + \langle \cos \theta_{ad} \rangle + 1 + 2\sigma_{\varphi}^2$$

$$\gamma_{pair} = s^2a^2\langle \cos \theta_{RL} \rangle + a(1-a)\langle \cos \theta_{LR} \rangle + a(1-a)\langle \cos \theta_{RR} \rangle + \langle \cos \theta_{LL} \rangle + s(1-s)\langle \cos \theta_{ab} \rangle + \langle \cos \theta_{ad} \rangle + 1 + 2\sigma_{\varphi}^2$$

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where \(\langle \cos (\varphi, \kappa) \rangle\) denotes the expected value of the cosines of the angles on a circle with weights given by the vMD with mean \(\varphi\) and variance given by \(\kappa = 1/(2\sigma^2)\). Since all splitting pseudopodia show the same variance this can be further reduced to

\[ \gamma_{\text{pair}} = s^2 \langle \cos (0, \kappa) \rangle [a^2 \cos (0) + 2a(1-a) \cos (\varphi) + (1-a)^2 \cos (2\varphi)]. \] (8)

In this equation \(\langle \cos (0, \kappa) \rangle\) is obtained by calculating the probabilities of all turn angles on a circle with the vMD using Eqs. 1 and 2 and then taking the weighted average of the cosines of these angles. Although this procedure is straightforward, Eq. 8 can be further simplified, because for \(s\kappa\) smaller than 50 degrees a good approximation is \(\langle \cos (0, \kappa) \rangle \approx \cos (2\kappa') / (\sqrt{\pi})\) (see Fig. S2). Finally, on a longer time scale and averaged over many steps, the correlation factor of pairs is related to the correlation factor of its underlying two steps by \(\gamma_{\text{pair}} = \gamma_{\text{step}}^2\). With these replacements we obtain the analytical expression for the correlation factor

\[ \gamma_{\text{step}} \approx s \cos (2\kappa') / (\sqrt{\pi}) \left[ a^2 + 2a(1-a) \cos (\varphi) + (1-a)^2 \cos (2\varphi) \right]. \] (9)

Thus, the correlation factor \(\gamma\) is the product of three terms: the splitting ratio \(s\), a noise term with the variance \(\sigma^2\), and a term with right/left bias \(a\) and angle \(\varphi\).

Finally, by considering movement in pairs of steps, Fig. 4 reveals that the step size of the displacement is given by

\[ \lambda = \lambda_p \cos (\varphi/2). \] (10)

Monte Carlo simulations of cell movement

We used Monte Carlo simulations to investigate how \(\lambda\) and \(\gamma\) depend on the pseudopod parameters size \(\lambda_p\), splitting fraction \(s\), alternating ratio \(a\), angle between split pseudopodia \(\varphi\) and

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**Figure 3. Dispersion.** The trajectories of wild type and mutant cells (see Fig. S1) were recorded as described in the method section. A. The mean square displacement was determined for ~20 cells (symbols). The data were fitted according Eq. 4 (lines) yielding the correlation factor of dispersion \(\gamma_{\text{obs}}\) as indicated in Table 1. B. The correlation factor of dispersion is plotted as a function of the fraction \(s\) of splitting pseudopodia, which was determined from the same movies. Symbols are closed circle for wild type (5 h starved in panel A; 1, 3, 5 and 7 h starved in panel B); closed triangle for sgc/gca-null cells, diamond for pla2-null cells; square for sgc/pla2-null cells. doi:10.1371/journal.pcbi.1000874.g003

**Figure 4. Bipedal amoeboid movement.** The diagram shows the probabilities, angles and sizes of pairs of pseudopod extensions. Dictyostelium cells frequently extend alternating right/left splitting pseudopodia. Pseudopodia to the left are in red, to the right in blue, and the movement after two pseudopodia is in black. Indicated are the four possible movements with two splitting pseudopodia after the cell has made a right and left pseudopod. The probability for alternating right/left or left/right is \((a)\), while the probability for consecutive right/right or left/left is \((1-a)\), yielding the probabilities of the four pseudopod pairs. The angle between pseudopodia is \(\varphi\). doi:10.1371/journal.pcbi.1000874.g004

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**Figure 8.** Pseudopod Model for Persistent Walk. The diagram shows the trajectories of wild type and mutant cells (see Fig. S1) were recorded as described in the method section. A. The mean square displacement was determined for ~20 cells (symbols). The data were fitted according Eq. 4 (lines) yielding the correlation factor of dispersion \(\gamma_{\text{obs}}\) as indicated in Table 1. B. The correlation factor of dispersion is plotted as a function of the fraction \(s\) of splitting pseudopodia, which was determined from the same movies. Symbols are closed circle for wild type (5 h starved in panel A; 1, 3, 5 and 7 h starved in panel B); closed triangle for sgc/gca-null cells, diamond for pla2-null cells; square for sgc/pla2-null cells. doi:10.1371/journal.pcbi.1000874.g003
The trajectories of 100,000 cells were obtained by Monte Carlo simulation; the displacement was analyzed with Eq. 4 to obtain the correlation \( \gamma_{MC} \) and step size \( \lambda \) (in units of pseudopod size and thus dimensionless). Simulations were performed with the parameter values as indicated on the x-axis, while the other parameters had the following standard values: \( \lambda_p = 1; s = 1; a = 1; \phi = 55 \) degrees; \( \sigma_p = 28 \) degrees. The data points denote the outcome of the Monte Carlo simulations; the lines are generated using Eq. 9, while the dotted line in panel C is generated using Eq. 8. The asterisks represent the predicted value for 5h starved Dictyostelium cells. A. The effect of angle \( \phi \) and right/left bias \( a \) (\( a = 1 \), all pseudopod alternating right/left; \( a = 0.5 \) right/left is random). B. The effect of the fraction of splitting pseudopodia (\( \eta \)). C. The effect of the variance of the angle between pseudopodia (\( \sigma_\phi \)).

Figure 5. Predicted correlation factor of persistence and step size. The trajectories of 100,000 cells were obtained by Monte Carlo simulation; the displacement was analyzed with Eq. 4 to obtain the correlation \( \gamma_{MC} \) and step size \( \lambda \) (in units of pseudopod size and thus dimensionless). Simulations were performed with the parameter values as indicated on the x-axis, while the other parameters had the following standard values: \( \lambda_p = 1; s = 1; a = 1; \phi = 55 \) degrees; \( \sigma_p = 28 \) degrees. The data points denote the outcome of the Monte Carlo simulations; the lines are generated using Eq. 9, while the dotted line in panel C is generated using Eq. 8. The asterisks represent the predicted value for 5h starved Dictyostelium cells. A. The effect of angle \( \phi \) and right/left bias \( a \) (\( a = 1 \), all pseudopod alternating right/left; \( a = 0.5 \) right/left is random). B. The effect of the fraction of splitting pseudopodia (\( \eta \)). C. The effect of the variance of the angle between pseudopodia (\( \sigma_\phi \)).

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Movement of the centroid of the cell

How does the movement of pseudopodia relate to the movement of the centroid of the cell? The data presented in Table 1 reveal that the observed correlation factor \( \gamma_{obs} \) of the
centroïd for different cell types correspond well with the deduced correlation factors of the pseudopods $\langle \gamma_{MC} \rangle$ and $\langle \gamma_{sep} \rangle$, but is always larger by $\sim 15\%$ (Table 1). Apparently, the observed turn angle of the cell's centroïd is smaller than the turn angle of the extending pseudopod. Inspection of movies of 5h starved AX3 cells confirm this notion: the average angle between splitting pseudopodia is $55 \pm 28$ degrees (Fig. 2A), while the centroïd moves during period at an angle of only $31 \pm 23$ degrees (mean and SD). Equation 9 reveals that the correlation factor $\gamma_{sep}$ increases by $15\%$ when $\phi = 55 \pm 28$ degrees for the pseudopod is replaced by $\phi = 31 \pm 23$ degrees for the cells centroïd.

Probably two phenomena are responsible for the difference between pseudopod and centroïd: extension of multiple pseudopodia and geometry of cells. When cells extend multiple pseudopodia it is likely that at any given instant of time, the front of the cell moves with a fixed fraction of the vector sum of velocities possessed by the pseudopodia active at that instant in time. The temporal overlap of two pseudopodia was deduced from the measured probability distributions of pseudopod extensions (Fig 2F in [26]), which reveal that $\sim 25\%$ of the pseudopodia overlap with another pseudopod during on average $\sim 40\%$ of their extension time. This suggest that the tip of the cell moves at an angle that is $\sim 6$ degrees smaller than 55 degrees. Secondly, geometry predicts that the rear of the cell makes smaller changes of direction than the tip of the cell, comparable to the differences in curvature made by the front and rear wheels of a car. Figure S3 indicates that for a stereotypic pseudopod at 55 degrees the directional change of the centroïd is $\sim 40$ degrees (see Fig. S3). Together, multiple pseudopodia and cell geometry can explain observed difference between pseudopod and centroïd changes of direction, leading to the small $15\%$ difference between deduced pseudopod correlation factor ($\langle \gamma_{MC} \rangle$ and $\langle \gamma_{sep} \rangle$) and observed centroïd correlation factor ($\langle \gamma_{ct} \rangle$).

Directional displacement

The directional displacement $\langle \hat{D}(0,n) \rangle$ is the displacement after n steps in the direction of the first step. An expression for the directional displacement is especially relevant when the organism is exposed to positional cues leading to a drift in one direction, such as during chemotaxis. The directional displacement of a cell after extending one pseudopod at an angle $\theta$ is $\hat{\lambda} \cos \theta$, and for a population of cells $\langle \hat{D}(0,1) \rangle = \hat{\lambda} \cos \theta$. By Eqs. 3 and 10, the displacement at the first step may be written as $\langle \hat{D}(0,1) \rangle = \gamma \hat{\lambda} \cos (\phi/2)$, and at the ith step $\langle \hat{D}(i-1,1) \rangle = \gamma \hat{\lambda} \cos (\phi/2)$, see Eq. 6 of reference [25]. The cumulative displacement after n steps is

$$\langle \hat{D}(0,n) \rangle = \sum_{i=0}^{n} \gamma \hat{\lambda} \cos (\phi/2),$$

which at $n \to \infty$ is given by

$$\langle \hat{D}(0,\infty) \rangle = \frac{\hat{\lambda} \cos (\phi/2)}{1-\gamma}, \quad 0 < \gamma < 1.$$  

In essence, this equation describes the displacement of a cell population in which all cells extend the first pseudopod in the same direction. Subsequent pseudopodia are extended with a bias, which reduces geometrically with each step; the correlation factor $\gamma$ indicates how many pseudopodia have correlated direction and therefore how far the population will disperse in the direction of the first pseudopod. Figure 6 presents the directional displacement as observed experimentally in wild type cells. The displacement in the direction of the first pseudopod slowly decreases at each subsequent pseudopod, approaching random movement after $\sim 10$ pseudopodia. On average a cell moves $\sim 15$ μm in the direction of the first pseudopod, which is the equivalent of about 3 pseudopodia (given a pseudopod size of $\sim 5$ μm). This figure also presents the directional displacement as modeled by Eq. 11a with observed data for $\lambda_{m}$, $\phi$ and $\gamma$, which is in very close agreement with experimental data, again suggesting that the movement of a cell is satisfactorily described by the model with five pseudopod parameters.

Cell shape, the variance in the direction of pseudopod extension, and dispersal

The variation in pseudopod direction $\sigma_{\phi}^{2}$ plays an important role in Eqs. 8–11 describing cell dispersal. Previously [12] we have shown that the next pseudopod emerges at a specific distance from the tip of the current pseudopod, and is then extended perpendicular to the cell surface (i.e. perpendicular to the tangent to the surface curvature at the position where the pseudopod emerges). The pseudopod direction is expected to have high confidence for cells with a smooth ellipsoid shape, because the local bending is very predictable. However, this confidence is much smaller for cells with a very irregular shape. We investigated the role of cell shape using three experiments. First we demonstrate that the variance $\sigma_{\phi}^{2}$ indeed depends on the variance of the tangent and the normal to the tangent. Second, we show that wild type or mutant cells with irregular shape exhibit increased variance $\sigma_{\phi}^{2}$. Finally we show that, due to the increased variance, the mutant exhibits poor dispersal.

Quimp3 was used to construct the tangent to the surface curvature at the position where the pseudopod emerges. We first determined for wild-type cells the angle $\alpha_{i}$ of this tangent relative to the previous pseudopod ($\alpha_{i} = 34.5 \pm 24.9$ degrees), and the angle $\beta$ of the new pseudopod relative to this tangent ($\beta = 89.1 \pm 13.3$ degrees). As mentioned above, the observed angle of the new pseudopod relative to the previous pseudopod is $\phi = 55.2 \pm 27.8$ degrees. We expect that the angle of the tangent relative to the previous pseudopod is independent from the angle of the pseudopod relative to the tangent; therefore we expect $\sigma_{\phi}^{2} = \sigma_{\alpha}^{2} + \sigma_{\beta}^{2} = 24.9^{2} + 13.3^{2} = 28.2^{2}$. Indeed, the observed standard deviation of 27.8 degrees is close to this expected value of

![Figure 6. Directional displacement. Pseudopod formation and trajectories were recorded for 5h starved Dictyostelium cells; 8 cells were followed during 15 min. The directional displacement is the distance moved after n pseudopodia in the direction of the first pseudopod. Data points are the means of $\sim 120$ measurements, the line is the outcome of Eq. 11 with pseudopod parameters as indicated in Table 1. doi:10.1371/journal.pcbi.1000874.g006](image-url)
28.2 degrees. Importantly, the largest contribution to $\sigma^2_\phi$ is derived from the variance of the tangent $\sigma^2_t$, which is related to the local shape of the cell.

In the collection of *Dictyostelium* mutants, we selected a strain with an irregular shape. Mutant *ddia2*-null with a deletion of the *forH* gene encoding the formin dDia2 has a star-like shape (Fig. 7C). In this mutant, new pseudopodia are extended at about the same frequency and distance from the present pseudopodia as in wild type cells, pseudopodia also grow perpendicular to the surface, and are extended roughly in the same direction of $\varphi = 55$ degrees as wild type cells (Fig. 7A). However pseudopodia exhibit much more variation in direction ($\sigma^2_\varphi = 47$ degrees compared to $\sigma^2_\varphi = 28$ degrees for wild type cells). Finally, we determined a shape parameter $Y$ that indicates how much the cell outline deviates from an ellipse (see method section and Fig. S4). Figure 7B reveals that cells with increased irregular shape, either being wild-type or mutant, exhibit strongly increased variance $\sigma^2_\varphi$. Importantly, the distance $d$ and angle $\varphi$ of the pseudopodia does not change with cell shape (Fig. 7A).

Using the observed values for $s$, $a$, $\varphi$, and $\sigma_\varphi$ for *ddia2*-null cells we expect from Eq. 9 to obtain $\gamma_{\text{step}} = 0.43$, significantly lower compared to $\gamma_{\text{step}} = 0.69$, for wild type cells. Fig. 7D shows that the dispersion of *ddia2*-null cells is strongly reduced. The observed mean square dispersion was fitted to Eq. 4 yielding a correlation factor $\gamma_{\text{disp}} = 0.33$ (Table 1), close to the value that was predicted from the extension of pseudopodia from an irregular surface.

In summary, these and previous results [12] suggest that a splitting pseudopod is induced at some distance $d$ from the tip of the current pseudopod, and then grows perpendicular to the surface. In a cell with a regular shape, the tangent and therefore pseudopod direction can be approximated using the distance $d$; alternating R/L extensions lead to a relative straight zig-zag trajectory, providing strong persistence of movement. In a cell with a very irregular shape, the local curvature of the membrane at distance $d$ is unpredictable. Consequently, alternating R/L splitting occur with large variation of directions, leading to frequent turns and poor persistence.

**Discussion**

The movement of many organisms in the absence of external cues is not purely random, but shows properties of a correlated random walk. The direction of future movement is correlated with the direction of prior movement. For organisms moving in two dimensions, such as most land-living organisms, this implies that movement to the right is balanced on a short term by movement to the left to assure a long-term persistence of the direction. In bipedal locomotion, the alternating steps with the left and right foot will yield a persistent trajectory. Amoeboid cells in the absence of external cues show ordered extension of pseudopodia: a new pseudopod emerges preferentially just after the previous pseudopod has stopped growth [12]. Importantly, the position at the cell
surface where this new pseudopod emerges is highly biased. When the current pseudopod has been extended to the left (relative to the previous pseudopod), the next pseudopod emerges preferentially nearby the tip at the right side of the current pseudopod. Since pseudopodia are extended perpendicular to the cell surface, this next pseudopod is extended at a small angle relative to the current pseudopod [12]. Therefore, this (imperfect) alternating right/left pseudopod splitting resembles bipedal locomotion. Cells may also extend a de novo pseudopod somewhere at the cell body, which is extended in a random direction. In starved *Dictyostelium* cells, the probability of extending a de novo pseudopod is ~10-fold lower than of pseudopod splitting (probability calculated per μm circumference of the cell [12]).

The model for pseudopod-based cell dispersion depends on five parameters, the pseudopod size ($\lambda_p$), the fraction of split pseudopodia ($\delta$), the alternating left/right bias ($a$), the angle between pseudopodia ($\varphi$) and the variance of this angle ($\sigma_\varphi^2$). With these parameters the experimental data on mean square displacement and directional displacement are well-explained using Eqs. 9 and 11, respectively. Pseudopodia are the fundamental instruments for amoeboid movement. The notion that the trajectories are described well by the five pseudopod parameters probably implies that we have identified the basic concept of the amoeboid correlated random walk: persistent alternating pseudopod splitting and formation of de novo pseudopodia in random directions.

The cells may modify one or more of these five pseudopod parameters in order to modulate the trajectories (see Table 1). Nearly all mutants, as well as wild type cells at different stages of starvation and development, have approximately the same average pseudopod size $\lambda_p$. In addition, the alternating right/left bias ($a$) fluctuates between 0.67 and 0.82, and the angle between splitting pseudopodia ($\varphi$) between 50 and 62 degrees. *pla2*-null cells are the only exception [12]; emerging pseudopodia in *pla2*-null cells exhibit longer growth periods (~27 s) than wild type cells (~13 s), and are thus longer. This suggests that all strains use the same mechanism for pseudopod splitting. In contrast to these constant properties of split pseudopodia, the fraction of split pseudopodia ($\delta$) changes predominantly de novo in random directions, leading to a nearly Brownian random walk [20]. Upon starvation, the appearance of cGMP and PL2A signaling weakens pseudopodia and suppresses de novo pseudopod extensions, which leads to more persistent movement. The important role of the fraction of splitting pseudopodia for cell movement is also depicted by the linear dependence of the correlation factor $\gamma$ on the fraction $\delta$ of splitting pseudopodia (Fig. 3B and Eq. 9).

The variance of the angle of pseudopod extension ($\sigma_\varphi^2$) plays an important role in movement. In wild type cells, as well as in many mutant strains, $\sigma_\varphi$ is about 28 degrees. The primary source of the variance of pseudopod angles lies in the variation of cell shape, by which the normal to the cell surface at a specific position on this surface will have significant variation. Since the direction of pseudopodia is given by this normal, it is predicted that a cell with irregular shape should have more variation in pseudopod direction, and consequently shows poor dispersion. The experiments with mutant *ddia2*-null cells strongly support this interpretation. Wild-type cells have a relatively regular spherical shape by which two nearby pseudopodia are extended in nearly the same direction (small $\sigma_\varphi$). In contrast, mutant *ddia2*-null cells have an irregular star-like shape; therefore, two nearby pseudopodia are often extended in very different directions (large $\sigma_\varphi$). The variance $\sigma_\varphi^2$ can be regarded as the noise of the system. It indicates how fast a cell that extends only alternating splitting pseudopodia ($a = 1$ and $s = 1$) will lose correlation of directionality. With $\sigma_\varphi = 28$ degrees for wild type cells it follows from Eq. 10 that all pseudapodia the correlation of direction is still $\sim 0.5$. In contrast, for *ddia2*-null cells we obtained $\sigma_\varphi = 46.5$ degrees, which implies that already after four pseudapodia the correlation of direction has declined to $\sim 0.5$. Supported by Monte Carlo simulations using the parameters of the mutant, we conclude that poor dispersion of *ddia2*-null cells is due to the increased variance of pseudopod angles, which is caused by its irregular shape.

The correlation factor $\gamma$ is the product of three terms (see Fig. 5 and Eq. 9), namely: splitting fraction ($\delta$), alternating pseudopod angles ($a$ and $\varphi$), and the SD of the pseudopod angle ($\sigma_\varphi$). Strong persistence of cell movement is attained when all three terms are large and about equal in magnitude. Starved wild type cells follow this strategy: each term is $\sim 0.9$, resulting in the observed correlation factor of 0.74. Mutants in which one of these terms is compromised, such as reduced splitting in *gca/pla2*-null cells or enhanced noise of *ddia2*-null cells, have poor dispersion.

In summary, the correlated random walk of amoeboid cells is well described by the balanced bipedal movement, mediated by the alternating right/left extension of splitting pseudopodia. Cells deviate from movement in a straight line due to noise and because cells occasionally hop or make random turns. The turns in particular are used by the cells to modulate the persistence time, thereby shifting between nearly Brownian motion during growth and strong persistent movement during starvation.

### Supporting Information

**Figure S1** Trajectories. Movies were recorded during 15 minutes and the trajectories of the centroid of ten cells were determined. A. Wild type *Dictyostelium* cells at different times after removing of food. The frequency and size of pseudopod extension is not very different, but starved cells extend predominantly splitting pseudopodia (see (16)). B. Trajectories of 5 hour starved mutant cells with deletions of genes encoding guanyl cyclases (sGC and GCA) and PL2A. C. Monte Carlo simulations calculated with the pseudopod parameters that were obtained experimentally for the mutants as indicated in Table 1.

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**Figure S2** Analysis of the noise equation $\langle \cos(\theta, k) \rangle \approx \cos(\frac{\sigma_\varphi}{\sqrt{\pi/2}})^2$. Pseudopodia are extended with a variance $\sigma_\varphi^2$.

In this equation, the notation $\langle \cos(\theta, k) \rangle$ is the average of the cosines of the angles on a circle with weights given by the von Mises Probability Distribution (vMD) with mean of 0 degrees and variance given by $\kappa = 1/\sigma_\varphi^2$. The figure reveals that $\langle \cos(\theta, k) \rangle$ deviates less than 2% from the simple expression $\cos(\frac{\sigma_\varphi}{\sqrt{\pi/2}})^2$ for values of $\sigma_\varphi < 40$ degrees.

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**Figure S3** Movement of pseudopod and centroid of a cell. A. The cell is drawn as an ellipse with short and long axes of 3 and 6 μm, respectively. A pseudopod of 5 μm is extended perpendicular to the ellipse at 55 degrees relative to the long axes of the ellipse, which define the starting point and direction of the pseudopod. The position of the centroid is indicated by an asterisk. B. In *Dictyostelium* cells a pseudopod usually extends during ~12 seconds, and then the cytoplasm moves into the pseudopod and the rear is retracted. The open headed arrows indicate that the front of the cell moves to the tip of the pseudopod, and the rear of the cell moves in the direction of the old axis of the cell.
Schematic after a few Right/Left pseudopod extensions. The centroid makes smaller turns than the pseudopod, ~40 degrees. 

**Figure S4** Determination of the shape parameter \( \Psi \). The cell outline is used to draw two ellipses, the largest possible ellipse inside the cell and the smallest possible ellipse outside the cell. Then an intermediate ellipse is constructed by interpolation of the inner and outer ellipse; the figure shows the intermediate ellipse. This ellipse intersects the outline, thereby forming the blue areas \( O \) of the cell that are outside the intermediate ellipse, and yellow areas \( I \) of the intermediate ellipsoid that do not belong to the cell. The intermediate ellipse was interpolated in such a way that \( O = I \).

The parameter of cell shape is defined as \( \Psi = (O+I)/T \), where \( T \) is the surface area of the cell (grey + blue). The minimal value is \( \Psi = 0 \) when the cell is an ellipse, and the maximal value is \( \Psi = 2 \) for a cell with extreme long extensions; the maximal value observed among ~600 Dictyostelium cells was \( \Psi = 0.92 \). The neuron cell is shown for comparison.

**Author Contributions**
Conceived and designed the experiments: PJMVH. Performed the experiments: PJMVH. Analyzed the data: PJMVH. Contributed reagents/materials/analysis tools: PJMVH. Wrote the paper: PJMVH.

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