Journal of Cellular Biochemistry 37:177–192 (1988)
Signal Transduction in Cytoplasmic Organization
and Cell Motility 157–172

"Dynamic Morphology System": A Method for Quantitating Changes in Shape, Pseudopod Formation, and Motion in Normal and Mutant Amoebae of *Dictyostelium discoideum*

David R. Soll, Edward Voss, Barbara Varnum-Finney, and Deborah Wessels

Department of Biology, University of Iowa, Iowa City, Iowa 52242

An automated, video-driven system was used to measure approximately 30 parameters of cell motion and accompanying changes in shape. This "Dynamic Morphology System" is based upon the Expertvision Motion Analysis System and is driven by a SUN computer. With the aid of this system, amoebic movement and shape changes were compared for vegetative wild-type *Dictyostelium discoideum* amoebae and a motility mutant, Mo-1. The measured parameters included speed, angle change, bearing, length, width, roundness, boundary flow, and curvature; and cell behavior was visualized monitoring amoebic tracks, difference pictures, and a newly developed ring expansion plot. Wild-type cells remained elongated, moved continuously and retained polarity throughout migration. In contrast, Mo-1 did not translocate, was round rather than elongated, formed bulges rather than elongated pseudopods, and exhibited no polarity. In contrast to the anterior f-action distribution in wild-type cells, f-actin in Mo-1 was distributed evenly as a shell just under the entire plasma membrane, a distribution consistent with the lack of polar cytoplasmic expansion.

Key words: chemotoxins, computer-assisted analyses, actin

The subtleties of amoebic behavior have long been the subject of intense investigation [see ref. 1, for a recent review] primarily because of the fundamental role of cellular movement in morphogenesis and the immune response. However, the dynamic changes in cell shape and pseudopod formation which accompany cellular translocation are difficult to quantitate, and reference to these parameters has therefore remained descriptive and in some cases anecdotal. A need therefore exists to obtain the most detailed quantitative descriptions of cellular behavior that are possible, and this need indeed becomes paramount in analyses of pseudopod behavior for cells in the process of chemotaxis [2,3] and for comparisons of normal cells and variant cells with alterations in select components of the cytoskeleton and plasma membrane. We have therefore

Received March 25, 1987; accepted September 11, 1987.

developed an automated, video-driven system, the "Dynamic Morphology System," for quantitatively analyzing a number of shape changes associated with amoebic motion. This system is based upon the Expertvision Motion Analysis System developed by Motion Analysis Corporation (Santa Rosa, CA) which was originally developed for tracking the centroid of a videorecorded moving object. The Dynamic Morphology System measures approximately 30 paremeters of motion and accompanying shape changes, and represents a new tool for exploring normal, as well as aberrant, cell behavior. In this report, we will describe this system and demonstrate its application in a comparison of a wild-type cell with a motility mutant, Mo-1, of *Dictyostelium discoideum*.

MATERIALS AND METHODS

Growth of Stock Cultures

Spores of the axenic strain Ax-3 (clone RC3) were stored on silica gel [4]. Every month, a fresh clone was generated for mass culture from silica gel. Clones were generated and cells were grown in suspension in the axenic medium HL-5 [5] according to methods previously described [6,7].

Isolation of the Motility Mutant Mo-1

Amoebae in the exponential phase of growth in suspension cultures were washed free of nutrient medium (HL-5), resuspended in a shaking flask containing buffer solution (40 mM phosphate buffer, pH 6.2), and 1.2 mg/ml 1-methyl-3-nitro-1-nitrosoguanidine, and incubated at 22°C for 8 min. Under these conditions, 6% of the amoebae survived. Survivors were plated on nutrient agar in association with *Klebsiella aerogenes* at a density of 20 amoebae per 150-mm petri dish. After 3 days, wild-type amoeba had cleared a circular "plaque" in the bacterial carpet roughly 15 mm in diameter and exhibited aggregation in the colony center. Mo-1 was chosen as a putative motility mutant because it produced a small plaque of 4.0 mm after 3 days and exhibited no aggregation. Cells from the original Mo-1 colony were then clonally plated and demonstrated to maintain the original Mo-1 phenotype in a heritable fashion.

Recording Amoebic Behavior for Computer Analysis

For analysis of amoebic behavior, wild-type and Mo-1 cells were grown in suspension in association with *Klebsiella aerogenes* in buffer solution (40 mM phosphate buffer, pH 6.2) at 22°C. Cells in the mid-log phase of growth were washed free of bacteria, resuspended in 1.1 ml of buffer solution, and inoculated into a Sykes-Moore perfusion chamber according to methods previously described in detail [8,9]. This chamber consisted of a rubber O-ring sandwiched between two circular glass coverslips, which in turn were held together by a stainless steel clamp. Cells were allowed to settle and attach to the bottom chamber wall, a process which took less than 5 min. The chamber was then inverted, and placed on the stage of a phase-contrast microscope fitted with a long-range condensor. The microscope lighting was adjusted so that it produced a high contrast image with the cell uniformly black and the background white. Amoebae were then perfused with buffer solution at a flow rate of 4 ml/min, resulting in the replacement of the chamber fluid every 15 sec. This flow rate was previously demonstrated to have no adverse effects on cell motility [9]. Buffer was perfused for 10 min prior to recording so that amoebae could adjust to the chamber conditions. Amoeboid movement was then videorecorded during a subsequent 10-min period (Fig. 1).

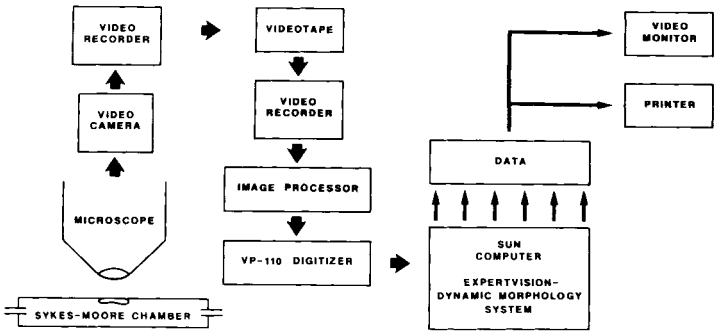

Fig. 1. Schematic drawing of the video-monitored perfusion chamber and computer analysis system. Amoebae were inoculated into the perfusion chamber and placed on the stage of a phase-contrast microscope fitted with a long-range condensor (lower left-hand side of screen). Amoebic behavior was videorecorded, the videotapes were replayed, and amoebae were image-processed to solid black images. By means of the VP-110 digitizer and the Expert Vision System, the x,y-coordinates of the pixels outlining the cells were recorded in a data file in the SUN 2/120 computer. Motion and cell shape parameters were then analyzed utilizing Dynamic Morphology System software (see detailed description in Materials and Methods). Data were visualized on a monitor and/or printed on a laser printer.

Computer Analysis of Cell Movement and Cell Shape

Videotapes were analyzed with the Expertvision Integrated Motion Analysis System (Motion Analysis Corp. Santa Rosa, CA), utilizing the Dynamic Morphology System (DMS), a software package developed by SVUI (Iowa City, IA) specifically for analyzing the dynamics of amoeboid movement and shape. Some aspects of this system have been previously described [2,3]. In this system, the video screen is divided into a 256 × 230 array of pixels which can be "on" or "off." The pixels which border the edge of the high-contrast cell image are then "on" and all others "off." The x,y-coordinates of all "on" pixels or the cell outlines were then recorded in a data file in a SUN 2/120 microcomputer. Utilizing the DMS software package, a path of cell movement is then generated. In any given video frame, the centroid (estimated center of the cell) is determined by calculating a mean for the x-coordinates and the y-coordinates, respectively, of the pixels making up the cells outline. These means provide the x- and y-coordinates of the centroid (represented by circles in Fig. 2A and B). The centroids in consecutive frames are then connected to generate a path of cell movement. To enhance the digitized cell outline, cubic splines are used to fit third-degree polynomial curves to a user-determined number of pixels in the cell's outline [10]. By means of the centroid data, speed of movement and angle change were determined by the central difference method [9,10]. For a cell in any one video frame (n), a vector was generated which originated at the centroid in the previous frame ($n-1$) and extended to the centroid in the subsequent frame ($n+1$). To calculate speed, the length of the vector was then divided by $2\Delta t$, where Δt is the time interval between frames. The angle of the vector was the direction of cell movement. The absolute difference in the direction between two consecutive frames ($|\text{direction}_n - \text{direction}_{n+1}|$) was the angle change.

With the aid of the cell outline data, length, width, area, roundness, and perimeter were determined. The length was the line connecting the two points in the cell outline

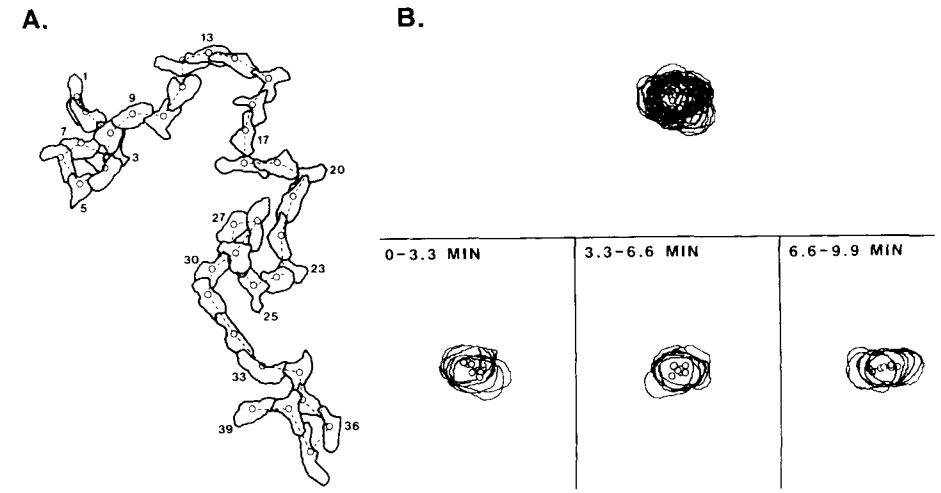

Fig. 2. Computer-generated tracks of cell movement for a wild-type (**A**) and Mo-1 (**B**) amoeba. The "smoothed" cell outline and centroid (○) were drawn for a representative wild-type (A) and Mo-1 (B, upper panel) amoeba at 30- and 15-sec intervals for 20 and 10 min, respectively, and for the same Mo-1 (B, lower panels) cell at 15-sec intervals for $6\frac{1}{3}$-min consecutive periods. Each outline of the wild-type cell is stacked over the preceding one; outlines of Mo-1 are simply stacked.

that were farthest apart. The width was the area (see below) divided by the length. The area was computed using the line integral

$$\left|\frac{1}{2}\int_C (ydx - xdy)\right|$$

over the contour C of the cell, where the contour C is obtained by connecting the boundary pixels with line segments. Perimeter was calculated by summing the lengths of the line segments that make up the contour. Roundness (circularity) was defined as follows:

$$\frac{4\pi \times \text{area}}{\text{perimeter}^2} \times 100$$

Roundness varies from 0% for a cell that is completely flat to 100% for a circle.

To provide graphic descriptions of how a cell's shape changed over time, cytoplasmic flow diagrams were generated. A description of these diagrams was presented previously [2]. Essentially, the cell outline from a later frame is superimposed on the cell outline of a preceding frame. The portion of the cell area in the later frame which has expanded relative to the cell area of the preceding frame is filled in black and the portion which has contracted is filled in grey. The cell area which is common for the consecutive frames remains unfilled, or white. An arrow extending from the centroid in the first frame through the centroid in the second frame indicates the direction of movement. These images are referred to as "difference pictures."

To provide a more quantitative description of cytoplasmic flow, flow plots were generated. As with difference pictures, the same cell was compared in two different frames. In each direction (0°–359°), the length of the radius (r) is computed. In this case, the radius begins at the centroid and extends to the boundary in a given direction. The rate of radial change $\Delta r/\Delta t$ is then plotted for each direction, where Δt is the time interval between each frame. To assess flow rhythms, flow plots were "wrapped" into concentric circles and stacked with the innermost circle representing the flow in the first frames and the outermost circle the flow in the last frames. Wrapped circles are calculated as follows: let $F(\theta,t)$ be the flow in direction θ at time t. It is defined as $F(\theta,t) = \Delta r/\Delta t$, the rate of change of the radius in direction with respect to time. It is plotted in polar coordinates (R,θ) with $R = t + cF(\theta,t)$, where c is a user-supplied scaling factor. This results in circles with radius t (time). The circles representing consecutive cell perimeters are drawn concentrically, earliest cell perimeter innermost and latest cell perimeter outermost. Closely stacked concentric lines represent perimeter regions of little shape change; sparsely stacked, open regions represent perimeter regions of active shape change.

To quantitate dynamic changes in cell shape further, curvature was plotted at different time intervals. Curvature is defined as the rate of change of the angle of the tangent line with respect to the perimeter along the cell's boundary [11]. A circle will have constant positive curvature. The tip of a cellular extension will exhibit large positive curvature, whereas the cell outline where the extension originates will exhibit negative curvature. Curvature is graphed as a function of perimeter (arc length). A circle is placed on each plot to indicate where the vector representing direction of travel intersects the perimeter.

Actin Staining With Tagged Phalloidin

Cells were washed twice in ice-cold phosphate buffer solution, then resuspended in cold phosphate buffer to a final density of 2×10^6 cells per ml. A drop of cell suspension was placed on a 12-mm circular coverslip which was placed in a 35 × 10 mm petri dish. After 5 min, 5 ml of phosphate buffer was gently added to the dish, followed by 0.5 ml of 37% formaldehyde. Cells were fixed for 30 min. Cells were then washed twice with 5 ml of phosphate buffer. The remaining solution was aspirated from the dish and a solution of 10 μM of fluorescein-conjugated phalloidin in 40 mM phosphate buffer pipetted over the coverslip. Cells were then incubated in the dark for 20 min and washed free of phalloidin with phosphate buffer. The coverslip was then inverted over a precleaned microscope slide and placed on a drop of mounting medium (1:1 mixture of glycerol and phosphate buffer). Slides were then photographed on a Zeiss fluorescence microscope.

RESULTS

Recording Cell Behavior

To record the behavior of wild-type and mutant cells, amoebae from growing cultures were washed free of nutrient medium, washed twice in buffered salts solution, then inoculated into a Sykes-Moore chamber. The chamber was immediately positioned on the stage of a compound trinocular microscope fitted with long-distance objectives and a long-distance condensor (Fig. 1). The chamber was then perfused with buffered salts solution at a velocity which resulted in the turnover of medium roughly four times

per minute, but which did not interfere with cellular behavior [9]. Cell behavior was recorded through a videocamera attached to a recorder (Fig. 1). The videotape of cellular behavior was subsequently analyzed in the Expertvision System employing the Dynamic Morphology System (see detailed description in Materials and Methods).

Path of Movement

In Figure 2, computer-generated cell tracks are presented for a typical wild-type and Mo-1 amoeba over a 20- and 10-min period, respectively. In this particular set of tracks, the "smoothed" perimeters of the amoebae are drawn at 30- and 15-sec intervals, respectively. In each cell tracing, the centroid of the cell is presented as a small circle, and the circles are connected by a dashed line to provide the centroid track. Tracings of the wild-type cell are overlaid, with the most recent tracing stacked over the preceding tracing.

It is clear from the wild-type track (Fig. 2A) that the amoeba is moving at a fairly rapid pace. Since there is no chemotactic gradient in the perfusion chamber, and no chemoattractant accumulation owing to rapid perfusion, the path of the wild-type cell is relatively random, and the average speed represents a basal measure of cellular translocation. It is clear from the track that movement is relatively continuous and that directional changes are common but in most cases are effected by continuous turning rather than by a reversal of polarity. The average speed of this particular cell was computed from half-minute measurements to be 22.4 μm per minute over a 10-min period (Table IA). Whenthe speeds calculated from centroid translocation every half minute are plotted as a function of time, it is clear that movement is relatively continuous (Fig. 3). The average angle change was computed from half-minute measurements to be 37.8° over a 10-min period (Table IA). All of the 10 wild-type amoebae examined gave results similar to those presented for the single exemplary cell.

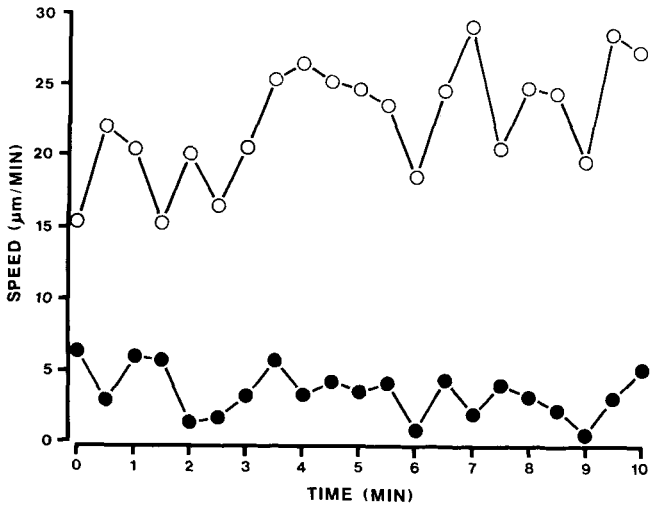

Fig. 3. Speed of cell movement for a wild-type and Mo-1 amoeba. From the centroid data, the speed was calculated using the central difference method every 30 sec over a 10-min period for a representative wild-type (○) and Mo-1 (●) amoeba.

In marked contrast, there was virtually no path, and therefore no translocation of Mo-1 over a 10-min period (Fig. 2B). The perimeters drawn every half minute over a 10-min period stack in a single area (Fig. 2B, upper panel). When the perimeter drawings are separated into consecutive 3.3 min periods (Fig. 2B, lower 3 panels from left to right), it is clear that the cell is not translocating significantly, although small changes in centroid position are occurring as a result of shape changes. The average speed of this particular Mo-1 amoeba was computed from half-minute measurements to be 3.5 μm per minute over a 10-min period (Table IB), but it is clear from the centroid plots in the lower panels of Fig. 2B that this speed does not represent true translocation. When the speeds calculated from centroid translocation every half minute are plotted as a function of time, it is clear that even though there is no true cellular translocation, the cell must be changing shape continuously in order to continuously change the position of the centroid (Fig. 3). The average angle change of this Mo-1 amoeba was computed from half-minute measurements to be 63.7° over a 10-min period (Table IB), twice the average angle change of the wild-type cell, indicating a more random track for the centroids of Mo-1. All of the 10 Mo-1 amoebae analyzed gave results similar to those presented for the single exemplary cell.

Length, Width, and Roundness

Drawings of the perimeters in Figure 2 indicate that the average shape of Mo-1 differs dramatically from that of the wild-type cell. This point is reinforced by measurements of length, width, and roundness computed every half minute over a 10-min period (Table I) and plotted in Figure 4. The average length of the wild-type cell was 20.4 μm and the average width 6.3 μm (Table IA). Throughout the entire 10-min period of migration, the wild-type amoeba maintained its elongated shape (Fig. 4A). In marked contrast, the average length of the Mo-1 cell was 17 μm and the average width 10 μm (Table IB). Throughout the entire 10-min period of analysis, the mutant amoeba maintained this rounder shape (Fig. 4B). The differences in shape between the wild-type mutant amoebae is underlined by comparing the parameter of "roundness." In this case, roundness is defined as

$$\frac{4\pi \times \text{area}}{\text{perimeter}^2} \times 100$$

in which a straight line is 0% and a circle is 100%. Average roundness of the wild-type cell was 55.2% and that of the mutant 91.6% (Table I). Again, there was little variation in the two cells over the 10-min period of analysis (compare panels C and 4D of Fig. 4).

Difference Pictures

Perhaps the most dramatic parameter measured by the Dynamic Morphology System is "difference," a measure of cytoplasmic flow. In this case, the system compares consecutive frames of an image, and generates an image with three discriminated regions: 1) the region of the later image not included in the earlier image, representing an area of expansion (this region is presented as the black area of each image in Fig. 5); 2) the region of the earlier image not included in the later image, representing an area of contraction (this region is presented as the gray area of each image in Fig. 5); and 3) the region of the later image common to the earlier image (this region is presented as the

TABLE I. Speed, Angle, Change, Length, Width, Area, Roundness, and Perimeter Measured Every Half Minute Over a 10-min Period for a Wild-Type Cell (A) and a Motility Mutuant, Mo-1 (B)*

Frame	Time (min)	Speed (μm/min)	Angle change (degrees)	Length (μm)	Width (μm)	Area (μm^2)	Roundness (%)	Perimeter (μm)
A. Wild-type								
1	0.0	15.3	0.0	21.6	5.8	126.0	56.6	52.9
7	0.5	22.0	14.1	19.7	5.8	115.0	58.4	49.7
13	1.0	20.4	20.8	18.3	6.2	113.6	60.5	48.6
19	1.5	15.2	99.3	21.1	6.1	128.7	53.7	54.9
25	2.0	20.1	16.8	19.6	6.5	126.5	63.2	50.2
31	2.5	16.5	89.8	19.7	6.1	120.8	43.5	59.0
37	3.0	20.5	76.4	16.9	6.4	108.4	64.2	46.1
43	3.5	25.3	71.1	18.8	6.6	123.8	60.0	50.9
49	4.0	26.5	5.1	15.0	8.3	124.9	71.7	46.8
55	4.5	25.2	44.1	18.7	6.7	124.9	74.2	46.0
61	5.0	24.6	6.6	19.0	7.7	145.1	59.2	55.5
67	5.5	23.5	44.7	19.9	7.0	139.1	59.0	54.4
73	6.0	18.5	18.0	28.2	5.1	144.6	42.7	65.2
79	6.5	24.5	50.4	21.6	6.0	128.7	56.3	53.6
85	7.0	29.0	22.7	23.9	5.1	122.1	49.1	55.9
91	7.5	20.4	46.9	19.4	6.3	122.4	40.2	61.8
97	8.0	24.7	47.1	19.9	6.0	118.8	35.8	64.6
103	8.5	24.3	15.0	18.6	6.0	112.0	55.2	50.5
109	9.0	19.5	61.2	26.6	5.2	138.8	42.9	63.7
115	9.5	28.4	27.3	20.8	6.5	134.7	57.3	54.4
121	10.0	27.2	15.6	21.5	6.1	130.1	54.8	54.6
$\bar{x} \pm sD$		22.4 ± 4.0	37.8 ± 28.0	20.4 ± 2.9	6.3 ± 0.7	126.1 ± 9.9	55.2 ± 9.7	54.3 ± 5.7

B. Mo-1

1	0.0	6.4	0.0	17.0	10.4	177.7	93.3	48.9
11	0.5	2.9	27.6	18.4	9.9	181.8	89.8	50.4
21	1.0	5.9	132.5	19.4	10.0	193.9	87.2	52.9
31	1.5	5.7	12.8	16.3	10.8	174.7	94.0	48.3
41	2.0	1.4	10.8	18.0	10.0	179.9	91.1	49.8
51	2.5	1.7	176.0	14.9	10.3	152.8	94.7	45.0
61	3.0	3.2	126.9	18.2	9.6	174.7	87.4	50.1
71	3.5	5.7	35.7	16.9	10.2	171.7	91.4	48.6
81	4.0	3.3	140.3	17.7	10.1	178.0	90.3	49.8
91	4.5	4.3	20.7	14.8	10.5	155.8	94.1	45.6
101	5.0	3.5	3.1	16.6	9.8	162.1	94.0	46.6
111	5.5	4.1	7.5	19.2	9.3	179.3	89.9	50.1
121	6.0	0.9	133.0	17.6	9.8	173.0	90.7	49.0
131	6.5	4.4	31.2	15.5	9.3	143.5	92.5	44.2
141	7.0	2.0	127.3	16.2	9.7	157.7	89.3	47.1
151	7.5	4.0	63.4	16.4	10.8	176.9	94.3	48.6
161	8.0	3.2	104.7	16.9	9.7	164.3	90.7	47.7
171	8.5	2.2	71.6	16.9	10.0	168.1	91.6	48.0
181	9.0	0.5	21.5	17.1	9.7	165.7	93.4	47.2
191	9.5	3.0	90.5	15.7	10.7	168.1	95.2	47.1
201	10.0	5.1	1.4	18.5	10.6	195.2	89.0	52.5
$\bar{x} \pm$ sD		3.5 ± 1.6	63.7 ± 55.8	17.1 ± 1.3	10.0 ± 0.4	171.2 ± 12.3	91.6 ± 2.3	48.5 ± 2.1

*Means ($\bar{x}$) and standard deviations (SD) are presented for each parameter. Total distance traveled by the centroid of each cell was 258 μm for wild-type and 45 μm for Mo-1.

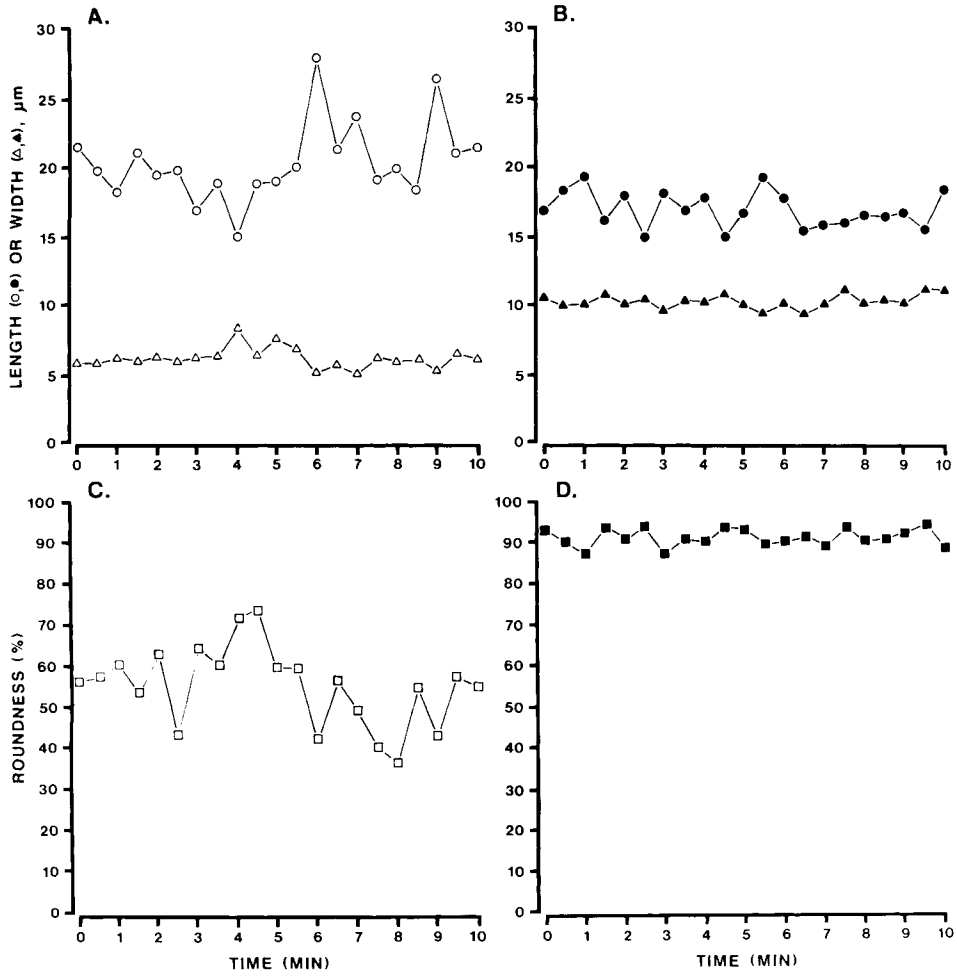

Fig. 4. Width, length and roundness for a wild-type (**A** and **C**) and Mo-1 (**B** and **D**) amoeba. Width (△,▲) and length (○,●) were plotted every 30 sec for a 10-min period for a wild-type (A) and Mo-1 (B) amoeba. Roundness was plotted for the same period for a wild-type amoeba (C) and Mo-1 amoeba (D).

white area of each image in Fig. 5). In each difference picture in Figure 5, the centroid of the later image is presented as an unfilled circle, and the direction of translocation is indicated by an arrow. In Figure 5, difference pictures were generated for 15-sec intervals (i.e., the time between the earlier and later image in each case was 15 sec); total time is presented below each image for a six-min period for wild-type (Fig. 5A) and Mo-1 (Fig. 5B). These difference pictures provide one with a visual representation of the expansion and contraction zones of a moving cell, and, therefore, the spatial and temporal dynamics of pseudopod formation.

The difference pictures of the wild-type cell (Fig. 5A) demonstrate several aspects of amoebic motion. First, there is a clear polarity of movement, with continuity between expansion zones. It is also clear that, at the anterior of a cell, pseudopods form and

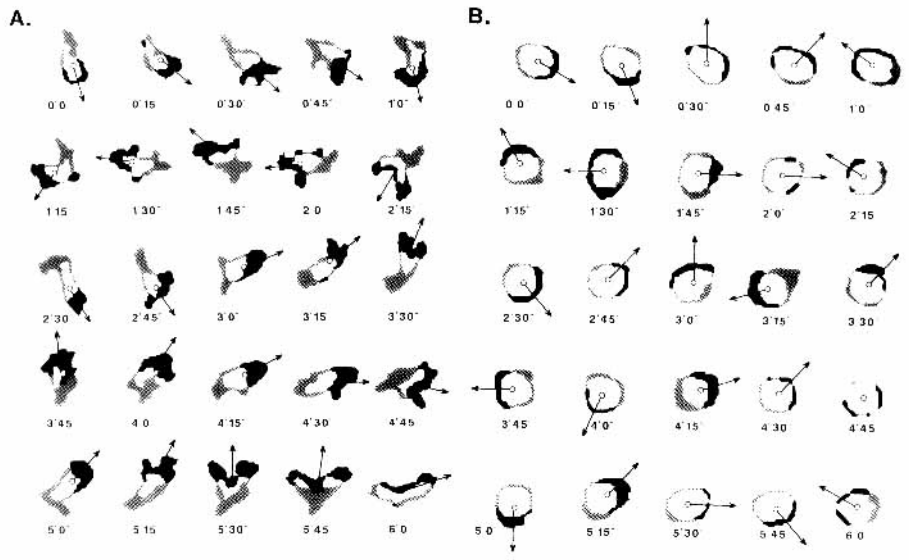

Fig. 5. Difference pictures for a wild-type (**A**) and a Mo-1 (**B**) amoeba. Cell outlines from two consecutive video frames 15 sec apart were superimposed to generate difference pictures for a representative wild-type (A) and a representative Mo-1 (B) amoeba. The black-filled regions represent areas of expansion in the second video frame relative to the first. The gray regions represent areas of contraction in the second video frame relative to the first. The white regions represent unchanged or common areas in both video frames. The open circles represent the centroids in the first video frames. The arrows represent the direction of cell movement.

branch and that the cell selects a particular pseudopod to channel into. Contraction regions appear to be most prominent at the posterior end of a cell, but significant contraction also occurs in a cell's midregion. Again, it is clear from the track of the wild-type amoeba that turning is effected from the front of the cell and that a sudden change in polarity does not occur. This is best realized by following the expansion zones in consecutive images.

The difference pictures of Mo-1 (Fig. 5B) differ dramatically from those of wild-type cells (Fig. 5A). To begin with, expansion zones appear to be far rounder than the elongate pseudopodial regions which rapidly extend from the anterior region of a wild-type amoeba. In addition, there is little polarity in the zones of expansion; within a 15-sec period, a new expansion zone may appear 180° from the previous region of expansion. This is best realized by comparing the expansion zones between the images of 0′15″ and 0′30″, 1′30″ and 1′45″, 3′15″ and 3′30″, 4′0″ and 4′15″, 5′0″ and 5′15″, or 5′45″ and 6′0″ (Fig. 5B).

Boundary flow plots were also used to quantitate expansion and contraction zones between consecutive images. In these plots, the change in radius (Δr) from the centroid to the cell perimeter is calculated between consecutive frames and the radial change $\Delta r/\Delta t$ is plotted around the entire cell perimeter. This results in a boundary flow plot (Fig. 6) in which peaks represent expansion zones and troughs contraction zones. The horizontal axis represents the degrees around the cell perimeter (0° to 360°) beginning with that point on the perimeter furthest right on the video screen (0°). The boundary flow plots at 15-sec intervals are then stacked in Figure 6A for the wild-type cell and Figure

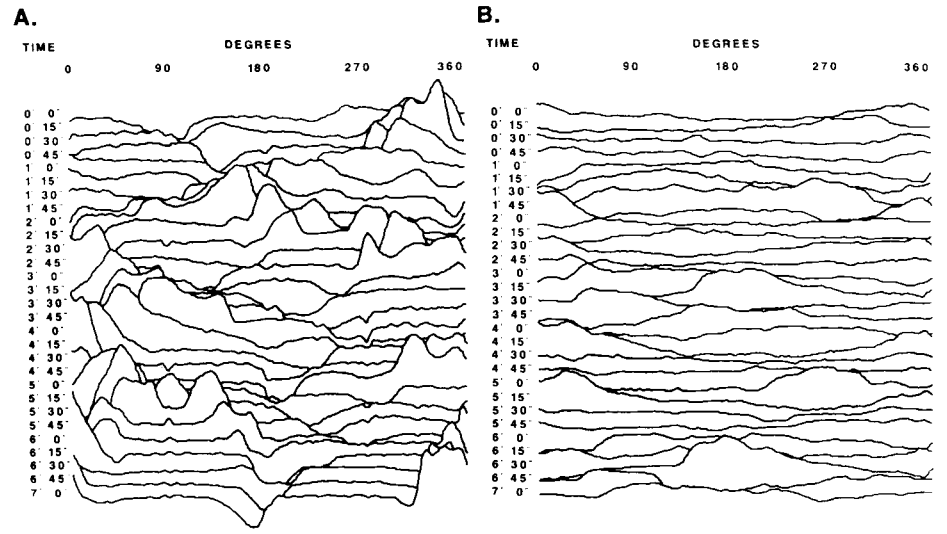

Fig. 6. Boundary flow plots for a wild-type (**A**) and a Mo-1 (**B**) amoeba. Boundary flow plots (see Materials and Methods, and Results sections for detailed descriptions of these plots) were generated every 15 sec for a 7-min period for a representative wild-type (A) and a Mo-1 (B) amoeba. Peaks represent expansion zones and troughs, contraction zones.

6B for Mo-1. Stacking allows one to scan the cell boundary with time in order to obtain a dynamic view of cytoplasmic flow. The boundary flow plots of the wild-type cell (Fig. 6A) exhibit far more peaks and valleys than those of Mo-1 (Fig. 6B). One can also observe how pseudopods (major peaks) move in a continuous fashion on the surface of wild-type cells, but expansion zones appear and disappear in a discontinuous fashion on the surface of Mo-1.

Curvature

Boundary flow plots are generated from difference measurements. A more direct way of visualizing curvature is to calculate the rate of change of the angle of the tangent line with respect to perimeter along the cell boundary (Fig. 7). Boundary length was measured beginning with that point on the perimeter furthest right on the video screen. This provides a curvature plot of a cell's boundary in which the tip of a narrow protrusion will result in large positive curvature while the surface of the cell from which the protrusion extends will result in negative curvature. The rate of change of the angle of the tangent is graphed as a function of boundary length, and the plots generated at different time points are stacked. As one would have predicted from the shape analyses in Figure 2 and Figure 5, there are far more protrusions in wild-type cells (Fig. 7A) than in Mo-1 cells (Fig. 7B), and the wild-type protrusions are more tapered, resulting in dramatic peaks in the curvature plots. The rounder protrusions from the surface of the Mo-1 cell result in flatter, wider peaks along the cell's surface.

Visualizing Rhythms in Pseudopod Formation

In order to test whether there is rhythmicity in pseudopod formation, the flow plots in Figure 6 were wrapped around one another, with "time" as the centripedal vector

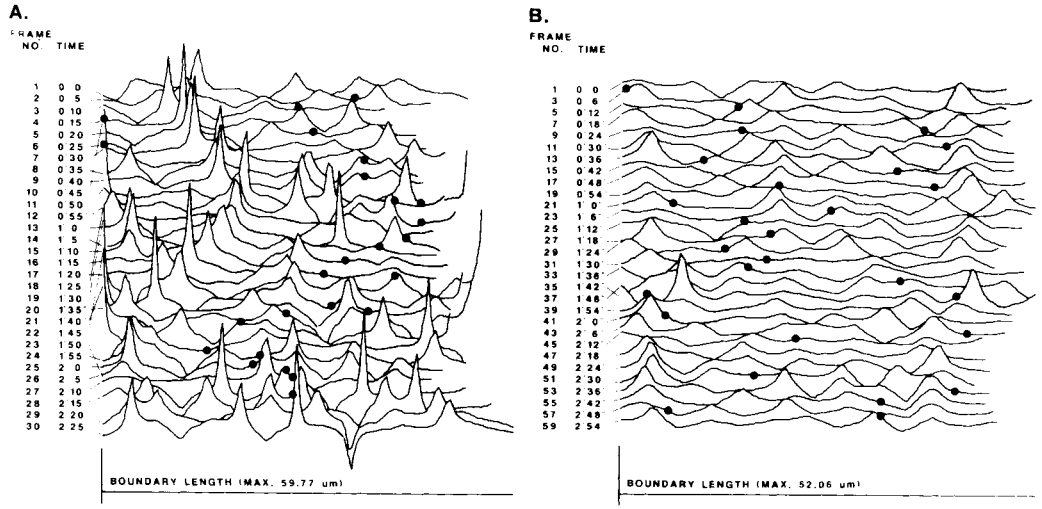

Fig. 7. Curvature plots for a wild-type (**A**) and a Mo-1 (**B**) amoeba. Curvature plots (see Materials and Methods, as well as Results, for a detailed description of these plots) were generated every 5 sec for $2\frac{1}{4}$min for a representative wild-type (A) and a Mo-1 (b) amoeba. Areas of large positive curvature represent expansion and areas of negative curvature, contraction. The dots represent the direction of cell movement.

(Fig. 8). The earliest flow plot is represented by the innermost "circle" and the last flow plot by the outermost circle. A technical description of "wrapping" is presented in the Materials and Methods section. Close concentric lines correspond to regions of low activity (little shape change or flow), while sparse areas correspond to regions of high activity (significant shape change or flow). In Figure 8A, wrapped flow plots for the wild-type cell are presented. It is immediately obvious that a rhythm of roughly 2.5 min as well as a faint indication of coiling can be distinguished. In Figure 8B, wrapped flow plots for Mo-1 are presented. Rhythmicity is less obvious in the Mo-1 plots.

F-Actin Distribution

The lack of polarity, the lack of meaningful translocation, and the rounded cytoplasmic protrusions of Mo-1 suggest that the cytoskeleton may be abnormally organized. This suggestion is reinforced by the pattern of f-actin organization. Wild-type cells stained with fluorescein-conjugated phalloidin exhibit heavy staining of f-actin in the anterior quadrant of the amoeba (Fig. 9A), the most active region of pseudopod formation [2,3]. They also exhibit f-actin granules in the posterior quadrant (Fig. 9A). In marked contrast, phalloidin stained Mo-1 cells exhibit an outer cortex of f-actin staining, with f-actin equally distributed in a shell just under the plasma membrane of the entire cell. These distributions are consistent with the polarity of pseudopod formation in the anterior quadrant of wild-type cells and the random bulging of Mo-1 around its entire perimeter.

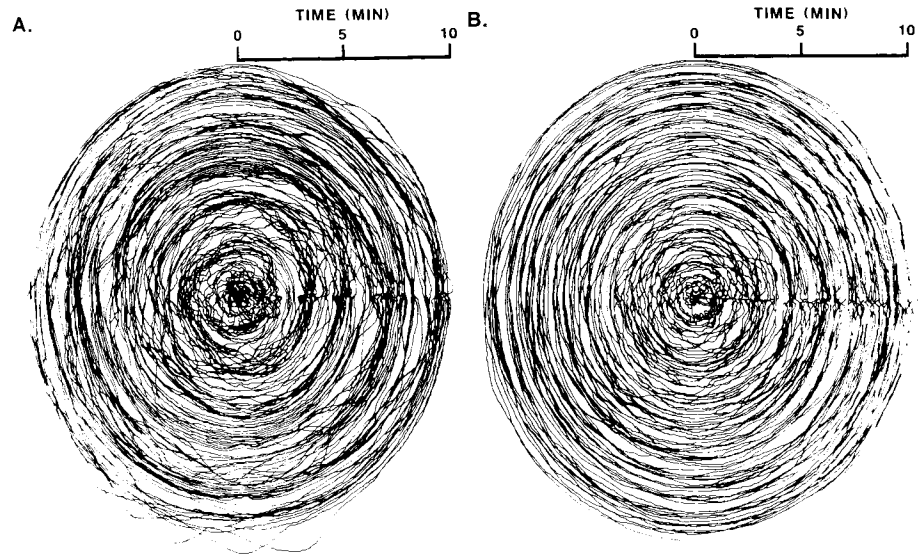

Fig. 8. Rhythms in pseudopod extension for a wild-type (**A**) and a Mo-1 (**B**) amoeba. To test for rhythmicity, the flow plots (see Fig. 6) were wrapped around one another, with "time" as the centrifugal vector. The earliest flow plot is the innermost "circle" and the last, the outermost "circle." Wrapped flow plots were generated for a representative wild-type (A) and Mo-1 (B) amoeba.

DISCUSSION

"DMS"

In this report, we have described a new video-driven system which automatically analyzes a number of parameters related to movement and shape change. In this system, a videorecording of any amorphous moving object is played into the machine, and over 30 parameters related to movement and cellular morphology are measured according to the dictates of the researcher. This can be as frequent as every video frame for fast-moving objects or as slow as is required for very slow moving objects. Not only does this system quantitate such characteristics as velocity, angle change, bearing, length,

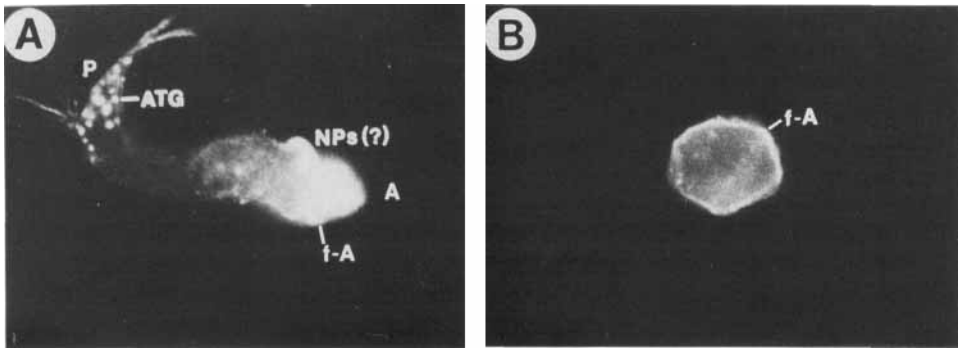

Fig. 9. The pattern of f-actin organization in a wild-type (**A**) and a Mo-1 (**B**) amoeba. A representative wild-type (A) and Mo-1 (B) amoeba were stained with phalloidin, which is specific for f-actin.

width, roundness, and boundary flow, but it also provides visualizations through "difference pictures," amoebic tracks, and the ring expansion plot which allows the researcher to obtain a dynamic view of the shape changes, especially pseudopod formation and cytoplasmic contraction zones, associated with movement. In addition, an object which is changing its shape need not exhibit any translocation to be critically analyzed by the Dynamic Morphology System, as evidenced by the analysis of Mo-1.

It should be emphasized that the Dynamic Morphology System is automated. Unlike its predecessors, which included laborious digitizing boards for analysis, once the video is recorded in the computer files, all measurements are automatically quantitated and either displayed or printed according to directions by a set of cascading menus. More importantly, the Dynamic Morphology System measures parameters with detail and speed which could not be achieved by a researcher with a digitizing pad.

A Comparison of Wild-Type and Mo-1 Behavior With DMS

We have compared the behavior of wild-type amoebae and Mo-1 amoebae to demonstrate the capabilities of DMS in comparative studies. Several aspects of the results reveal a number of points related to amoebic movement and the deficiencies of the mutant cell line. To begin with, the amoebae examined were grown in the presence of bacteria, then analyzed immediately. Therefore, the motility of these amoebae reflect the capabilities of cells moving for feeding purposes, but not necessarily the capabilities of developing amoebae [12]. We previously demonstrated that bacterially grown amoebae of *D. discoideum* are very motile on the glass wall of a Sykes-Moore chamber [12]. In the example of a cell track analyzed in this study, the representative wild-type amoeba moved at an average speed of 22 μm per minute, movement was relatively continuous, the polarity of the cell never changed, and the average angle change was 38°. The wild-type cell remained elongated throughout its migration, with an average roundness of 53%. By visualizing the cell path, it was clear not only that speed was continuous, but also that turning occurred in a relatively continuous fashion, at least for the interval times between measurements. When flow plots were wrapped around one another with time as the centrifugal vector, a rhythm of roughly 2.5 min was observed for boundary flow at any point on the cell periphery, and spiraling usually was observed to the right of the cell axis. The significance of the rhythm has not been fully considered at this time.

Virtually every aspect of Mo-1 motility and shape differed from wild-type, but particular differences were more revealing than others. To begin with, the representative Mo-1 amoeba showed no significant translocation, and the average rate of 3.5 μm per minute which was calculated from the centroid position simply reflected the changes in surface contour owing to bulging. Indeed, there was no real path of motion, and an attempt at generating a path in Figure 2B led to stacking of the cell perimeters with time. The shape of the Mo-1 amoeba also differed dramatically from that of wild-type. Whereas the wild-type cell was elongated and exhibited an average roundness measure of 55%, the Mo-1 cell was far rounder and exhibited an average roundness measure of 92%. However, the most dramatic difference in behavior was observed both in the polarity of cytoplasmic expansion and in the shape of the expansion zones. The wild-type cell formed normal pseudopodial extensions from the anterior quadrant of the cell body. In fact, all expansion was limited to the anterior half of the cell and all turning occurred in the direction of a new pseudopodial extension. Difference pictures demonstrated the relatively tapered morphology of many new pseudopods, and contour plots

of the perimeter exhibited steep peaks in the anterior region of the cell. In marked contrast, the Mo-1 cell exhibited cytoplasmic bulges at the periphery with no obvious polarity. Bulging on one side of the cell was followed by bulging on the opposite side of the cell in a relatively random fashion. In addition, the contour plots of the Mo-1 periphery exhibited very broad hills rather than distinct peaks, demonstrating the dramatic difference in the morphology of the expansion zones between wild-type pseudopods and Mo-1 "bulges." When the amount of cytoplasmic expansion or contraction was plotted over time for wild-type and Mo-1 (data not shown), it was also clear that wild-type was more active than Mo-1. Finally, no clear rhythm or spiraling was observed when flow plots of Mo-1 were wrapped.

A Consideration of Behavior and Cytoskeletal Organization

In wild-type amoebae, f-actin is densely localized in the anterior region of the cell active in pseudopod formation, and actin granules are apparent in the posterior region. The localization of f-actin affects the polarity of an actively translocating amoeba, and pseudopodia, which appear to be intimately involved not only in translocation, but also in chemotactic behavior [2,3], are the main repository. In marked contrast to wild-type amoebae, nontranslocating Mo-1 amoebae appear to have f-actin wrapped around the cortex of the cell, just under the plasma membrane, in a completely unpolarized fashion. This may explain why no polarity is apparent in cytoplasmic expansion zones, and why these cells cannot translocate.

ACKNOWLEDGMENTS

The authors are indebted to N. Schroeder for technical assistance, to K. Drollinger for assembling the manuscript, and to D. Morice and M. Lohman for developing the figures. The authors are indebted to Dr. W. Hand of Motion Analysis Corporation, Santa Rosa, California, for assistance in developing the Dynamic Morphology System. This work was supported by grants GM25832 and HD18577 from the National Institutes of Health awarded to D. R. Soll, and by funds from the Office of the Vice President for Research and Development, the University of Iowa. B.Varnum was supported by training grant HD07216 from the National Institutes of Health.

REFERENCES

1. Trinkaus JP: Exp Biol Med 10:130–173, 1985.
2. Varnum-Finney B, Voss E, Soll DR: Cell Motil Cytoskel 8:7–17, 1987a.
3. Varnum-Finney B, Voss E, Soll DR: Cell Motil Cytoskel 8:18–26, 1987b.
4. Sussman M: In D. Prescott (ed) "Methods in Cell Physiology." New York: Academic Press, 1966, pp 397–410.
5. Cocucci S, Sussman M: J Cell Biol 45:399–407, 1970.
6. Yarger J, Stults K, Soll DR: J Cell Sci 14:681–690, 1974.
7. Soll DR: Methods Cell Biol 28:413–431, 1987.
8. Soll DR, Herman M: J Gen Microbiol 129:2809–2824, 1983.
9. Varnum B, Edwards KB, Soll DR: J Cell Biol 101:1–5, 1985.
10. Maron MJ: "Numerical Analysis." New York: MacMillan Publishing Co., 1982.
11. Do Carmo MP: In "Differential Geometry of Curves and Surfaces." New Jersey: Prentice-Hall Inc., 1976, pp 16, 25.
12. Varnum B, Edwards KB, Soll DR: Dev Biol 113:218–227, 1986.