Abstract. Ponticulin is a 17-kD glycoprotein that represents a major high affinity link between the plasma membrane and the cortical actin network of Dictyostelium. To assess the role of ponticulin in pseudopod extension and retraction, the motile behavior of two independently generated mutants lacking ponticulin was analyzed using computer-assisted two- and three-dimensional motion analysis systems. More than half of the lateral pseudopods formed off the substratum by ponticulin-minus cells slipped relative to the substratum during extension and retraction. In contrast, all pseudopods formed off the substratum by wild-type cells were positionally fixed in relation to the substratum. Ponticulin-minus cells also formed a greater proportion of both anterior and lateral pseudopods off the substratum and absorbed a greater proportion of lateral pseudopods into the uropod than wild-type cells. In a spatial gradient of cAMP, ponticulin-minus cells were less efficient in tracking the source of chemoattractant. Since ponticulin-minus cells extend and retract pseudopods with the same time course as wild-type cells, these behavioral defects in ponticulin-minus cells appear to be the consequence of pseudopod slippage. These results demonstrate that pseudopods formed off the substratum by wild-type cells are positionally fixed in relation to the substratum, that ponticulin is required for positional stabilization, and that the loss of ponticulin and the concomitant loss of positional stability of pseudopods correlate with a decrease in the efficiency of chemotaxis.
tend a pseudopod and crawl, but are required for the orderly and highly regulated temporal and spatial dynamics of anterior and lateral pseudopod extension and retraction (for review see Soll, 1995). The selective absence of such proteins, effected by gene disruption, can result in abnormalities in the frequency, order, rate of growth and 3-D geometry of pseudopod extension and retraction (Wessels et al., 1988, 1989, 1991, 1996; Wessels and Soll, 1990; Titus et al., 1993). These abnormalities, in turn, can impact significantly on the efficiency of translocation, chemotaxis, and multicellular morphogenesis (DeLozanne and Spudich, 1987; Knecht and Loomis, 1987; Wessels et al., 1988; Condeelis, 1993; Doolittle et al., 1995; Sheldon and Knecht, 1995).

To assess directly the role of ponticulin in single cell motility and chemotaxis, we have used computer-assisted two- and three-dimensional motion analysis systems (Soll, 1988, 1995; Soll et al., 1988; Murray et al., 1992; Wessels et al., 1994; Chandrasekhar et al., 1995) to characterize the motile behavior of aggregation-competent ponticulin-minus cells in buffer and in spatial gradients of cAMP. We have discovered that all lateral pseudopods formed off the substratum by wild-type cells in buffer are fixed positionally in relation to the substratum during extension and retraction, while more than half of the lateral pseudopods formed by ponticulin-minus cells in buffer are not fixed and slip posteriorly along the cell axis. Slippage is very rapid, and results in an abnormally high proportion of pseudopods that are retracted into the uropod rather than into the main cell body. Ponticulin-minus cells also form an abnormally high proportion of both anterior and lateral pseudopods off the substratum, and these cells are less efficient at chemotaxing in a spatial gradient of the chemoattractant, cAMP. Our results demonstrate that ponticulin plays a novel role in the control of pseudopod position relative to the substratum, and that this spatial stability is an important component of normal cell motility and chemotaxis.

Materials and Methods

Ponticulin-Minus Mutants

The genesis of the ponticulin-minus mutants, Tfl.1 and T24.1, in Dictyostelium discoideum strain Ax3K was described in detail in a previous report (Hitt et al., 1994a). In brief, mutants were generated by homologous recombination with a construct in which a neomycin-resistance cassette was inserted after nucleotide 9 of the ponticulin coding sequence. The single-copy gene encoding ponticulin was disrupted in both of the independently generated transformant cell lines, as demonstrated by Southern blot hybridization and by the absence of a diagnostic 2.5 kb PCR product (Hitt et al., 1994a). Northern and Western analyses of Tfl.1 and T24.1 confirmed the absence of detectable ponticulin message and ponticulin protein, respectively (Hitt et al., 1994a). Both mutant cell lines were subcloned twice in HL-5 medium (Cocucci and Sussman, 1970) and stored as frozen stocks in HL-5 medium containing 10% dimethylsulfoxide.

Development of Wild-type and Mutant Strains

Cells from frozen cultures were grown in suspension in HL-5 medium at 22°C and serially transferred for no longer than 3 wk. To initiate development, cells from the mid-log phase of growth were washed free of nutrient medium with a buffered salt solution (BSS) consisting of 20 mM KCl, 2.4 mM MgCl2, 0.34 mM streptomycin sulfate, 20 mM KH2PO4, and 20 mM Na2HPO4, pH 6.4 (Sussman, 1987). Cells in BSS were dispersed at high density (5 x 105 cells per cm2) on a black filter (29; Whatman Inc., Clifton, NJ) supported by two prefilters (Millipore Corp., Bedford, MA) saturated with the same solution (Soll, 1987). These conditions, in which cells are multilayered, have been shown to result in highly synchronous development and highly reproducible developmental timing of Ax3 cells (Soll, 1979). Unless otherwise noted, cells of both wild-type and mutant strains were removed at the ripple stage, which represents the onset of aggregation under these conditions (Soll, 1979), by gently pipetting BSS across the surface of the development filter. The cells were pelleted and washed, and the final cell suspension, in BSS, was gently mixed and adjusted to a concentration of 3 x 106 cells per ml for inoculation into a perfusion or chemotaxis chamber. Growth cultures of strain Ax2 and strain Ax3-clone RC3 were initiated from desiccated cultures of spores and then developed in the same manner as described above.

Two-Dimensional Analysis of Cell Behavior and Pseudopod Dynamics

A 0.3-ml aliquot of cell suspension was inoculated into a Dvorak-Stoter chamber (Nicholson Precision Instruments, Inc., Gaithersburg, MD), the chamber closed, and the cells allowed to settle to the bottom glass chamber wall to reinitiate motile behavior. The resulting density on the chamber wall was ~35 cells/mm2. The chamber was then clamped to the stage of an inverted microscope (ICM 405; Carl Zeiss, Inc., Thornwood, NY) equipped with differential interference contrast (DIC) optics for high magnification (>630) recordings. The chamber was perfused with BSS, and the behavior of single cells not in contact with each other video-recorded on half or three-quarter inch tape according to methods previously described (Wessels et al., 1989; Wessels and Soll, 1990). Low magnification videorecorded cell images were automatically digitized and high magnification cell images were manually digitized into the data file of the DIAS program (Wessels et al., 1992; Sylwestter et al., 1993, 1995). 2-D motility and morphology parameters were computed according to formulas and methods previously described (Soll, 1988, 1995; Soll et al., 1988). Instantaneous velocity for a cell in frame (n) was computed by first drawing a line from the cell centroid in the previous frame (n-1) to the cell centroid in the subsequent frame (n+1), and dividing the length of the line by Δt, where Δt is the time interval between analyzed frames. Positive flow was computed as the percentage of area contained in the expansion zones of different pictures, generated by overlapping the images in frames n and n-1 (Soll, 1995). Roundness was computed as (4π x area x perimeter2) x 100. A perfect circle has a roundness measure of 100% and a straight line a roundness measure of 0%. L-2 centriod tracks were analyzed for turning frequency by dividing total translocation time by the number of turns persisting for >32° that caused the direction of the cell centroid to change by >30°.

Definition of a Pseudopod

In the 2-D analysis of the spatial and temporal dynamics of pseudopod extension and retraction, an anterior pseudopod was defined as a cell extension that formed at the anterior end of the main cell body in the approximate direction (+ 30°) of the long cell axis, which attained a minimum area of 4 μm2 and which was initially free of particulate cytoplasm. The anterior end of a cell was determined from the long axis of the cell established during the preceding translocation step in the behavior cycle (Wessels et al., 1994). A lateral pseudopod was defined as an extension which formed from the side of the cell body in a direction 30° to 90° from the long cell axis, which attained a minimum area of 4 μm2 and which was initially free of particulate cytoplasm. Again, the angle of pseudopod expansion was determined from the long axis of the cell established during the preceding translocation step. Comparisons of the videotaped segments of cells recorded through DIC optics and the digitized images of the same cells demonstrated that in all cases pseudopods defined by the preceding criteria in digitized images represented cell extensions in which the apical zones were free of particles. As pseudopods grew, the apical ends remained particle free, but the proximal regions filled with particulate cytoplasm. The apical zones of particle-free cytoplasm corresponded to the F-actin-filled zones of pseudopods, as determined by staining with fluorescein-conjugated phallolidin (Wessels et al., 1989). During pseudopod retraction, the pseudopods were primarily filled with particulate cytoplasm.
Three-Dimensional Analysis of Cell Behavior and Pseudopod Dynamics

The methods for optically sectioning living cells, reconstructing 3-D images and wrapping the images for analysis with 3-D DIA software have been described elsewhere (Murray et al., 1992; Wessels et al., 1994; Soll, 1995). In brief, cells were inoculated into a Dvorak-Stotler chamber, and the chamber was positioned on the stage of a Zeiss ICM405 inverted microscope equipped with DIC optics and perfused with BSS. To obtain optical sections, the plane of focus was automatically raised in 1-μm increments at 0.2-s intervals using a newly developed microstepper motor regulated by a Macintosh-based operating program. Sectioning of a cell was complete in 2 s and repeated every 5 s. Perimeters of the in-focus portion of each section were manually digitized into the 3-D DIA data file.

To illustrate this technique, the original videorecorded DIC images and the manually digitized perimeters of in-focus portions are presented in Fig. 1 A for one 3-D image (i.e., at a single time point). Pseudo-3-D representations of the stacked optical sections are presented at progressive angles in Fig. 1 B. A more representative rendition of the reconstructed cell was then generated by reinserting the in-focus portion of each image into its digitized perimeter, stacking these images and presenting them as pseudo-3-D renditions at progressive angles in Fig. 1 C. Noise removal and dilation-erosion techniques were used to unify perimeters, when necessary, in each optical section, and final shapes were generated using β-splines (Soll, 1995). The final shapes in the stack of optical sections were first connected to form transparent caged images (Fig. 1 D), then wrapped and smoothed to generate nontransparent 3-D reconstructions that most closely reflect the original images (Fig. 1 E). Smoothed images of cells reconstructed at 5-s intervals were then viewed as dynamic animations on the polarizing screen of a stereo monitor (4337; Tektronix, Beaverton, OR). The final 3-D reconstructions were used to quantify 3-D parameters.

In 3-D analyses of pseudopod extension and retraction, the criteria used to define anterior and lateral pseudopods were similar to those used in 2-D analyses. However, instead of using minimum area as a criterion, a minimum vol of 6 μm³ was used, and instead of using 2-D shape criteria, 3-D shape criteria were used to interpret extensions. Comparisons of 3-D reconstructions with the original DIC optical sections demonstrated that pseudopods defined by 3-D criteria conformed to the same criteria used in defining a pseudopod in 2-D images.

Pseudopod volume was measured in μm³ by encapsulating the 3-D projection in a box which abutted the cell body and then summing the faceted blocks contained in a faceted image of the pseudopod (Wessels et al., 1996). Rates of expansion or contraction were computed in μm³ per 5 s.

Error Estimates

To estimate the error in 3-D measurements due to movement of the cell or pseudopod during optical sectioning, we first computed the distance an average cell would move in the course of 2 s, the time required to generate a 3-D image. For an average cell moving 10 μm/min, the distance moved in 2 s is 0.33 μm. Since elongate cells are, on average, 20 μm in length, the directional skew (the distance error between the bottom and top sections due to movement during optical reconstruction) is ~2%. For a pseudopod growing at an average rate of 30 μm/min with an average height of 4 μm, the average number of optical sections necessary for reconstruction is four, and the average time for reconstruction is 0.8 s. In this case, the directional skew would be ~10% between the bottom and top sections of the reconstruction.

To estimate volume errors due to missing portions of the pseudopod in optical sections, we modeled a pseudopod as a parabola, then positioned the parabola in the worst positions for analysis (i.e., with the highest and lowest optical sections just missing the top and bottom edges of the pseudopod, respectively). For an average-sized pseudopod 5 μm in length and 4 μm in height, the average error is 12%. This low error is due to an automatic interpolation algorithm in the 3D-DIAS program which completes shapes for volume measurements.

To test the reliability and accuracy of in-focus outlining, an Ax3K cell was fixed on a gridded coverslip and optically sectioned at 1-μm increments using the DIC method. The cell was then stained with FITC-conjugated phalloidin, which stains F-actin, but more importantly, provides a high resolution image of F-actin just under the plasma membrane and, therefore, a very accurate outline of the cell. The original cell optically sectioned by DIC was found on the gridded coverslip and optically reconstructed by confocal microscopy. In the confocal microscopy sectioning procedure, 40 sections at 0.2-μm increments were collected. The in-focus perimeters of the DIC sections and the perimeters of the confocal sections were then both digitized into the DIA data bank. Both the reconstructed DIC and confocal pseudo-3-D images were then generated, and their volumes compared. The reconstructed images were visually similar and the difference in computed volume was only 0.4%. The only difference between the two reconstructions was the resolution of a small number of stained filopodia in the confocal reconstruction.

Analysis of Chemotaxis in a Gradient Chamber

To assess the chemotactic index (CI) of individual parental Ax3K and mu-

Figure 1. Optical sectioning and computer-generated reconstruction of a translocating cell at a single time point. (A) The original sequence of optical sections obtained by differential interference contrast microscopy and manually drawn perimeters of the in-focus image. Sections were recorded at 0.2-s intervals and were separated in the z-axis by 1 μm. 0 is at the glass substratum and 9 is 9 μm above the substratum. (B) The digitized perimeters of the in-focus portions of the optical sections are filled and stacked to create a pseudo 3-D reconstruction. This reconstruction is then rotated for views initially from the side and progressively toward the dorsal surface. (C) Each digitized perimeter of the in-focus portion of the optical sections are refilled with only that portion of the section which was in focus, and these filled perimeters are stacked and viewed from progressively dorsal angles. This provides a more realistic reconstruction. (D) The plates in the image in B are connected by facets. This transparent image is used for computing 3-D morphology and motility parameters (Soll, 1995). Two views are presented of the faceted image. (E) The faceted image is then smoothed and wrapped to obtain a nontransparent image which most closely represents the original cell. Two views are presented of the wrapped image. a, anterior end of cell; u, uropod.
tant cells in a spatial gradient of the chemoattractant cAMP, a dilute sus-

Results

Developmental Regulation of Motility

Since the rate of single cell motility is regulated by the de-

velopmental program of *Dictyostelium* (Varnum et al.,

1986), we first identified conditions that permitted com-

parison of wild-type Ax3K and ponticul-in-minus mutant

cells at comparable developmental stages. In a previous

report (Hitt et al., 1994b), ponticul-in-minus cells were
demonstrated to exhibit a shorter preaggregation period than

Ax3K cells when developed at low density on agar contain-
ing Sorensen’s buffer (14.6 mM KH$_2$PO$_4$, 2.0 mM

Na$_2$HPO$_4$, pH 6.0). We found that when cells were plated

four deep on development filters saturated with BSS (Fig.

2) or were developed on agar with Sorensen’s buffer con-

taining 2.4 mM MgCl$_2$ (data not shown), the preaggrega-
tion period and timing of subsequent stages of develop-

ment were similar for wild-type and mutant cells. In BSS,

Ax3K and Tf24.1 cells progressed simultaneously through the

ripple (R), loose aggregate (LA) and tight aggregate (TA)
stages at 7.0, 8.5, and 9.5 h, respectively (Fig. 2). This

latter developmental protocol, therefore, provided the

temporal parallelism needed for a valid comparison of be-

havioral phenotypes, and was used in all studies reported

here.

The developmental regulation of motility in both wild-
type and mutant cells was also similar through the first 9 h

of development under these conditions. In Fig. 2, the mean

instantaneous velocity of individual Ax3K and Tf24.1 cells

are plotted as functions of developmental time. The mean

instantaneous velocity of both cell types was ~2 μm/min
during the first 6 h of development, increased to peak val-

ues of 9.5 and 11.5 μm/min, respectively, at 7 h of develop-

ment, and then decreased to 3.5 μm/min at 9 h of develop-

ment. These results are highly similar to those originally

reported for wild-type strain Ax3-clone RC3 (Varnum et al.,

1986). Subsequent comparisons of wild-type and ponti-
cul-in-minus cells were performed with ripple stage cells re-

moved from filters after 7 h of development (Fig. 2, R), at

peak instantaneous velocity for all three strains.

Two-Dimensional Motility and Shape Parameters

We initially considered two possible outcomes of a ponti-
cul-in-minus phenotype. First, we considered the possibility

that a cell lacking ponticulin and, therefore, most of the

high affinity linkages between actin and the plasma mem-

brane (Hitt et al., 1994b), would translocate at a slower ve-

locity than wild-type cells if the ponticulin-based connec-
tions were required for pseudopod extension. Alternatively,

we considered it possible that a cell lacking ponticulin might

translocate at a faster velocity than wild-type cells if ponti-
culin-actin connections have to be disrupted for normal

pseudopod extension.

A 2-D analysis of general motility parameters suggests

that ponticul-in-minus cells translocate at instantaneous ve-

locities similar to or slightly higher than those of wild-type

Ax3K cells (Table I). Both the mean instantaneous veloc-

ty and the mean positive flow, a measure of area displace-

ment with time (Soll et al., 1988; Soll, 1988, 1995), were

slightly greater for mutant cells than for wild-type cells, al-

though the differences were statistically significant only

for Tf1.1 cells vs. wild-type cells (Table I). There were no

significant differences in the velocity cycle (Wessels et al.,

1994). The mean period between velocity peaks of Ax3K

cells was 1.23 ± 0.29 min (n = 24), and that of Tf1.1 and

Tf24.1 cells 1.06 ± 0.20 (n = 33) and 1.44 ± 0.75 (n = 21)

min, respectively. The differences between mutant and

wild-type values were in both cases insignificant (P > 0.35).

There was also no significant difference between wild-type

and mutant cell shape (Table I). Mean maximum length,

mean maximum width, mean area, and mean roundness of

wild-type and mutant cells were statistically indistinguish-

able, with values very similar to those previously reported

for other wild-type strains of *Dictyostelium* (Wessels et al.,

1991; Cox et al., 1992). We, therefore, conclude that ponti-

cul-in-minus and wild-type cells crawl in buffer with similar

instantaneous velocities, velocity cycles, and general shape.
Table I. Motility and Morphology Measurements of Parental Ax3K and Ponticulin-Minus Transformants (Tf1.1, Tf24.1) Translocating in Buffer

<table>
<thead>
<tr>
<th></th>
<th>Mean Inst. Vel. (μm/min)</th>
<th>Mean Pos. Flow (% area)</th>
<th>Mean Max. Len. (μm)</th>
<th>Mean Max. Wid. (μm)</th>
<th>Mean Area (μm²)</th>
<th>Mean Roundness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax3K (parental)</td>
<td>9.2 ± 2.2</td>
<td>15.9 ± 3.1</td>
<td>19.2 ± 3.8</td>
<td>9.7 ± 1.8</td>
<td>97.9 ± 26.9</td>
<td>43.0 ± 9.8</td>
</tr>
<tr>
<td>(n = 26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tf1.1</td>
<td>11.1 ± 3.0</td>
<td>19.5 ± 6.4</td>
<td>21.7 ± 4.1</td>
<td>9.6 ± 1.6</td>
<td>105.9 ± 25.7</td>
<td>38.8 ± 9.4</td>
</tr>
<tr>
<td>(n = 23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tf24.1</td>
<td>10.9 ± 4.4</td>
<td>16.1 ± 5.7</td>
<td>21.5 ± 6.6</td>
<td>9.3 ± 1.0</td>
<td>115.8 ± 38.1</td>
<td>44.7 ± 6.8</td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>P values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ax3K vs. Tf1.1</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ax3K vs. Tf24.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Determinations were made from 2-D cell perimeters manually digitized at 4-s intervals.

See Materials and Methods for explanations of Parameters. (Mean Inst. Vel.), Mean Instantaneous Velocity; (Mean Pos. Flow), Mean Positive Flow; (Mean Max. Len.), Mean Maximum Length; (Mean Max. Wid.), Mean Maximum Width; Mean Area; Mean Round, (Mean Roundness). All values represent the mean and standard deviation for the number of cells (n) noted.

The Student t test was used to assess significance. A P value >0.05 was considered not significant.

Formation of Lateral and Anterior Pseudopods

Computer-generated 2-D tracks of the digitized perimeters of ponticulin-minus cells translocating in buffer gave the impression of being slightly wider, on average, than tracks of wild-type cells. Because the width of a perimeter track is affected by the number, shape, and dynamics of lateral pseudopods, and because the majority of lateral pseudopods formed by wild-type cells in buffer are extended off the substratum and are then retracted (Wessels et al., 1994; Soll, 1995), aberrations in the dynamics of lateral pseudopods could affect the width of a perimeter track without significantly affecting instantaneous velocity. To explore the possibility of abnormalities in the shape or position of lateral pseudopods formed by ponticulin-minus cells, we analyzed pseudopod formation in three dimensions (Murray et al., 1992; Wessels et al., 1994, 1996; Soll, 1995).

In dynamic 3-D reconstructions, we observed that wild-type Ax3K cells and ponticulin-minus cells of strain Tf1.1 and Tf24.1 extended lateral pseudopods at comparable frequencies (Table IIA). However, lateral pseudopods formed by Tf1.1 and Tf24.1 cells were significantly more likely to be formed off the substratum than were lateral pseudopods formed by wild-type cells. Only 10–15% of the lateral pseudopods of ponticulin-minus cells were formed on the substratum, i.e., with their ventral surface in contact with the substratum (Table IIA). By contrast, 40% of the lateral pseudopods of wild-type cells were formed on the substratum. This latter proportion is very similar to that previously reported for cells of the wild-type strain Ax3, clone RC3 (Wessels et al., 1994). Therefore, the proportion of lateral pseudopods formed on the substratum by ponticulin-minus cells was approximately three times smaller than that of wild-type cells.

Since lateral pseudopods formed on the substratum have a greater propensity for initiating sharp turns than do lateral pseudopods formed off the substratum (Wessels et al., 1994), one would expect wild-type cells translocating in

Table II. The Proportion of Lateral and Anterior Pseudopods Formed on and off the Substratum by Ax3K, Tf1.1, and Tf24.1 Cells Translocating in Buffer

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. cells</th>
<th>No. lateral</th>
<th>Lateral per 5 min.</th>
<th>Proportion formed off substratum</th>
<th>Proportion formed on substratum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Lateral Pseudopods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ax3K</td>
<td>15</td>
<td>93</td>
<td>6.2 ± 2.4</td>
<td>60 ± 23%</td>
<td>40% ± 23</td>
</tr>
<tr>
<td>Tf1.1</td>
<td>4</td>
<td>31</td>
<td>7.8 ± 3.6</td>
<td>90 ± 8%</td>
<td>10% ± 8</td>
</tr>
<tr>
<td>Tf24.1</td>
<td>5</td>
<td>39</td>
<td>7.8 ± 0.4</td>
<td>85 ± 14%</td>
<td>15% ± 14</td>
</tr>
<tr>
<td>P values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ax3K vs. Tf1.1</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>0.02</td>
</tr>
<tr>
<td>Ax3K vs. Tf24.1</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>0.04</td>
</tr>
</tbody>
</table>

| B. Anterior Pseudopods | | | | | |
| Ax3K    | 6         | 22          | 3.8 ± 0.7          | 40 ± 25%                         | 60% ± 25%                       |
| Tf1.1   | 4         | 18          | 4.2 ± 1.1          | 73 ± 15%                         | 27% ± 15%                       |
| Tf24.1  | 6         | 25          | 4.5 ± 1.0          | 80 ± 12%                         | 22% ± 12%                       |
| P values | | | | | |
| Ax3K vs. Tf1.1 | | | | NS | 0.02 |
| Ax3K vs. Tf24.1 | | | | NS | 0.04 |

Determinations were made from dynamic three-dimensional reconstructions generated at 5-s intervals.

*Student t-test was used to assess significance; similar results were obtained with the nonparametric Mann-Whitney test. A P value >0.05 was considered not significant.
buffer to turn more often than mutant cells, since a greater proportion of their lateral pseudopods are formed on the substratum. We, therefore, counted the number of sharp turns in centroid tracks which persisted for $>32$ s. The results supported the above prediction. While centroid tracks of Ax3K cells contained turns at a frequency of $0.74 \pm 0.20$ per min ($n = 15$), the centroid tracks of ponticulin-minus cells (Tf1.1 and Tf24.1) contained turns at a frequency of $0.45 \pm 0.34$ ($n = 25$). The difference was significant, with a $P < 0.01$.

We also examined the proportion of anterior pseudopods formed on and off the substratum. Again, the proportion of anterior pseudopods formed on the substratum by ponticulin-minus cells was only about one-third that observed for wild-type cells (Table IIB). These results suggest that at least some of the defects described above for the more easily characterized lateral pseudopods also are present in anterior pseudopods.

**Fates of Lateral Pseudopods**

The ultimate fates of lateral pseudopods formed off the substratum differed between wild-type and ponticulin-minus cells. The majority of the lateral pseudopods formed off the substratum by Ax3K cells were retracted back into the main cell body. An example of normal pseudopod retraction by an Ax3K cell is presented in the sequence of wrapped 3-D reconstructions shown in Fig. 3. At 0 s, this cell had no lateral pseudopods, a tapered anterior end which had lifted off the substratum, and a slightly bifurcated uropod. Between 0 and 5 s, the cell extended a lateral pseudopod ($r_l$) from the right side of the anterior portion of the main cell body. As is evident in the horizontal view at 5 s (bottom), the ventral surface of pseudopod $r_l$ was not in contact with the substratum. Pseudopod $r_l$ was fully extended at 10 s (not shown) and was retracted into the main cell body between 10 and 20 s, as the cell again extended the original anterior pseudopod along the substratum. Since retraction was completed at the main cell body, the uropod retained its original shape. Throughout the process of pseudopod extension and retraction, the ventral surface of pseudopod $r_l$ did not contact the substratum.

Although the majority of lateral pseudopods formed off the substratum by Tf1.1 and Tf24.1 cells also were extended from the anterior portion of the main cell body, significantly fewer completed retraction into the main cell body when compared with wild-type cells. Instead, the majority completed retraction at the uropod. A representative example of this phenomenon is presented in Fig. 4. At 0 s, the beginning of a lateral pseudopod ($r_l$) was evident on the right side of this Tf1.1 cell. The horizontal views of the cell (bottom) demonstrate that pseudopod $r_l$ was formed off the substratum. Between 0 and 10 s, this pseudopod continued to expand, and a second pseudopod ($II$) formed on the left side of the cell body, again off the substratum. Between 10 and 15 s, both pseudopods began to retract but they were now positioned at the uropod. By 20 and 30 s, respectively, pseudopods II and $r_l$ were com-

**Figure 3.** Lateral pseudopods formed off the substratum by Ax3K cells usually complete retraction at the main cell body. (Top) 3-D reconstructions of an Ax3K cell viewed from an oblique frontal angle after downward rotation of the anterior end of the 3-D image. (Bottom) Horizontal views from the vantage point noted by the black line in the upper panel at time 0. The positions of the anterior end ($a$) and the uropod ($u$) of the cell were determined from the translocation dynamics of the cell before this time series. The cell extended a pseudopod ($r_l$) from the right side of the cell body between 0 and 5 s, then retracted it into the cell body between 15 and 20 s. Note in the top panels that the uropod retained its bifurcated shape throughout the time series. In the bottom panels, note that the lateral pseudopod remained off the substratum (i.e., its ventral surface did not make contact with the substratum during extension or retraction).

**Figure 4.** Lateral pseudopods formed off the substratum by ponticulin-minus cells usually complete retraction at the uropod. In the top panel at each time point, the 3-D reconstruction of a Tf1.1 cell is viewed from an oblique frontal angle and in the bottom panel at each time point, the cell is viewed horizontally at the vantage points noted by the black line in the top 0-s panel. The position of the anterior end ($a$) and uropod ($u$) of the cell were determined from the translocation dynamics of the cell prior to the presented time series. This cell extended one pseudopod to the right ($r_l$) and one to the left ($II$) between 0 and 10 sec. Both were formed off the substratum, and both completed retraction at the uropod.
were always observed concomitant with absorption at 25 ± 15, and 45 ± 21%, and at the uropod 17 ± 14, 75 ± 15, and Tfl.1, and 33 Tf24.1 pseudopods. (B) A 2-D analysis of 58 Ax3K, formed off the substratum by wild-type (Ax3K) and mutant Figure 5. Completely absorbed. The uropod, in this case, changed its position along the cell body at which pseudopod retraction (Fig. 5) is that pseudopods in ponticulin-minus cells may be positionally unstable, (i.e., more inclined to slip posteriorly relative to the cell body and/or the substratum), and, therefore, more likely to complete retraction at the uropod. In wild-type cells, we discovered that the position of every lateral pseudopod formed off the substratum remained fixed in relation to the substratum through the entire extension and retraction process, even during translocation of the cell (Fig. 6; Table IV). This positional invariance was observed both for the majority of lateral pseudopods, which were retracted into the main cell body (Fig. 6 A, r2), and for the majority of lateral pseudopods, which completed retraction at the uropod (Fig. 6 A, r1). Positional invariance of each lateral pseudopod formed off the substratum was observed not only for the Ax3K parental strain in this study, but also for strain Ax3, clone RC3 (Table IV, Fig. 6 B), and strain Ax2 (Table IV), two other laboratory strains of Dictyostelium discoideum that have been used in previous computer-assisted analyses of cell motility (Wessels et al., 1989, 1992, 1994). Of 104 lateral pseudopods formed by 14 wild-type axenic cells, 100% remained fixed in relation to the substratum, i.e., none showed positional slippage (Table IV).

In marked contrast, lateral pseudopods formed off the substratum by ponticulin-minus cells were not positionally fixed relative to the substratum (Fig. 7; Table IV). Half or more of these lateral pseudopods changed positions in relation to the substratum during expansion and retraction (Table IV). Most of these unfixed pseudopods slipped posteriorly; none were observed to slip towards the front of the cell. In the case of pseudopod r1, formed by the representative Tfl.1 cell in Fig. 7, the distance that the base of the pseudopod slipped in relation to the substratum (noted by a dotted line at 10 s) was 6.25 μm, which represents a minimum retrograde velocity of 75 μm/min. Pseudopod II on this same cell slipped 5 μm in the same 5-s period and an additional 5 μm in the next 5-s period, which represents a minimum retrograde velocity of 60 μm/min. Of 76 lateral pseudopods formed by 11 ponticulin-minus cells, over half slipped and in all cases they did so in the posterior direction (Table IV). Every mutant cell analyzed for a period of 3–10 min exhibited slippage of one or more pseudopods (Table IV).

Another explanation for the positional difference in lateral pseudopod retraction (Fig. 5) is that the lateral pseudopods of ponticulin-minus cells may be longer-lived pseudopods. For a cell rapidly translocating in buffer, a longer-lived lateral pseudopod would be more likely to remain extended as the cell translocated anteriorly, and the consequence would be completion of retraction at the uropod. To test this possibility, we analyzed the rates and periods of expansion and retraction of lateral pseudopods (Table III). The results showed no significant differences between Ax3K and ponticulin-minus cells in the rate of expansion, the rate of retraction, or the longevity of lateral pseudopods formed off the substratum.

Pseudopods of Ponticulin-Minus Cells Slip Posteriorly

One explanation for the positional difference in lateral pseudopod retraction (Fig. 5) is that pseudopods in ponticulin-minus cells may exhibit a longer average lifespan due to a decrease in the rate of pseudopod extension and/or retraction. For a cell rapidly translocating in buffer, a longer-lived lateral pseudopod would be more likely to remain extended as the cell translocated anteriorly, and the consequence would be completion of retraction at the uropod. To test this possibility, we analyzed the rates and periods of expansion and retraction of lateral pseudopods (Table III). The results showed no significant differences between Ax3K and ponticulin-minus cells in the rate of expansion, the rate of retraction, or the longevity of lateral pseudopods formed off the substratum.
Table IV. Lateral Pseudopod Slippage in Ponticulin-Minus (Tf1.1, Tf24.1) Cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. cells</th>
<th>No. lateral pseudopods</th>
<th>Percentage pseudopod slippage</th>
<th>Percentage cells exhibiting slippage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax3K</td>
<td>5</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ax3(RC3)</td>
<td>4</td>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ax2</td>
<td>5</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tf1.1</td>
<td>6</td>
<td>47</td>
<td>49</td>
<td>100</td>
</tr>
<tr>
<td>Tf24.1</td>
<td>5</td>
<td>29</td>
<td>55</td>
<td>100</td>
</tr>
</tbody>
</table>

The relationship of the base of each pseudopod to the substratum was determined during extension and retraction from 2-D perimeters hand-digitized at 4-s intervals.

*The proportion of cells exhibiting at least one case of lateral pseudopod slippage during the period of analysis, which was between 3 and 10 min per cell.

Chemotactic Inefficiency of Ponticulin-Minus Cells

Lateral pseudopods that are extended off the substratum have been suggested to play a key role in sensing chemotactic gradients (Varnum-Finney et al., 1987b; Wessels et al., 1994; Soll, 1995) and in initiating turns in the correct direction (i.e., in the direction of increasing cAMP concentration). Thus, cells with lateral pseudopods that frequently slip posteriorly and/or dorsally might be expected to be less efficient in tracking a spatial gradient (see Discussion).

Figure 6. The base of each lateral pseudopod formed by wild-type cells remains fixed relative to the substratum during both extension and retraction. (A) A representative Ax3K cell; (B) A representative Ax3-clone RC3 cell. The reconstructions are viewed dorsally so that the position of the base of each pseudopod can be established and marked by an arrow on the underlying grid. The positions of the anterior end (a) and uropod (u) of the cell at 0 s of each time sequence were determined from the translocation dynamics of the cell before the presented time series. The Ax3K cell in A had already formed a lateral pseudopod to its right (r1) before 0 s and another to its right (r2) between 0 and 5 s. The Ax3-RC3 cell in B formed a lateral pseudopod to its left (l1) between 0 and 5 s.

minus cells appeared to be absorbed into the main cell body when viewed dorsally (Fig. 8, top). However, when the same cells were viewed from a horizontal vantage point, it was clear that the pseudopod had rotated to the dorsal surface of the cell and then slipped posteriorly to the uropod (Fig. 8, bottom). Thus, the top views of the representative Tf24.1 cell in Fig. 8 failed to discriminate between pseudopod retraction and circumferential slippage to the dorsal surface of the cell body. In the case of the cell in Fig. 8, the pseudopod on the dorsal surface of the cell slipped 7.5 μm toward the posterior end of the cell (Fig. 8, dotted line) between 15 and 25 s, which represents a retrograde velocity of 45 μm/min. At 25 s, this dorsal pseudopod was positioned at the uropod. Since the proportion of pseudopods which slipped posteriorly (Table IV) was determined from 2-D reconstructions in which dorsal slippage such as that demonstrated in Fig. 7 would not have been detected, the reported proportions of slippage by lateral pseudopods of Tf1.1 and Tf24.1 cells in Table IV are probably underestimates.

Figure 7. Lateral pseudopods formed by ponticulin-minus cells are not anchored to the substratum and slip posteriorly. The anterior end (a) and uropod (u) of this representative Tf1.1 cell were determined from the translocation dynamics before the presented time series. The reconstructions are viewed dorsally. Arrows denote the original position of the base of each pseudopod in relation to the substratum on the underlying grid. This representative Tf1.1 cell formed one pseudopod to the right (r1) and one pseudopod to the left (l1) between 0 and 5 s. Both pseudopods then slipped posteriorly, and the distances that they slipped are noted by dotted lines. This figure represents the same cell and time series viewed from oblique and horizontal angles in Fig. 3, which showed that neither r1 nor l1 contacted the substratum.
The mean CI of ponticulin-minus cells was significantly less than that of wild-type cells (Fig. 9). The mean CI for Ax3K cells was $+0.45 \pm 0.29$ ($n = 66$), while that for Tf1.1 was $+0.30 \pm 0.29$ ($n = 48$) and that for Tf24.1 was $+0.30 \pm 0.30$ ($n = 52$). The difference between the mean CIs of Ax3K and Tf1.1 cells, and the CIs of Ax3K and Tf24.1 cells were highly significant. In the former case, the $P$ value was 0.006 and in the latter case 0.007. In contrast, the $P$ value for the mean CIs of Tf1.1 and Tf24.1 was 0.986, demonstrating an extremely high similarity between the two mutant strains. The difference in the distributions of CIs of wild-type and mutant cells is demonstrated in the histogram in Fig. 9, in which the pooled data for the two mutants is compared with that of wild-type cells. Perhaps even more important than the shift in the average CI is the dramatic decrease in the proportion of mutant cells that achieve high end CIs. While 33% of wild-type cells exhibited CIs of 0.6–0.8, only 17% of mutant cells did so, and while 8% of wild-type cells exhibited CIs of 0.8–1.0, no mutant cells were this efficient.

The decrease in the chemotactic efficiency of ponticulin-minus cells appears to be due to an increase in the frequency of sharp turns away from the direction of the gradient (i.e., away from the direction of increasing concentration of chemoattractant). In Fig. 10, $A$ and $B$, the perimeter and centroid tracks of a wild-type cell and a ponticulin-minus cell with comparable instantaneous velocities and very high CIs are compared. Although both cells were moving up the spatial gradient, the mutant cell made two small lateral deviations that lowered its chemotactic index. In each case, the deviation was due to a mistaken turn (i.e., a turn away from the direction of the source) generated by extension of a lateral pseudopod. These deviations generated kinks in the centroid track (Fig. 10 $B$). An examination of the tracks of the 10 Ax3K and 10 ponticulin-minus cells with the highest CIs demonstrated that mutant cells, on average, made more lateral turning mistakes than the wild-type cell. An examination of the 10 Ax3K and 10 ponticulin-minus cells with CIs closest to the mean of each group demonstrated that mutant cells, on average, made more lateral turning mistakes.

**Discussion**

We demonstrate here that lateral pseudopods formed off the substratum by wild-type Dictyostelium amoebae translocating in buffer remain fixed in relation to the substratum as they are extended and retracted, even though the main cell body may continue to translocate anteriorly. Because the ventral surfaces of lateral pseudopods formed off the substratum are not in contact with the substratum,
these pseudopods must be anchored to the substratum through interactions between the substratum and cortical and/or plasma membrane proteins in that portion of the cell body from which the pseudopod extends. Presumably, these interactions involve actin-based supramolecular cytoskeletal structures that connect the substratum with the plasma membrane, the cortex of the cell body and the cortex of the pseudopod.

We also have demonstrated here that ponticulin, a transmembrane protein responsible for most of the high affinity binding between F-actin and the Dictyostelium plasma membrane (Wuestehube and Luna, 1987; Wuestehube et al., 1989; Chia et al., 1991; Hitt et al., 1994a,b), is required for positionally fixing lateral pseudopods relative to the substratum, since the lateral pseudopods formed off the substratum by these mutant cells undergo frequent and dramatic slippage. Slippage can occur during both extension and retraction, and occurs primarily in the posterior direction. Pseudopods can also slip circumferentially to the top of a cell, and then slip posteriorly. Such circumferential slippage may be responsible for the significant increase in the proportion of both lateral and anterior pseudopods formed off the substratum by ponticulin-minus cells, since pseudopods that slip dorsally have less chance of contacting and subsequently extending along the substratum (Wessels et al., 1994).

This basic behavioral defect of ponticulin-minus cells is quite specific to this mutant. Ponticulin-minus cells translocating in buffer still extend pseudopods with the same frequency and growth dynamics as wild-type cells, and retract pseudopods with the same dynamics as wild-type cells. They exhibit the same periodicity in their velocity cycle as wild-type cells and can readily sense a gradient of chemoattractant, although with less efficiency. Their basic behavioral defect is, specifically, the loss of the positional stabilization of lateral pseudopods in relation to the substratum and subsequent pseudopod slippage. This mutant phenotype leads to a number of additional behavioral defects and contrasts markedly with that of myosin IA and B mutants, which are defective primarily in the frequency of pseudopods formed on the substratum (Wessels et al., 1991, 1996; Titus et al., 1993). It also contrasts with that of ABP-120 mutants, which are defective in the frequency of pseudopod formation and the rate and extent of pseudopod expansion (Cox et al., 1992; Cox, D., D. Wessels, D. R. Soll, J. Hartwig, and J. Condeelis, manuscript submitted for publication), with that of myoII mutants, which are defective in polarity, the original position of pseudopod extension, and the rate and extent of pseudopod growth (Wessels et al., 1988; Wessels and Soll, 1990), and with that of the discoidinless mutants, which are capable of rapid translocation in spite of their aberrant blunt morphology and loss of a conventional tapered uropod (Alexander et al., 1992).

Pseudopod slippage in ponticulin-minus cells correlates with a number of behavioral defects for cells translocating in buffer and for cells chemotaxing in a spatial gradient of cAMP. All of these defects may be consequences of slippage. First, ponticulin-minus cells translocating in buffer complete the process of pseudopod retraction at a more posterior cellular position than wild-type cells. In wild-type cells, most lateral pseudopods formed off the substratum extend from the anterior portion of the main cell body and are retracted primarily into the middle or posterior portion of the main cell body, just anterior to the uropod. This appears to be a consequence of the forward movement of the cell and the fixed position of the pseudopod in relation to the substratum. In wild-type cells, the morphological integrity of the uropod is, therefore, maintained throughout the 3-D behavior cycle of most cells (Wessels et al., 1994; Soll, 1995). In marked contrast, most pseudopods formed off the substratum by ponticulin-minus cells complete the retraction process at the uropod, with an accompanying characteristic alteration of uropod morphology. Pseudopod slippage obviously accounts for posteriorization of the retraction process in these mutants.

Ponticulin-minus cells translocating in buffer also exhibit altered turning behavior. Pseudopods formed by wild-type cells off the substratum have a greater propensity for being retracted and are less likely to initiate a sharp turn than pseudopods formed on the substratum (Wessels et al., 1994). Ponticulin-minus cells form a far greater proportion of their pseudopods off the substratum, which most likely explains why they translocate in buffer with a lower frequency of turning.

Finally, ponticulin-minus cells exhibit a decrease in the efficiency of chemotaxis. This decrease is due to an increase in turning mistakes made by ponticulin-minus cells moving up a spatial gradient of the chemoattractant cAMP. Superficially, this observation appears to contradict the observa-
tions made on turning in buffer, namely that slippage leads to a decrease in turning. However, inappropriate pseudo-
pod slippage would be expected to lead to a depression in chemotactic efficiency since there is strong reason to be-
lieve that the spatial stability of lateral pseudopods is nec-
ecessary for sensing a spatial gradient of chemotactic and 
reorienting (Varnum-Finney et al., 1987b; Soll et al.,
1993; Soll, 1995). Cells moving towards the source of a spa-
tial gradient form lateral pseudopods at roughly one-third
the frequency of cells moving in buffer, and the fewer lat-
eral pseudopods which do form generate turns at a lower
frequency (Varnum-Finney et al., 1987b), suggesting that
they are formed primarily off the substratum and, there-
fore, have a greater propensity for being retracted. In ad-
dition, cells oriented at an angle towards the source of
chemoattractant form as many lateral pseudopods towards
as away from the source, but turn into the former two to
three times as frequently as they do into the latter (Var-
num-Finney et al., 1987b). These results suggest that lat-
eral pseudopods formed by cells in a spatial gradient sense
the direction of the gradient and either fall to the substra-
tum to initiate a turn when sensing a positive gradient or
remain off the substratum and are retracted when sensing
a negative gradient (Soll, 1995). Within this context, the
loss of the positional stability of pseudopods by ponticulin-
minus cells would diminish the efficiency of chemotaxis by
three possible mechanisms. First, if a lateral pseudopod
rotates to the dorsal surface of a cell, it would be perpen-
dicular to the gradient of chemoattractant and, therefore,
incapable of sensing it. This outcome would depress the
average chemotactic index because cells veering off track
would produce fewer compensating sharp turns (Soll et al.,
1993). Second, if a lateral pseudopod slipped posteriorly
along the cell axis, it would also move through the gradient
in a direction of decreasing concentration, which would
confuse a temporal and/or spatial mechanism of gradient
assessment (Soll et al., 1993; Soll, 1995). Again, this out-
come would depress the average chemotactic index by de-
creasing the efficiency of reorientation. Third, if a lateral
pseudopod slipped to the substratum during sensing, it
would initiate an inappropriate turn, and result in a de-
pression in chemotactic index. It is likely that all three out-
comes contribute to the decrease in chemotactic efficiency
observed in ponticulin-minus cells.

Therefore, the loss of the positional stability of pseudo-
pods and the corresponding decrease in chemotactic ef-
ciency of ponticulin-minus cells suggests that ponticulin is
involved in at least one step in the chemotactic signal-
response pathway of Dictyostelium. One intriguing possi-
bility is that ponticulin is involved in stabilizing a sensing
pseudopod as it extends through a spatial gradient of at-
tractant off the substratum, and that a pseudopod which
senses an increasing concentration of attractant (i.e., is ex-
tending up a concentration gradient towards the source of
attractant) falls to the substratum to initiate a turn by de-
stabilizing the pseudopod through the regulation of pon-
ticulin.

Posterior slippage of lateral pseudopods along the long
axis of cells shares some morphological similarities to the
capping of cell surface receptors. However, these two phe-
nomena occur at dramatically different velocities. Lateral
pseudopods formed by ponticulin-minus mutants slip pos-
teriorly at ~50-60 μm/min, which is about an order of
magnitude faster than the velocity with which Dictyostel-
ium amoebae cap beads coated with lectin (Pasternak et
al., 1989; Jay and Elson, 1992). The velocity of lateral
pseudopod slippage is also at least five times faster than
the mean instantaneous velocity of cellular translocation.
The velocity of pseudopod slippage is, however, compara-
tive to the instantaneous velocities of intracellular particles
moving within the central cytoplasm of rapidly translocat-
ing wild-type Dictyostelium amoebae (Wessels and Soll,
1990), but these particles were observed to move primarily
towards the front of the cell. Since the velocity of these
forward moving particles was roughly five times that of
cellular translocation, there would either have to be disso-
lution of the particles at the anterior edge of the particu-
late cytoplasm or a retrograde compensatory current of
particles, perhaps in the cytoplasmic cortex, to account for
the fact that particles do not accumulate in the front of a
cell. The absence of posterior slippage of lateral pseudo-
pods in wild-type cells suggests that ponticulin is integral
to a plasma membrane-associated cytoskeletal structure
that normally resists the suggested rearward flow of corti-
cal cytoplasm. Posterior pseudopod slippage in ponticulin-
minus cells is consistent with the contraction-hydraulic
(Mast, 1926; Jahn, 1964) and cortical flow (Bray and White,
1988) models of cellular translocation, in which fountains
of submembranous cytoplasm (Grebecki, 1994) move rear-
ward in regions of the cell not attached to the substratum.

The absence of posterior pseudopod slippage in wild-
type cells suggests that ponticulin is involved in a plasma
membrane-associated cytoskeletal structure (membrane
skeleton) that normally resists the putative rearward flow
of cytoplasm. Ponticulin-based anchorage of lateral pseudo-
pods relative to the substratum is consistent with the gen-
eral role played by membrane skeleton proteins in separ-
ating regions of the plasma membrane into discrete func-
tional domains (for review see Luna and Hitt, 1992).
Another implication of our results is that pseudopods,
though dynamic in nature, may be thought of as plasma
membrane domains that are stabilized and regulated by
membrane skeleton proteins, such as ponticulin. In con-
junction with other recently described regions of mem-
brane specialization in motile cells (for review see Sheetz,
1995), our observations suggest that the membrane skele-
tons of motile cells are at least as complex as, though much
less well understood than, the membrane skeletons of
more static cells such as erythrocytes and epithelial cells.

Because the formation of pseudopods on and off the
substratum and the periodicity of the motility cycle of Dic-
tyostelium amoebae translocating in buffer are strikingly
similar to several behavioral aspects of polymorphonu-
clear leukocytes (Murray et al., 1992) and of T cells and gi-
ant HIV-induced T cell syncytia (Sylwester et al., 1993,
1995), a final implication of our results is that precise tem-
poral and spatial regulation of pseudopod dynamics may
be a general requirement for the taxis of many chemotacti-
cally responsive mammalian cells. Therefore, proteins with
structural and functional similarities to Dictyostelium pon-
ticulin may play critical roles in morphogenesis, the cellu-
lar immune system, and other biological processes in which
amoeboid cells must move in a directed fashion through
extension of actin-based pseudopods.
References


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