Quantitative Analysis of Cell Motility and Chemotaxis in *Dictyostelium discoideum* By Using an Image Processing System and a Novel Chemotaxis Chamber Providing Stationary Chemical Gradients

P. R. Fisher, R. Merkl, and G. Gerisch

Max Planck Institut für Biochemie, D-8033 Martinsried bei München, Federal Republic of Germany

Abstract. An image processing system was programmed to automatically track and digitize the movement of amebae under phase-contrast microscopy. The amebae moved in a novel chemotaxis chamber designed to provide stable linear attractant gradients in a thin agarose gel. The gradients were established by pumping attractant and buffer solutions through semipermeable hollow fibers embedded in the agarose gel. Gradients were established within 30 min and shown to be stable for at least a further 90 min. By using this system it is possible to collect detailed data on the movement of large numbers of individual amebae in defined attractant gradients. We used the system to study motility and chemotaxis by a score of *Dictyostelium coideum* wild-type and mutant strains, including "streamer" mutants which are generally regarded as being altered in chemotaxis. None of the mutants were altered in chemotaxis in the optimal cAMP gradient of 25 nM/mm, with a midpoint of 25 nM. The dependence of chemotaxis on cAMP concentration, gradient steepness, and temporal changes in the gradient were investigated. We also analyzed the relationship between turning behavior and the direction of travel during chemotaxis in stable gradients. The results suggest that during chemotaxis *D. discoideum* amebae spatially integrate information about local increases in cAMP concentration at various points on the cell surface.

DURING differentiation in response to starvation, amebae of the cellular slime mould *Dictyostelium discoideum* become chemotactically sensitive to cAMP which they now synthesize and secrete. Although the identity of the attractant as cAMP has been known for 20 years (Konijn et al., 1967), chemotaxis studies have been hampered by the difficulty of providing the amebae with a defined chemotactic stimulus, and by the tedious procedures necessary to collect quantitative data on the movement of large numbers of individual amebae.

For these reasons, semiquantitative assays have been favored of chemotaxis in which the chemical gradients are undefined and unstable, and the movement of individual amebae is not measured (e.g., the droplet assay; Konijn, 1970). While simple to perform, such assays provide only limited information for making inferences about the mechanism of chemotaxis or the nature of defects induced by mutations or pharmacological agents.

To provide stable, defined chemical gradients, several kinds of chemotaxis chambers have been designed and used to study chemotaxis by ameboid cells. The most successful and most widely used of these are the Boyden chamber (Boyden, 1962) and the Zigmond chamber (Zigmond, 1977), both of which have been extensively used to study leucocyte chemotaxis (Wilkinson, 1982). Only in the case of the Zigmond chamber is the actual movement of the cells recorded to provide detailed information on chemotaxis. However, the Zigmond chamber does not provide truly stable linear gradients because of diffusion of the attractant from one reservoir to the other. In this paper, we describe a novel chemotaxis chamber in which a stable attractant source and sink are provided by pumping solutions through hollow fibers embedded in a thin agarose gel.

Like the Zigmond chamber, our chamber allows quantitative measurement of the movement of the amebae during chemotaxis. Until recently it was necessary to collect such data by recording the movement on time-lapse video film and measuring it later from the video monitor, frame by frame, cell by cell (e.g., Alcantara and Monk, 1974; Futrelle, 1982; Fisher et al., 1985; Varnum et al., 1985). Here we describe the use of a programmeable digital image processor to automate this process, so that quantitative data on large numbers of cells can be collected during the course of the experiment for immediate statistical analysis at its conclusion. This system was used to measure motility and spontaneous turning by *D. discoideum* folate chemotaxis mutants (Segall et al., 1987). Varnum et al. (1986) and Varnum-Finney et al. (1987*a*,*b*) recently reported the use of Motion Analysis Corp. (Santa Rosa, CA) equipment to automate tracking of smaller numbers of *Dictyostelium* amebae.

P. R. Fisher's present address is Department of Microbiology, La Trobe University, Bundoora, Melbourne, Victoria 3083, Australia.

By using our system we have analyzed unstimulated motility by growth phase *D. discoideum* amebae, and chemotactic motility by aggregation competent amebae from 17 different *D. discoideum* strains, including a series of "streamer" mutants, which are putative chemotaxis mutants (Ross and Newell, 1981). We have characterized chemotaxis by *D. discoideum* amebae in this chamber with respect to the effects on chemotaxis of varying the midpoint gradient concentration, the gradient steepness, and temporal changes in the attractant concentration during and after gradient formation. Our results support the hypothesis that during chemotaxis, *D. discoideum* amebae spatially integrate information about temporal increases in attractant concentration at different points on the cell surface.

Materials and Methods

Strains

The *D. discoideum* strains used are listed in Table II in Results. Three wild isolates were used: NC4, WS526, and WS584 (Welker et al., 1985). All other strains used are ultimately derived from NC4, and can be grouped into the following categories. (*a*) Streamer mutants derived from XP55 representing each of the defined *stm* loci (Ross and Newell, 1981). (*b*) Mutants altered in behavior (phototaxis, thermotaxis) of the multicellular slug stage of the *D. discoideum* life cycle, derived from strain X22 (Fisher and Williams, 1982). (*c*) Agip-53, a mutant unable to relay cAMP signals, derived from the axenically growing NC4 derivative, AX2 (Darmon et al., 1975).

Media and Culture Conditions

With the exception of the axenically growing strains, amebae were grown in a shaken suspension containing 9×10^{10} *Klebsiella aerogenes* (Williams and Newell, 1976) per milliliter in pH 6.5 phosphate buffered salt solution (PBSS)[1] (PBSS: 10 mM KCl, 10 mM NaCl, 1.35 mM $CaCl_2$, 8 mM Na_2HPO_4, 16 mM KH_2PO_4, pH~6.5). PBSS was prepared by aseptically mixing sterile Bonner's salt solution (SS) (Bonner, 1947) and PBS (Deering et al., 1970) in a 1:1 ratio.

The bacterial suspension was prepared by harvesting, washing, and resuspending the bacteria in PBSS after growth as a lawn on standard medium nutrient agar plates (Sussman, 1966). 1-ml aliquots of the suspension were stored frozen (−30°C) in presterilized 2.25-ml Eppendorf tubes (Brinkmann Instruments, Inc., Westbury, NY) and thawed immediately before use. Viable and total cell counts showed bacterial viability after storage to be always >50%, while total cell counts remained unchanged.

For culture of growth phase *D. discoideum* amebae, we normally inoculated 10^6 amebae into a 1.0-ml bacterial suspension in a well of a Costar 24-well tissue culture plate (Cambridge, MA). During growth at 21°C shaken at 175 rpm, the amebae grew with a doubling time of 5–6 h and reached counts of $\sim 6 \times 10^7$ cells per milliliter by the end of the growth phase. We routinely harvested and washed the amebae in PBSS when the cell counts had reached $\sim 1 \times 10^7$ amebae per milliliter. The amebae were harvested and washed at room temperature by brief centrifugation in an Eppendorf centrifuge (total spin time ~20 s; Brinkmann Instruments, Inc.) before being resuspended in 1 ml of PBSS. In studies of the motility of growth phase cells, amebae from washed suspensions were used immediately, while for chemotaxis experiments they were incubated further in a plate well (Costar) at 21°C and 175 rpm for 6 h unless otherwise specified.

AX2 and Agip-53 which grow axenically were cultured in axenic medium with 1.8% maltose at 23°C, harvested in the growth phase at $\sim 10^6$ cells/ml, and allowed to develop in Sørensen's phosphate buffer at 23°C as previously described (Gerisch et al., 1985). In the case of strain Agip-53, the suspension was pulsed with 20 nM cAMP at 5-min intervals to stimulate development (Gerisch et al., 1975; Darmon et al., 1975). Both bacterially and axenically grown amebae were resuspended for development at a density of 10^7 cells/ml.

1. *Abbreviation used in this paper*: PBSS, phosphate-buffered salt solution (10 mM KCl, 10 mM NaCl, 1.35 $CaCl_2$, 8 mM Na_2HPO_2, 16 mM KH_2PO_4, pH ~6.5).

Image Processing and Computing Hardware

The system consisted of a TV-11-35 High Resolution Camera with an XQ 1397 Resistron Tube connected to a VTE Digitalvideo (Herrsching, Federal Republic of Germany) Modular Image Processor (MBVAR) interfaced with a DEC PDP11/23 microcomputer. The image contained 512 rows of 512 image points (pixels) with grey levels ranging from 0 to 255 (8 bits). The microscope was a Zeiss Axiomat in inverted format with a long range phase-contrast condenser. To obtain stable illumination for image processing, we used a separate power supply providing stable voltage/current to the microscope lamp.

Image Processing Software

Individual low level image processing operations were carried out by means of Fortran-callable subroutines from VTE. The image processing software that we developed in this work used these subroutines and was menu driven. Under phase-contrast microscopy, after slightly defocussing the microscope, the amebae were easily distinguished from the background on the basis of brightness. Images were accepted at a time-lapse interval specified by the user (usually 1.5 min) and the image was processed between consecutive pictures to smooth edges and reduce noise in the grey levels, detect the cells, find their edges and clean them.

From the cell outlines, parameters of cell movement and form were calculated. The position, movement and shape parameters were stored and used to identify the cells from picture to picture by means of a dissimilarity measure derived from the Canberra metric (Clifford and Stephenson, 1975). The results were analyzed statistically at the end of the experiment. The cell outlines and paths were stored in the graphics memory and superimposed on the unprocessed image to enable the experimenter to directly observe the tracking process during the experiment. The processing of each image to find and clean the edges of the amebae and store them in the graphics memory took ~45 s. Searching for, measuring the properties of, and identifying each cell took ~0.3 s. With a time-lapse interval of 1.5 min we were thus able to track up to 150 amebae at the same time. We routinely used cell densities of 30–100 amebae per image, and tracked them for 1–3 h in 30-min segments. Information on the cell tracking and identification methods can be obtained from the first author.

By measuring the apparent movement of 4.5-μm Latex beads between consecutive images we showed that the x,y coordinates of the centers of amebae were accurate to within ± 0.5 μm. The digitizing errors contributed <0.1 rad^2 to the variance of directions for displacements of at least 1 pixel (2.85 μm). To ensure that the digitizing errors were well within these values, we routinely excluded data involving displacements <2.9 μm from the directional statistics analysis.

Statistical Analysis

Directions were analysed using directional statistics as previously described (Fisher et al., 1981; Fisher et al., 1983). Directional statistics were based on the von Mises distribution, a bell shaped "normal" distribution for directional data. The distribution has two parameters: a mean direction, μ, and a concentration parameter, κ, that measures how strongly the individual directions are clustered around the mean. In all of the chemotaxis experiments reported here the mean direction was not significantly different from 0°, the direction towards the cAMP source. κ was calculated from the average cosine of the observed directions ($\bar{C}$). As $\bar{C}$ ranges from 0 to 1, κ ranges from 0 (no orientation) to infinity (perfect orientation). $\bar{C}$ is equivalent to the chemotactic index (C.I.) used by Varnum-Finney et al. (1987*b*), Futrelle et al. (1982), and others (Wilkinson, 1982). Accuracies of chemotaxis (κ) in the range observed here (up to ~1.0) are roughly equal to twice C.I.

To estimate spontaneous turning rates, κ was converted to the variance (σ^2) of the wrapped normal distribution, the familiar Gaussian distribution wrapped around a circle. The spontaneous turning rates were measured as the variance of the changes in direction from one time-lapse interval to the next, divided by the length of the time-lapse interval (Fisher et al., 1983; Segall et al., 1987). The spontaneous turning rate is numerically equal to twice the reciprocal of the persistence time measured by Potel and MacKay (1979).

Nondirectional data analysis and regression analysis were performed using the usual parametric and nonparametric statistical methods (Neter and Wasserman, 1974; Siegel, 1956).

Chemotaxis and Motility Experiments

All chemotaxis and motility experiments were conducted using the che-

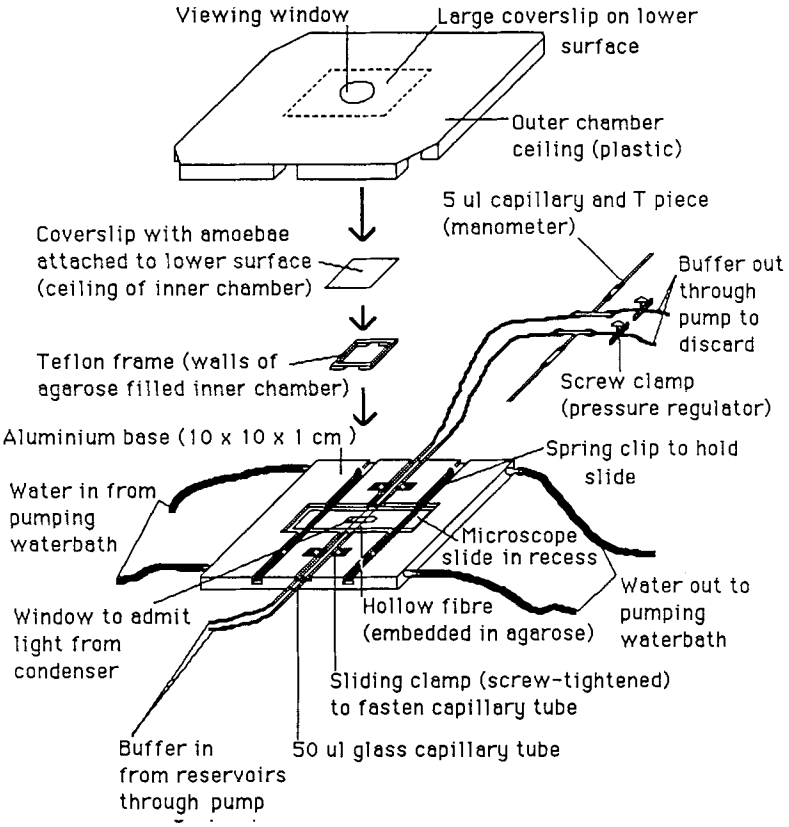

Figure 1. Chemotaxis chamber for tracking *Dictyostelium* amebae in stable, linear chemical gradients. The gradient was formed by pumping attractant and buffer solutions through Enka PF296 hollow fibers embedded in the agarose gel. The 50-μl glass capillaries were clamped into grooves in the chamber base by sliding clamps tightened with screws. The fibers were 2.5 mm apart center to center, so that 2.0 mm separated source and sink (i.e., from the internal wall of one fiber to the other).

motaxis chamber illustrated in Fig. 1. The base of the chamber is an aluminum block measuring 10 × 10 × 1 cm, with holes drilled through two sides to allow the passage of controlled temperature water from pumping water baths. This provides for constant uniform temperature in the chamber, or for the creation of temperature gradients for thermotaxis experiments. The bottom of the internal chamber is a clean microscope slide which is inserted into a recess in the aluminum base. A fresh slide is used for each experiment.

In chemotaxis experiments, attractant gradients were established by pumping attractant or buffer solutions at 20 ml/h through hollow fibers that were embedded in a thin gel of agarose. Hollow fibers were cut into short lengths of ~5 cm and their ends were glued into half-lengths of 50-μl glass micropipettes with 40–60°C melting point paraffin wax. To prevent pressure differences developing between the inside and the outside of the hollow fiber, the glass capillaries were connected with rubber tubing to the peristaltic pump on both entry and exit sides so that "pushing" and "pulling" were balanced. Fine tuning of the pressures was achieved with a clamp placed next to an open T-junction close to the chamber. Pressures at this T-junction were adjusted to atmospheric with the clamp so that neither air entry nor fluid extrusion through the open arm of the T-junction occurred.

Before preparing the chamber, 100 μl from a suspension of 5×10^4 amebae per milliliter was spread over the surface of a clean coverslip. The amebae were allowed to settle onto the glass during the preparation of the chamber.

For chemotaxis experiments hollow fibers were mounted in the chamber across the microscope slide, 2.5 mm apart at the centers, and a 1-mm high Teflon frame was placed over them to form the walls of the inner chamber. To embed the hollow fibers in the agarose gel, a hemocytometer coverslip was placed on top of the Teflon frame and the inner chamber formed in this way was then filled with fresh molten PBSS agarose (0.5%). After the gel had set, the hemocytometer coverslip was replaced with the coverslip carrying the amebae, from which excess liquid had been removed. The distance from the bottom surface of the coverslip to the surface of the agarose was estimated to be ~20 μm (based on differences in focussing distance). At this point the lid and walls of the outer chamber were placed onto the aluminum base and the whole unit was inverted and mounted onto the microscope stage for the experiment. For motility and spontaneous turning experiments with vegetative amebae, hollow fibers were not embedded in the agarose gel.

All experiments were carried out using the 10× objective, which provided an image field measuring 1.459 × 0.973 mm. For technical reasons the "active" area for cell tracking was slightly smaller: 1.419 × 0.945 mm. The chemotaxis chamber and tracking software can also be used at higher magnifications. During all chemotaxis experiments we tracked amebae both during the gradient formation period (30 min) and for at least 30 min (and for as long as 2.5 h) thereafter in the stable gradient. Unless otherwise stated all results presented here are for chemotaxis in the stable gradient, during the second 30-min period in the chamber.

Measurement of Diffusion of Small Molecules through Hollow Fiber Walls

The steepness of the attractant gradient at steady state in the agarose gel in the chemotaxis chamber depends upon how freely the attractant can diffuse through the hollow fiber walls. To measure the diffusion constants for various dyes and attractants in the walls of different hollow fiber types, we prepared hollow fibers as above but mounted them in a 250-ml beaker with 200-ml distilled water which was stirred constantly. Dye or attractant solution was pumped through the hollow fiber as usual. Samples were taken at regular time intervals and the concentration of dye or attractant was measured photometrically at the appropriate wavelength. The rate of concentration increase, and the dimensions of the hollow fiber enabled us to calculate the diffusion constants. Steady-state gradients were calculated from the diffusion constants and the distance separating source and sink fibers.

Results

Motility and Turning Behavior by Growth Phase and Aggregation Competent Amebae

Based on the displacement of individual amebae over 1.5-min time intervals, we measured the speed of movement of both growth phase and aggregation-competent amebae during unstimulated motility and chemotaxis. In all experiments we observed an increase in the motility of the amebae during

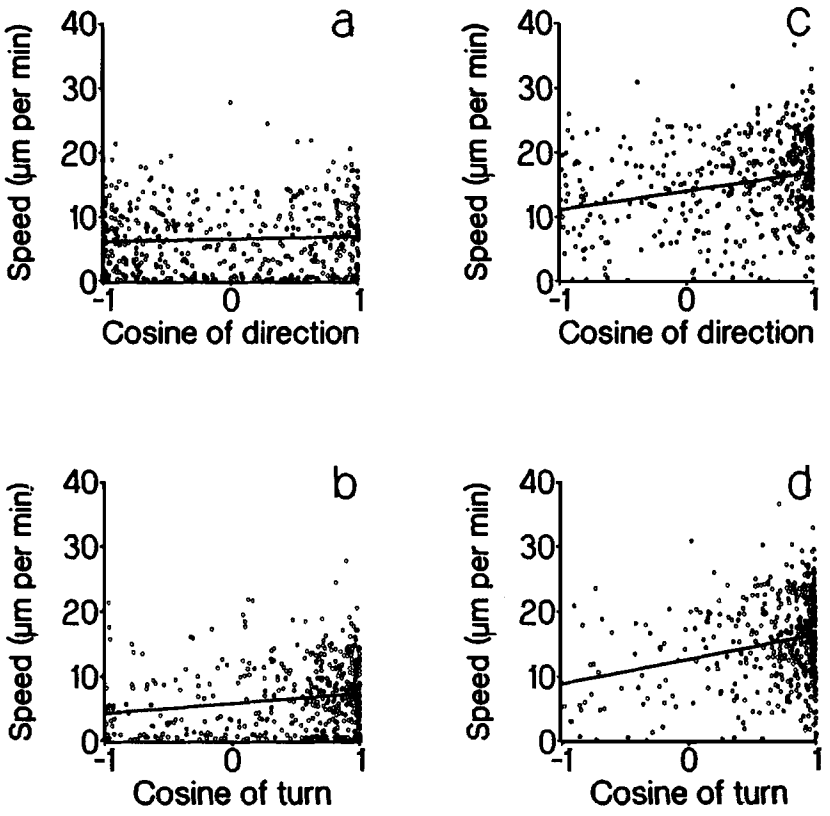

Figure 2. Correlation between the speed of migration of individual amebae and their direction of travel (*a* and *c*), or the magnitude of their most recent turn (*b* and *d*). Unstimulated motility by vegetative X22 amebae was measured (*a* and *b*), or chemotactic motility by aggregation competent X22 amebae in a 25 nM/mm gradient of cAMP with midpoint concentration 25 nM (*c* and *d*). Cosines are plotted on the *x*-axis to remove the distinction between directions or turns to the left and the right. Turns ranged from 0° (no turn, cosine = 1), through ±90° (cosine = 0), to ±180° (cosine = −1). The direction towards the right of the image was 0° and corresponded to the direction towards the cAMP source in the chemotaxis case. The slope of the regression line was significantly different from 0 ($P < 0.01$) in each of *b–d*. Regression lines were fitted by the least squares method.

their first 30-min in the chamber. During subsequent 30-min periods only slight increases in speed were observed. For routine measurements of motility we therefore used data collected during a 30-min period starting after the amebae had been in the chamber for 30-min.

Depending upon the straightness of the path taken by an ameba, its final displacement during any given time interval underestimates the actual distance traveled. Furthermore, turning amebae genuinely seem to move more slowly (Varnum-Finney et al., 1987*b*). An unbiased measure of motility needs to be independent of how frequently the cells turn. To estimate the magnitude of the bias in our speed measurements, we carried out a regression analysis on the relationship between the speed and the cosine of the change in direction. Typical results are shown in Fig. 2 *b*. As expected there was a highly significant correlation between the magnitude (cosine) of direction changes and the measured speed ($P << 0.01$). No correlation with the actual direction of travel was observed (Fig. 2 *a*, $P > 0.1$).

Extrapolation of the regression line provides an estimate of the mean speed for amebae traveling along a perfectly straight path. This value (7.8 μm/min in the example in Fig. 2 *b*) provides the best measure of motility, but it is not as simple to calculate as the mean (7.0 μm/min in the example). The extent of the underestimation involved in using the mean was small compared with the variation between experiments. For routine measurements we therefore used the mean speed measured over 1.5-min time intervals. For growth phase X22 amebae the mean speed in different experiments ranged from ~6 to 12 μm/min with an average ~9 μm/min. Our mean speed measurements agree well with those of others (e.g., Varnum-Finney et al., 1987*a*,*b*; Varnum et al., 1985; Varnum and Soll, 1984; Futrelle et al., 1982; Potel and Mackay, 1979).

We also measured the speeds of individual amoebae during chemotaxis in stable cAMP gradients. Fig. 2, *c* and *d* shows that the amebae moved faster up-gradient than down-gradient and, as expected, measured speeds were smaller for turning cells. Both effects were tested in a multiple regression analysis and shown to be highly significant ($P << 0.01$). In the example shown, the estimated mean speeds were: (*a*) amebae moving straight ahead up-gradient, 17.1 μm/min; (*b*) amebae moving straight ahead down-gradient, 13.9 μm/min; (*c*) amebae moving up-gradient having turned 180°, 11.3 μm/min; (*d*) amebae moving down-gradient having turned 180°, 8.1 μm/min. The overall mean speed was 15.1 μm/min. These results indicate that motility is stimulated by the temporal increases in attractant concentration experienced by amoebae during up-gradient movement. This is consistent with the observations of Varnum et al. (1985) who demonstrated that temporal increases in cAMP concentration stimulated motility by aggregation-competent amebae in the absence of spatial gradients. Varnum-Finney et al. (1987*b*) observed that amoebae moving up a spatial gradient with temporally increasing concentrations migrate faster than amebae moving down-gradient.

Spontaneity of Random Turns

The following observations show that random turning observed under our experimental conditions is spontaneous and

not elicited by randomly directed external stimuli such as secretory products from other amebae, or transient thermal gradients.

(*a*) In 35 experiments, the measured rates of spontaneous turning for growth phase X22 amebae showed no significant correlation ($r = 0.21$) with cell density in the range from 16 to 160 cells per image (1,200 to 12,000 cells/cm^2). The mean spontaneous turning rate in these experiments was 0.9 rad^2/min. The values for individual experiments were usually between 0.6 and 1.2 rad^2/min.

(*b*) Spontaneous turning rates by growth phase amebae were unaltered in several phototaxis/thermotaxis mutants of X22 and XP55 and in Agip-53, a mutant of AX2 that is unable to secrete cAMP in response to exogeneous cAMP (see Table II).

(*c*) Turning rates during chemotaxis by aggregation-competent amebae of these mutants, in particular Agip-53, were also normal compared with control strains (Table II).

(*d*) Even during chemotaxis in externally generated cAMP gradients, most turning is spontaneous and not elicited by the external gradient (discussed in greater detail below).

(*e*) Aggregation-competent amebae allowed to remain in the chemotaxis chamber overnight in the absence of an external cAMP gradient failed to aggregate, although they remained motile and viable.

(*f*) Chemotaxis by aggregation-competent X22 amebae was unaltered in the presence of 5 mM caffeine (accuracy of chemotaxis [κ] = 0.63 $\pm$ 0.1), which inhibits cAMP relay (Brenner and Thomas, 1984), and in the mutant Agip-53 (Table II).

At the low cell densities used, individual amebae, even if aggregation competent, were clearly too widely separated for intercellular chemical signaling to significantly affect their behavior.

Formation of Stable Linear Gradients in the Chemotaxis Chamber

In our chemotaxis chamber the attractant must diffuse through the walls of the hollow fibers in order to enter or be removed from the agarose gel. Too low a diffusion constant in the fiber wall would result in too flat a gradient in the agarose at steady state, and would also extend the period of time needed to reach steady state. We therefore measured the diffusion constant for the attractant cAMP in the walls of different hollow fibres as outlined in Materials and Methods. We also measured the diffusion constant for various dyes, in particular, for bromophenol blue which we used to demonstrate the formation of stable linear gradients in the chamber. The results, which are shown in Table I, revealed that the best choice of hollow fiber from among those tested was fibre type PF296 from Enka AG (Wuppertal, Federal Republic of Germany). For PF296 fibers the estimated diffusion constants approached those expected for these molecules in distilled water, indicating that the PF296 fiber walls do not present an appreciable barrier to diffusion of small molecules.

Fig. 3 illustrates the formation of a stable linear bromophenol blue gradient in the agarose in the chemotaxis chamber. Fig. 3 *a* shows that the concentration at the midpoint of the gradient rapidly increased and reached steady state within 30 min. Fig. 3 *b* shows that the gradient as measured optically with the image processor was approximately linear after 30 min and was stable over a further 90-min period. The relatively small deviations from linearity and stability, particularly at the edges of the image, arise from the far from ideal optical conditions inherent in the use of the chemotaxis chamber, bright field microscopy, and the image processor. The gradients in Fig. 3 *b* ranged from a grey level of ~9 through a midpoint grey level of ~36 to a value of ~63. These values suggest that the gradient in the image, ranging over ~54 grey levels, represented ~75% of the total gradient (72 grey levels). This corresponds to a gradient in the agarose from 93 to 7% of source concentration, which agrees very well with the expected values based on the diffusion constant for the bromophenol blue in the PF296 fiber walls (see Table I).

Development Regulation of Chemotactic Sensitivity

Fig. 4 shows the acquisition of cAMP chemotaxis by amebae of strain X22 during development in suspension in PBSS in a 24-well plate Costar (see Materials and Methods). Chemotaxis was maximal after 6 h of development. This is consistent with earlier observations using semiquantitative chemotaxis assays for amebae differentiating on a solid surface

Table I. Gradient Formation Using Different Kinds of Hollow Fibers

Tube type	Internal diameter	External diameter	Diffusion constant			Gradient in agarose		
			Bromophenol blue	Lucifer Yellow	cAMP	Bromophenol blue	Lucifer Yellow	cAMP
	μm	μm	cm^2/s	cm^2/s	cm^2/s	%	%	%
Enka PF296	500	650	2.0×10^{-6}	3.9×10^{-6}	4.8×10^{-6}	92–8	95–5	96–4
Enka plasmaphan	300	640	$<6.8 \times 10^{-10}$	ND	ND	<50.04–>49.96	ND	ND
Enka cuprophan	175	315	5.2×10^{-8}	ND	3.6×10^{-7}	65–35	ND	87–13
Amicon HIP10-8	200*	386*	6.4×10^{-11}	2.4×10^{-10}	6.4×10^{-10}	53–47	60–40	70–30

Diffusion constants refer to diffusion through the hollow fiber wall. Dimensions of the Enka fibers were measured from fiber sections under the light microscope using the image processor. Amicon fiber dimensions were measured on a scanning electron micrograph. Calculations of diffusion constants were based on these dimensions and the measured flux of dye or cAMP molecules through the fiber walls. The gradients in the agarose at steady state were calculated using a distance of 2.5 mm from the center of one hollow fiber to the other, the dimensions of the fibers, the diffusion constants for diffusion through the fiber wall, and a presumed diffusion constant of 5×10^{-6} cm^2/s for diffusion in the agarose gel. Gradients are expressed as a percentage of the concentration difference between source and sink fibers.

* In the case of the Amicon hollow fibers the real diffusion barrier was an inner membrane layer ~0.3 μm thick. This formed the basis for calculations for this fiber type.

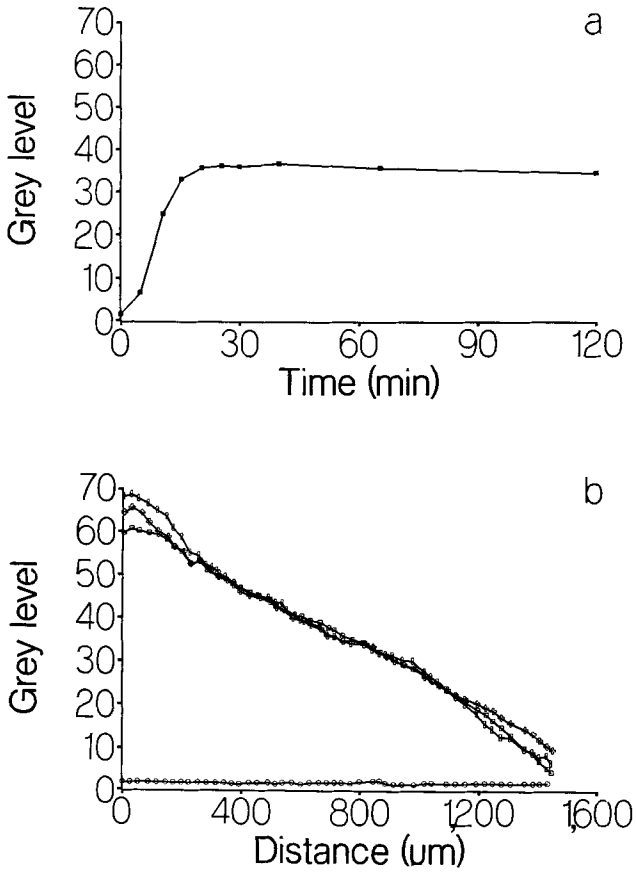

Figure 3. Formation and stability of a bromophenol blue gradient in the chemotaxis chamber. Grey levels represent the difference from a reference image taken immediately before gradient formation began at 0 min. The mean grey level at the center of the gradient (center column of image pixels) is plotted as a function of time in *a*. In *b* the entire grey level gradient at 0 (○), 30 (✣), 60 (□), and 120 (▯) minutes is plotted using the mean grey level of every 10th column of 512 pixels in the image. The grey levels plotted are an approximate measure of the concentration of the dye (unpublished data). Nonzero grey levels at 0 min reflect electrical noise in the grey levels of individual pixels.

(Konijn, 1970; Varnum and Soll, 1981; Futrelle et al., 1982). During further incubation, both chemotaxis and adenylate cyclase activity (used here as a developmental marker) declined. Roos et al. (1977) reported identical changes in adenylate cyclase activity during 8 h of development in suspension by axenically grown AX2 amebae.

Concentration Dependence of Chemotaxis

Fig. 5 shows how the accuracy of cAMP chemotaxis by t_6 (6 h of development) X22 amebae depended on the concentration of cAMP at the midpoint of the gradient. In all cases the cAMP concentration in the sink was zero, so that the relative steepness of the gradient was constant (50% per millimeter), but the absolute steepness changed with the source concentration. The inset shows the paths taken by t_6 X22 amebae during chemotaxis in the optimum gradient of 25 nM/mm, with midpoint 25 nM. The accuracy of chemotaxis varied only twofold in the 100-fold concentration range from

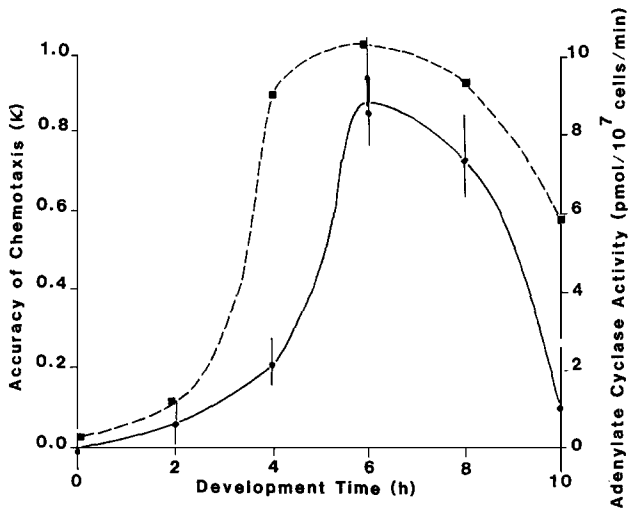

Figure 4. Developmental regulation of chemotactic responsiveness to cAMP gradients. Chemotaxis (●) in stable 25 nM/mm gradients with midpoint 25 nM was measured for X22 amebae developing at 21°C in suspension in 1 ml of PBSS in a plate well (Costar). Bars represent 90% confidence intervals. In a control experiment the activity of adenylate cyclase (■) was measured (Gerisch et al., 1985) in X22 amebae developing under the same conditions.

2.5 nM to 250 nM and was detectable down to a midpoint concentration of 25 pM. Chemotaxis became insignificant at midpoint concentrations >2.5 μM or as low as 2.5 pM. The results are consistent with the view that chemotaxis is mediated by either or both of the high affinity A and B forms of the receptor (K_d = 60 and 12 nM; van Haastert and de Wit, 1984; van Haastert et al., 1986). Zigmond (1977) observed

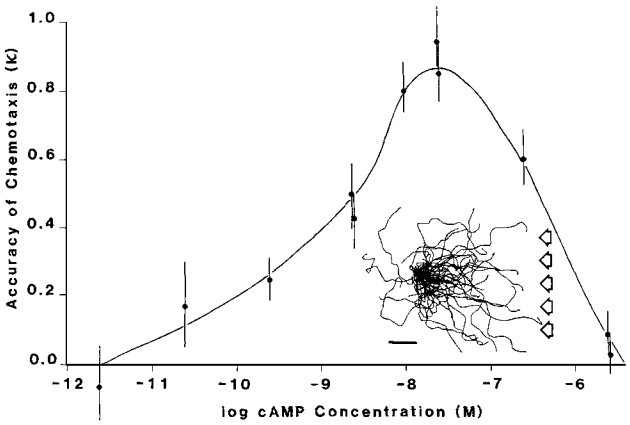

Figure 5. Dependence of chemotaxis on cAMP concentration. The accuracy of chemotaxis by aggregation competent (t_6) X22 amebae in cAMP gradients was measured. Source concentration ranged from 5 pM to 5 μM, while sink concentration was 0 in all cases. The concentrations at the midpoints of the gradients are plotted. Absolute gradient steepness (nanomoles per millimeter) varied with source concentration, while relative gradient steepness was constant. Bars represent 90% confidence limits. The inset shows tracks of amebae during chemotaxis in the optimum gradient of 25 nM/mm with midpoint 25 nM. Arrows indicate the cAMP source. Each trail is plotted from the same start point. Bar, 50 μm.

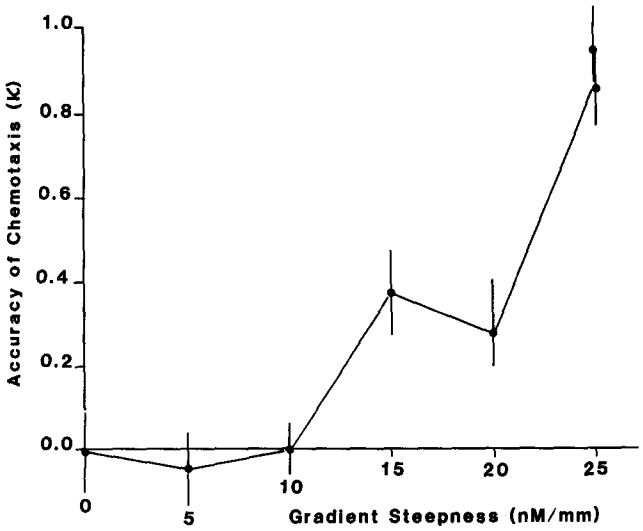

Figure 6. Dependence of chemotaxis on gradient steepness. The accuracy of chemotaxis by aggregation competent X22 amebae was measured in cAMP gradients of varying steepness with a constant midpoint concentration of 25 nM. Bars represent 90% confidence limits.

a similarly shaped curve for the concentration dependence of chemotaxis towards f-met-leu-phe by polymorphonuclear granulocytes.

The optimum gradient for chemotaxis in our chamber by aggregation competent *D. discoideum* amebae is comparable to the best gradient for chemotaxis by such amebae in the Zigmond chamber (Varnum and Soll, 1984). The accuracy of chemotaxis by amoebae in the two cases is similar (Varnum and Soll, 1984). Vicker et al. (1984) found optimum chemotaxis at slightly higher concentrations during gradient formation in their chambers, but did not detect chemotaxis in stable gradients (see Discussion).

Dependence of Chemotaxis on Relative Gradient Steepness

In the experiments described above, the relative steepness of the gradient was kept constant over a range of cAMP concentrations. For an ameba 10 μm in diameter, the gradient across the cell always ranged from ~0.6% at the high concentration end of the image to ~3% at the low concentration end (gradient expressed as a percentage of the concentration at the midpoint of the cell). To examine the dependence of chemotaxis on relative gradient steepness we prepared gradients with cAMP in the sink buffer as well. The gradient midpoint was kept constant at 25 nM while the steepness was varied from 0 to 25 nM/mm. The results in Fig. 6 show that chemotaxis was reduced in shallower gradients and became insignificant for gradients of 10 nM/mm or less. In the 10 nM/mm gradient, the gradient across a 10-μm ameba ranged from ~0.3 to ~0.6% of the concentration at the midpoint of the cell. The results support the earlier suggestion that *D. discoideum* amebae are able to detect concentration differences as small as 1% across their surface (Mato et al., 1975). Similarly, polymorphonuclear leukocytes can detect 1% concentration differences in attractant concentration during chemotaxis in the Zigmond chamber (Zigmond, 1977).

Chemotaxis in Gradients with Temporally Changing cAMP Concentrations

The results in Figs. 5 and 6 show that in a 10 nM/mm gradient chemotaxis was strong if the gradient midpoint was 10 nM but not detectable if the midpoint was 25 nM. This and the other results of Figs. 5 and 6 are consistent with *D. discoideum* amebae "measuring" relative not absolute differences in attractant binding during chemotaxis. Other organisms achieve this by comparing current concentrations with a time average of concentrations experienced during the recent past.

To investigate whether such temporal sensing of cAMP concentrations plays a role in *D. discoideum* chemotaxis, we examined the behavior of the amebae during and after gra-

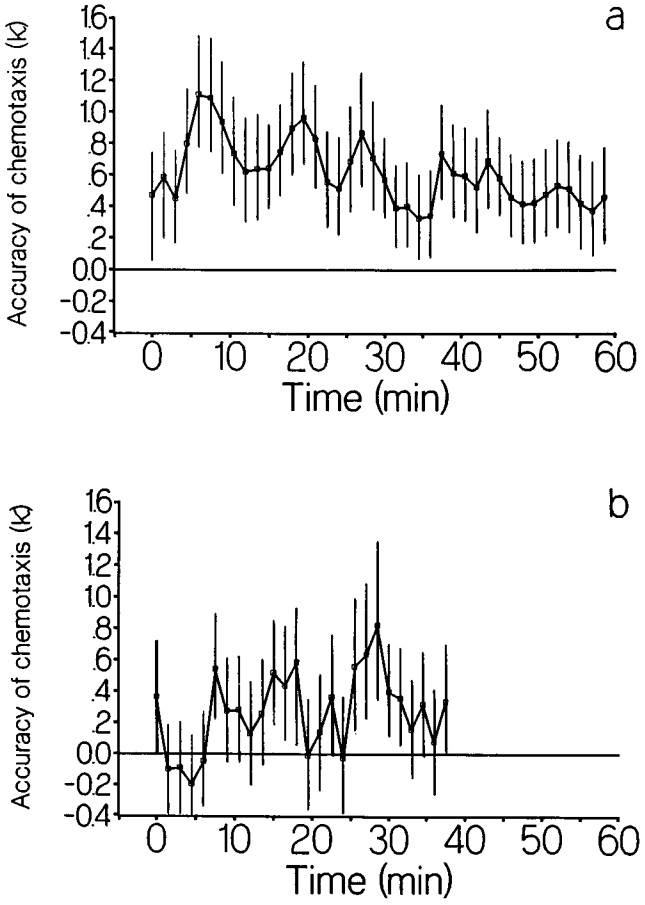

Figure 7. Chemotaxis in cAMP gradients with temporally changing concentrations. Chemotaxis by aggregation competent X22 amebae was measured at 1.5-min intervals in gradients with temporally increasing (*a*) or decreasing (*b*) cAMP concentrations. In each case the steady-state gradient after ~30 min was 25 nM/mm with midpoint 25 nM. (*a*) The starting concentration in the agarose was 0 and the concentration increased everywhere during gradient formation by diffusion from the source fiber. (*b*) The cAMP concentration was initially 50 nM in the agarose and decreased to steady-state levels during gradient formation by diffusion into the sink fiber. Bars represent 90% confidence limits. Each point represents chemotaxis during the 1.5-min time interval starting at the time shown. (*a*) The amebae were also tracked for 15 min and (*b*) for 39 min before beginning gradient formation (not shown). The amebae showed no significant preference for any particular direction during this period (κ not significantly greater than 0).

dient establishment in the chemotaxis chamber. Fig. 7 *a* shows that with a starting concentration of zero and formation of a gradient by addition of cAMP at the source, chemotaxis was already detectable within the first 1.5-min time interval (plotted at 0 min in Fig. 7) and increased rapidly to a maximum within ~10 min. Thereafter the accuracy of chemotaxis declined to approach a stable value after ~30 min.

However, if the gradient was formed by starting from a uniform concentration of 50 nM and then removing cAMP at the sink, the amebae were unable to respond chemotactically for several minutes (Fig. 7 *b*). Chemotaxis then approached a stable value that was somewhat lower than in Fig. 7 *a*. We conclude that chemotaxis is strongest in gradients where the concentration is increasing over time, and weakest in gradients where the concentrations are decreasing everywhere.

During gradient formation with temporally increasing concentrations, we observed apparently periodic changes in the accuracy of chemotaxis (Fig. 7 *a*). For reasons outlined previously, we do not believe these oscillations are coordinated by intercellular interactions, e.g., oscillatory secretion of cAMP by the amebae. Indeed we saw the same phenomenon in experiments with Agip-53, which is unable to relay cAMP signals, and with X22 in the presence of 5 mM caffeine, which inhibits relay but not chemotaxis (unpublished data).

Relationship between the Turning Behavior and Direction of Migration during Chemotaxis in a Stable Gradient

The results described in previous sections support the hypothesis that *D. discoideum* amebae are able to sense temporal changes in cAMP concentration during chemotaxis. One possible mechanism of orientation in stable gradients would involve the suppression of random turns by temporal increases in cAMP concentration as amebae move up gradient. We therefore examined the relationship between turns and the direction of movement during chemotaxis in a stable gradient.

The results in Fig. 8 *a* show that during chemotaxis there

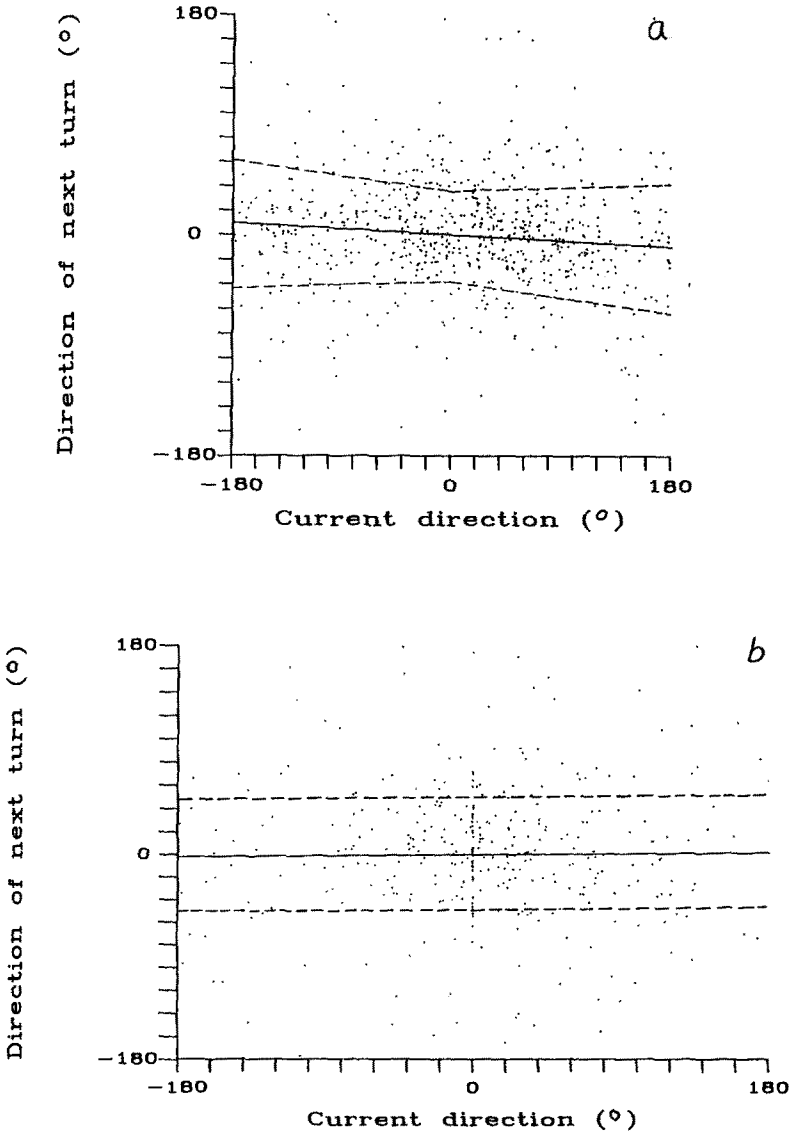

Figure 8. Correlation between the direction of the next turn and the direction of travel by X22 amebae during chemotactic (*a*) and unstimulated (*b*) motility. The chemotaxis gradient was 25 nM/mm cAMP with midpoint 25 nM. Aggregation competent (t_6) amebae were used in each case. Regression lines were fitted by least squares (*solid lines*). Dashed lines represent the mean residual magnitude, residuals being the differences between actual observed turns and the expected turns based on the regression line. Both the observed turns and the residual magnitudes were significantly dependent on the direction of travel during chemotaxis ($P < 0.01$, F tests) but not during unstimulated motility. A current direction of 0° represents the direction towards the cAMP source in chemotaxis (*a*) and the starting direction in unstimulated motility (*b*). A turn of 0° means no change in direction from the current time lapse interval to the next. The accuracy of chemotaxis (κ) in *a* was 0.55, while in *b* there was no significant preference for any particular direction. In *b* the amebae deviated from their random starting direction with a spontaneous turning rate of 0.5 rad²/min.

was a highly significant ($P << 0.01$) negative correlation between the direction of travel and the direction of the next turn. This correlation was not present ($P > 0.1$) during persistent motility in the starting direction in the absence of an attractant gradient (Fig. 8 *b*). The negative correlation in the chemotaxis case demonstrates that amebae currently traveling to the right of the correct direction tend subsequently to turn left, and vice versa.

To completely account for the observed accuracy of chemotaxis (κ) it can be shown that the slope of the regression line in Fig. 8 *a* would need to be ~ -0.1. In fact the slope of the regression line in Fig. 8 *a* was only ~ -0.05, a 1° correction for a 20° deviation from the correct direction. This accounts for only ~1% of the total sum of squares, and the difference between the observed slope and the expected value of -0.1 was highly significant ($P << 0.01$, F test).

We therefore tested whether the magnitude of the residuals (remaining random component of the turns) was significantly correlated with the deviation of the current direction of travel from the correct direction. In every case, including that illustrated in Fig. 8 *a*, random turns were smaller if the amebae were traveling in directions closer to the correct direction. Quantitatively, this effect represented a 1° decrease in the magnitude of random turns for a 20° decrease in the "aiming error" (the deviation from the correct direction). It accounted for a further 1% of the total sum of squares of turns and is illustrated in Fig. 8 *b* with dashed lines relating the mean residual magnitude to the direction of travel. Analysis of amebal behavior during gradient formation where cAMP concentrations are increasing everywhere with time yielded analogous results (not shown). We conclude that two mechanisms contribute about equally to chemotaxis in a stable gradient. (*a*) Error correction: amebae that have deviated to the left of the correct direction tend to correct the error and subsequently turn right. (*b*) Suppression of random turns when the amebae are traveling up gradient and experiencing temporally increasing cAMP concentrations.

Together these two mechanisms are sufficient to account quantitatively for the observed chemotaxis ($\kappa = 0.55$). It is worth noting that 98% of the total turning activity (sum of squares of turns) is nonchemotactic. This means that the straightness of the paths taken by the amebae is primarily a function of spontaneous turning rather than chemotactic turning.

Motility and Spontaneous Turning Rates of Growth Phase Amebae, and Chemotaxis by Aggregation Competent Amebae of D. discoideum Mutants

Previously we used the tracking program to measure unstimulated motility and spontaneous turning by folate chemotaxis mutants (Segall et al., 1987). Here we measured the motility and spontaneous turning rates of growth phase amebae of a number of other *D. discoideum* mutants that were potentially altered in amebal behavior. For each mutant, we also measured motility, chemotaxis, and turning rates of aggregation competent amebae in a 25 nM/mm gradient, with midpoint of 25 nM. In each case the control strain was the immediate parent of the mutant tested.

The results, listed in Table II, showed that growth phase amebae of NC4-derived strains (X22 and mutants, XP55 and mutants), with the possible exception of NP387, moved at average speeds of 5–10 μm/min. Growth phase amebae of strain AX2 and Agip-53 moved at ~3–5 μm/min. Spontaneous turning rates were generally in the range 0.6–1.4 rad²/min, and none of the mutants examined gave values significantly outside this range.

During chemotaxis, aggregation-competent amebae of most strains moved at speeds between 7 and 13 μm/min, a little faster than the growth phase amebae. Varnum et al. (1986) have reported that amebal motility in the absence of a cAMP gradient increases transiently at the onset of aggregation. AX2 amebae and those of its derivative, Agip-53 were again somewhat slower than other strains. The aggregation competent amebae of all strains showed normal chemotaxis towards cAMP with an accuracy (κ) in the range 0.4–0.8. Turning rates during chemotaxis were in the range of 0.5–1.1 rad²/min for all strains examined. We conclude that the genetic alterations in the mutants examined are not important for normal chemotaxis in optimal gradients.

Streamer mutants altered in the *stm*F locus on linkage group II (Coukell and Cameron, 1985) lack the cGMP phosphodiesterase activity responsible for terminating the increase in intracellular cGMP which occurs soon after cAMP stimulation of aggregation competent amebae (Ross and Newell, 1981; van Haastert et al., 1982; Coukell et al., 1984). Their phenotype has been used as evidence for a role of cGMP in the processing of chemotactic signals (Ross and Newell, 1981). Our results for strain NP368 in Table II would not support this conclusion, but we cannot exclude possible alterations in chemotaxis by such mutants under less than optimum gradient conditions.

Discussion

In the past, investigation of the chemotactic behavior of ameboid cells has been hampered by the inability to track and analyze the behavior of individual cells in stable defined attractant gradients. Gradients in the Zigmond chamber are approximately linear but are unstable (Zigmond, 1977). The recent study of *D. discoideum* chemotaxis by Varnum-Finney et al. (1987*b*) used a Zigmond chamber and tracked the amebae in this chamber from 9 to 21 min after gradient formation started. Like our chamber, the Zigmond chamber used by Varnum-Finney et al. (1987*b*) has a 2-mm distance between source and sink. The kinetics of gradient formation will be similar in both chambers at this early time before, in the Zigmond chamber, there is substantial accumulation of attractant in the sink reservoir and depletion of the source. Our results show that chemotaxis is maximal at this time when attractant concentrations are still increasing rapidly in the chamber. The results of Varnum-Finney et al. (1987*b*) therefore pertain to spatial gradients with temporally increasing concentrations at the time when chemotactic accuracy is maximal. Our results establish that even in stable gradients over long time periods, *D. discoideum* amebae migrate faster and randomly turn less frequently when moving up-gradient than down, and that they correct aiming errors by turning up gradient.

D. discoideum amebae synthesize and secrete cAMP in response to temporal increases in extracellular cAMP concentration and the kinetics of the adaptation processes involved have been characterized (Dinauer et al., 1980*a*,*b*). Temporal decreases in cAMP concentration do not elicit a response in

Table II. Motility and Chemotaxis by Wild-Type and Mutant D. discoideum Amebae

Strain	Growth phase (t_0) amebae Unstimulated motility		Differentiated (t_6) amebae Chemotaxis		
	Speed	Spontaneous turning	Speed	Accuracy	Turning
	(μm/min)	*(rad²/min)*	*(μm/min)*	*(κ)*	*(rad²/min)*
X22	8.3	0.89 (0.78, 0.99)	7.8	0.53 (0.42, 0.62)	0.83 (0.74, 0.92)
HU120	8.5	0.99 (0.82, 1.16)	11.8	0.48 (0.42, 0.55)	0.76 (0.69, 0.81)
HU411	7.6	0.99 (0.84, 1.14)	7.9	0.54 (0.47, 0.62)	0.86 (0.78, 0.92)
HU410	5.8	0.55 (0.45, 0.69)	10.7	0.55 (0.48, 0.64)	0.68 (0.62, 0.74)
HU409	7.1	0.72 (0.63, 0.80)	13.6	0.61 (0.52, 0.71)	0.70 (0.62, 0.77)
XP55	9.7	1.20 (1.10, 1.27)	8.8	0.67 (0.60, 0.76)	0.74 (0.69, 0.79)
NP368	10.2	0.71 (0.59, 0.82)	8.9	0.40 (0.32, 0.5)	0.90 (0.81, 0.97)
NP387	2.6	0.96 (0.84, 1.08)	6.1	0.43 (0.32, 0.56)	0.89 (0.76, 1.03)
NP383	4.6	1.19 (1.04, 1.34)	8.6	0.62 (0.54, 0.71)	0.84 (0.76, 0.91)
NP370	6.5	1.43 (1.15, 1.80)	12.1	0.57 (0.49, 0.65)	1.00 (0.90, 1.10)
NP371	6.0	0.71 (0.63, 0.76)	10.0	0.64 (0.58, 0.72)	0.76 (0.70, 0.80)
NP294	8.9	1.34 (1.18, 1.50)	9.0	0.41 (0.35, 0.48)	1.00 (0.94, 1.12)
AX2	3.0	1.21 (1.08, 1.33)	7.6	0.81 (0.70, 0.93)	0.53 (0.47, 0.60)
Agip-53	3.2	1.18 (1.08, 1.29)	5.1	0.66 (0.60, 0.74)	0.65 (0.59, 0.70)
NC4	6.6	0.96 (0.86, 1.04)	ND	ND	ND
WS526	6.8	0.83 (0.72, 0.93)	ND	ND	ND
WS584	3.1	0.90 (0.78, 1.01)	ND	ND	ND

The gradient for chemotaxis was 25 nM/mm with a midpoint of 25 nM. All but a small proportion of the turning during chemotaxis can be regarded as spontaneous (see text). Numbers in parentheses are lower and upper 90% confidence limits. Strains with designations beginning with HU are slug phototaxis mutants derived from X22 (Fisher and Williams, 1982). Strains with designations beginning with NP are streamer mutants derived from XP55 representing each of the defined *stm* loci. The *stm*F locus that is mutant in NP368 has been associated with cGMP phosphodiesterase activity. Agip-53 is a mutant of AX2 unable to synthesize and secrete cAMP and therefore normally unable to aggregate. With the exception of the two independent wild-isolates WS526 and WS584, all strains are ultimately derived from NC4.

the sense that they do not suppress cAMP synthesis and secretion to below basal levels (Devreotes and Steck, 1979).

A number of investigators have attempted, in the past, to determine whether chemotaxis by *D. discoideum* amebae is similarly based on sensing of temporal increases in attractant concentration (Futrelle, 1982; van Haastert, 1983; Vicker et al., 1984; Varnum et al., 1985, 1986; Varnum-Finney 1987*a*,*b*). However, the conclusions reached by these authors appear to be contradictory. Futrelle (1982) opted for spatial sensing of the gradient, van Haastert (1983) for a temporal mechanism, and Vicker et al. (1984) denied both for stable gradients while remaining noncommittal for gradients where concentrations are increasing temporally. Varnum-Finney et al. have investigated motility and turning behavior by *D. discoideum* amebae in both temporal and spatial gradients (Varnum et al., 1985, 1986; Varnum-Finney et al., 1987*a*,*b*). Their results led them to support a temporal-sensing model.

Spatial mechanisms imply that the amebae "read" the gradient by comparing attractant concentrations "measured" simultaneously at different points on the cell surface. Having detected the gradient the ameba is able to correct directional errors by turning towards the attractant source. We have demonstrated here that *D. discoideum* amebae do indeed correct directional errors during chemotaxis in stable linear gradients. Varnum-Finney et al. (1987*b*) reported similar results

for chemotaxis in spatial gradients with temporally increasing concentrations.

Temporal mechanisms imply that the cells read the gradient by comparing concentrations measured at different times during randomly directed movement in the gradient. Having detected a concentration increase during movement up-gradient, the amoeba reduces random turning that could lead it to stray from the correct direction. We demonstrated here that this does occur during *D. discoideum* chemotaxis in stable gradients. Varnum et al. (1985) reported that purely temporal gradients inhibit random turning by *D. discoideum* amebae and that extension of new pseudopodia was suppressed during up-gradient movement of amebae in temporally increasing spatial gradients (Varnum-Finney et al., 1987*b*).

Our results are not easily accommodated with those of Vicker et al. (1984) who used chemotaxis chambers with source and sink reservoirs to provide essentially stable linear gradients. Instead of measuring the movement of individual cells, they measured the asymmetric distribution of amebae resulting from chemotaxis. Assays with stable gradients revealed that the amebae were chemotactic initially, but their distribution in the gradient subsequently became uniform. These authors concluded that *D. discoideum* amebae are not chemotactic in stable gradients. They suggested that suppression of random turns by absolute attractant concentrations leads transient asymmetric distributions of amebae (Lapidus, 1980; Futrelle, 1982; Vicker et al., 1984).

While unable to detect chemotaxis in stable gradients Vicker et al. (1984) did detect chemotaxis if concentrations in the gradient are increasing temporally. Apart from differences in the magnitude of the responses, we observe amebae to behave in similar fashion whether migrating in stable gradients, or in gradients with temporally increasing concentrations. We tracked uniformly distributed amebae for periods up to 2.5 h in demonstrably stable gradients and observed no significant changes in chemotaxis (Fig. 7 *a* and unpublished data). Under these conditions Vicker et al. predict that chemotaxis will not occur. The results of Vicker et al. (1984) might be explained by their use of nonoptimal conditions. These could have influenced the behaviour and gradient sensing ability of the amebae sufficiently to obscure responses to stable gradients, yet allow detection of the stronger responses to gradients with temporally increasing concentrations.

Futrelle (1982) claimed to have demonstrated spatial sensing of gradients by showing that aggregation-competent amebae still moved towards a cAMP source even when the concentration was decreasing everywhere with time. Futrelle's experiment involved moving a cAMP-filled pipette with a micromanipulator to create a pulse of attractant as the pipette tip approached and moved past individual amebae. Van Haastert (1983) used the droplet method to assay chemotaxis by postvegetative amebae in response to cAMP gradients formed by a droplet of cAMP phosphodiesterase on agar containing 10 μM cAMP. He observed normal chemotaxis when the amebal droplet was placed on the agar after gradient formation, but found no chemotaxis if the amoebae were already present before gradient formation. He concluded that chemotactic responsiveness is modified by adaptation processes and that the amoebae respond only to temporal increases in concentration above the level to which they are adapted.

Our results support those of both Futrelle (1982) and Van Haastert (1983) and resolve the apparent discrepancy between them. We found, in support of van Haastert, that *D. discoideum* amebae do not orient during the first several minutes of gradient formation in our chamber if the gradient is formed by removal of cAMP at the sink. The rate at which the cAMP concentrations decrease is greatest during this period. Subsequently chemotaxis was observed, although it was somewhat weaker than in the gradient formed by addition of cAMP through the source fiber. During this period our results are similar to those of Futrelle (1982).

Waves of cAMP propagate outward from the aggregation center during aggregation. The inability of *D. discoideum* amebae to orient in gradients with rapidly decreasing cAMP concentrations helps to explain why they do not reverse and follow the receding cAMP wave as it passes them. During chemotaxis, changing concentrations at various points on the cell surface would be generated both by changes in the gradient itself, and by the movement of the ameba in the gradient, including localized extension of filopodia and pseudopodia. Our results suggest that the amebae are unable to respond chemotactically to decreasing concentrations, a circumstance that arises only if the gradient concentrations fall more rapidly than can be accommodated by the motility of the cell. This hypothesis explains the discrepancy between Futrelle's (1982) and van Haastert's (1983) observations.

We suggest that *D. discoideum* amebae spatially integrate information about temporal increases in attractant concentration at points distributed over the cell surface. More specifically, we envisage that competition among individual filopodia and pseudopodia would favor pseudopodium growth in those areas where local concentration increases were greatest. This mechanism, suggested by Gerisch et al. (1975*b*), is temporal at the level of individual filopodia, but spatial at the level of the whole cell (Fisher et al., 1984). Varnum-Finney et al. (1987*b*) reported that initial extension of pseudopodia was not biased in favor of the up-gradient direction, but that growth of up-gradient pseudopodia leading to a turn was favored. These authors were also led by their results to propose local temporal sensing on pseudopodia as well as a "decision-making system along the entire cell body."

The pseudopodium/cell body pattern of an amoeba can be explained by coupled pseudopodium autoactivation and inhibition processes (Meinhardt and Gierer, 1974). It is common for such spatial patterning mechanisms to be capable also of generating temporal patterns (oscillations). Bumann et al. (1984) observed oscillations in extracellular Ca^{2+} concentrations in the absence of measurable changes in cAMP concentration. Our observation of possible oscillations in the accuracy of chemotaxis during gradient formation is consistent with this and suggests that the initial cAMP stimulus may have synchronized an intracellular oscillatory system. Chemotaxis itself would be a result of signals from the cAMP receptor interacting with the pseudopodium activation/inhibition system to activate pseudopodium growth in the correct direction and inhibit it in inappropriate directions. Such a model could explain the morphological polarity of amebae, oscillations, persistence, turning behavior not only in chemotaxis but also in thermotaxis and multidirectional pho-

totaxis, and sign reversals during phototaxis and thermotaxis (Fisher et al., 1984, 1985; Hong et al., 1981, 1983; Häder and Poff, 1979*a*,*b*).

We are grateful to Dr. J. Hemberger, Enka Research Institute Obenburg, Obenburg, Federal Republic of Germany for the gift of hollow fibers, to Dr. J. Segall for helpful comments on the manuscript and for showing us how to adjust the diffusion constant calculations for the cylindrical shape of the hollow fibres, and to Dr. J. Hagmann for carrying out the adenylate cyclase assays.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

Received for publication 19 November 1987 and in revised form 31 October 1988.

References

Alcantara, F., and M. Monk. 1974. Signal propagation in the cellular slime mould *Dictyostelium discoideum*. *J. Gen. Microbiol.* 85:321-324.

Brenner, M., and S. D. Thoms. 1984. Caffeine blocks activation of cyclic AMP synthesis in *Dictyostelium discoideum*. *Dev. Biol.* 101:136-146.

Bonner, J. T. 1947. Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. *J. Exp. Zool.* 106:1-26.

Boyden, S. V. 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *J. Exp. Med.* 115:453-466.

Bumann J., D. Malchow, and B. Wurster. 1984. Oscillations of Ca^{2+} concentration during the cell differentiation of *Dictyostelium discoideum*. Their relation to oscillations in cyclic AMP and other components. *Differentiation.* 3:85-91.

Clifford, H. T., and W. Stephenson. 1975. An Introduction to Numerical Classification. Academic Press., Inc., New York.

Coukell, M. B., and A. M. Cameron. 1985. Genetic locus (*stm*F) associated with cGMP phosphodiesterase activity in *Dictyostelium discoideum* maps in linkage group II. *J. Bacteriol.* 162:427-429.

Coukell, M. B., A. M. Cameron, C. M. Pitre, and J. D. Mee. 1984. Developmental regulation and properties of the cGMP specific phosphodiesterase in *Dictyostelium discoideum*. *Dev. Biol.* 103:246-257.

Darmon, M., P. Brachet, and L. H. Pereira da Silva. 1975. Chemotactic signals induce cell differentiation in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA.* 72:3163-3166.

Deering, R. A., M. S. Smith, B. K. Thompson, and A. C. Adolf. 1970. Gamma-ray-resistant and -sensitive strains of slime mould (*Dictyostelium discoideum*). *Radiat. Res.* 43:711-728.

Devreotes, P. N., and T. L. Steck. 1979. Cyclic 3',5' AMP relay in *Dictyostelium discoideum*. II. Requirements for the initiation and termination of the response. *J. Cell Biol.* 80:300-309.

Dinauer, M. C., T. L. Steck, and P. N. Devreotes. 1980*a*. Cyclic 3'5'-AMP relay in *Dictyostelium discoideum*. IV. Recovery of the cAMP signaling response after adaptation to cAMP. *J. Cell Biol.* 86:545-553.

Dinauer, M. C., T. L. Steck, and P. N. Devreotes. 1980*b*. Cyclic 3'5'-AMP relay in *Dictyostelium discoideum*. V. Adaptation of the cAMP signaling response during cAMP stimulation. *J. Cell Biol.* 86:554-561.

Fisher, P. R., and K. L. Williams. 1982. Thermotactic behaviour of *Dictyostelium discoideum* slug phototaxis mutants. *J. Gen. Microbiol.* 128: 965-971.

Fisher, P. R., E. Smith, and K. L. Williams. 1981. An extracellular chemical signal controlling phototactic behaviour by *D. discoideum* slugs. *Cell.* 23: 799-807.

Fisher, P. R., U. Dohrmann, W. N. Grant, and K. L. Williams. 1983. Spontaneous turning behaviour in *Dictyostelium discoideum* slugs. *J. Cell Sci.* 62: 161-170.

Fisher, P. R., U. Dohrmann, and K. L. Williams. 1984. Signal processing in *Dictyostelium discoideum* slugs. *Mod. Cell Biol.* 3:197-248.

Fisher, P. R., D. P. Häder, and K. L. Williams. 1985. Multidirectional phototaxis by *Dictyostelium discoideum* amoebae. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 29:43-47.

Futrelle, R. P. 1982. *Dictyostelium* chemotactic response to spatial and temporal gradients. Theories of the limits of chemotactic sensitivity and of pseudochemotaxis. *J. Cell. Biochem.* 18:197-212.

Futrelle, R. P., J. Traut, and W. G. McKee. 1982. Cell behavior in *Dictyostelium discoideum*: preaggregation response to localized cyclic AMP pulses. *J. Cell Biol.* 92:807-821.

Gerisch, G., H. Fromm, A. Huesgen, and U. Wick. 1975*a*. Control of cell-contact sites by cyclic AMP pulses in differentiating *Dictyostelium* cells. *Nature (Lond.).* 255:547-549.

Gerisch, G., D. Huesler, D. Malchow, and U. Wick. 1975*b*. Cell communication by periodic cyclic-AMP pulses. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 272:181-192.

Gerisch, G., J. Hagmann, P. Hirth, C. Rossier, U. Weinhart, and M. Westphal. 1985. Early *Dictyostelium* development: control mechanisms by-passed by sequential mutagenesis. *Cold Spring Harbor Symp. Quant. Biol.* 50:813-822.

Häder, D. P., and K. L. Poff. 1979*a*. Photodispersal from light traps by amebas of *Dictyostelium discoideum*. *Exp. Mycol.* 3:121-131.

Häder, D. P., and K. L. Poff. 1979*b*. Light-induced accumulation of *Dictyostelium discoideum* amoebae. *Photochem. Photobiol.* 29:1157-1162.

Hong, C. B., M. A. Häder, D. P. Häder, and K. L. Poff. 1981. Phototaxis in *Dictyostelium discoideum* amoebae. *Photochem. Photobiol.* 33:373-377.

Hong, C. B., D. R. Fontana, and K. L. Poff. 1983. Thermotaxis of *Dictyostelium discoideum* amoebae and its possible role in pseudoplasmodial thermotaxis. *Proc. Natl. Acad. Sci. USA.* 80:5646-5649.

Konijn, T. M. 1970. Microbiological assay of cyclic 3',5'-AMP. *Experientia (Basel).* 26:367-369.

Konijn, T. M., J. G. C. van de Meene, J. T. Bonner, and D. S. Barkly. 1967. The acrasin activity of adenosine-3',5'-cyclic phosphate. *Proc. Natl. Acad. Sci. USA.* 58:1152-1154.

Lapidus, I. R. 1980. "Pseudochemotaxis" by microorganisms in an attractant gradient. *J. Theor. Biol.* 86:91-103.

Mato, J. M., A. Losada, V. Nanjundiah, and T. M. Konijn. 1975. Signal input for a chemotactic response in the cellular slime mold *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA.* 72:4991-4993.

Meinhardt, H., and A. Gierer. 1974. Applications of a theory of biological pattern formation based on lateral inhibition. *J. Cell Sci.* 15:321-346.

Neter, J., and W. Wasserman. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, IL.

Potel, M. J., and S. A. Mackay. 1979. Preaggregative cell motion in *Dictyostelium*. *J. Cell Sci.* 36:281-309.

Roos, W., D. Malchow, and G. Gerisch. 1977. Adenylyl cyclase and the control of cell differentiation in *Dictyostelium discoideum*. *Cell Differ.* 6:229-239.

Ross, F. M., and P. C. Newell. 1981. Streamers: chemotactic mutants of *Dictyostelium discoideum* with altered cyclic GMP metabolism. *J. Gen. Microbiol.* 127:339-350.

Segall, J. E., P. R. Fisher, and G. Gerisch. 1987. Selection of chemotaxis mutants of *Dictyostelium discoideum*. *J. Cell Biol.* 104:151-161.

Siegel, S. 1956. Nonparametric Statistics: For the Behavioural Sciences. McGraw-Hill Inc., Tokyo.

Sussman, M. 1966. Biochemical and genetic methods in the study of cellular slime mold development. *Methods Cell Physiol.* 2:397-410.

van Haastert, P. J. M. 1983. Sensory adaptation of *Dictyostelium discoideum* cells to chemotactic signals. *J. Cell Biol.* 96:1559-1565.

van Haastert, P. J. M., and R. J. W. de Wit. 1984. Demonstration of receptor heterogeneity and affinity modulation by nonequilibrium binding experiments. The cell surface cAMP receptor of *Dictyostelium discoideum*. *J. Biol. Chem.* 259:13321-13328.

van Haastert, P. J. M., M. M. van Lookeren Campagne, and F. M. Ross. 1982. Altered cGMP-phosphodiesterase activity in chemotactic mutants of *Dictyostelium discoideum*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 147:149-152.

van Haastert, P. J. M., R. J. W. de Wit, P. M. W. Janssens, F. Kesbeke, and J. DeGoede. 1986. G-protein-mediated interconversions of cell-surface cAMP receptors and their involvement in excitation and desensitization of guanylate cyclase in *Dictyostelium discoideum*. *J. Biol. Chem.* 261:6904-6911.

Varnum, B., and D. R. Soll. 1981. Chemoresponsiveness to cAMP and folic acid during growth, development and dedifferentiation in *Dictyostelium discoideum*. *Differentiation.* 18:151-160.

Varnum, B., and D. R. Soll. 1984. Effects of cAMP on single cell motility in *Dictyostelium*. *J. Cell Biol.* 99:1151-1155.

Varnum, B., K. B. Edwards, and D. R. Soll. 1985. *Dictyostelium* amoebae alter motility differently in response to increasing versus decreasing temporal gradients of cAMP. *J. Cell Biol.* 101:1-5.

Varnum, B., K. B. Edwards, and D. R. Soll. 1986. The developmental regulation of single-cell motility in *Dictyostelium discoideum*. *Dev. Biol.* 113: 218-227.

Varnum-Finney, B. J., K. B. Edwards, E. Voss, and D. R. Soll. 1987*a*. Amebae of *Dictyostelium discoideum* respond to an increasing temporal gradient of the chemoattractant cAMP with a reduced frequency of turning: evidence for a temporal mechanism in ameboid chemotaxis. *Cell Motil. Cytoskeleton.* 8:7-17.

Varnum-Finney, B. J., E. Voss, and D. R. Soll. 1987*b*. Frequency and orientation of pseudopod formation of *Dictyostelium discoideum* amebae chemotaxing in a spatial gradient: further evidence for a temporal mechanism. *Cell Motil. Cytoskeleton.* 8:18-26.

Vicker, M. G., W. Schill, and K. Drescher. 1984. Chemoattraction and chemotaxis in *Dictyostelium discoideum*: myxamoeba cannot read spatial gradients of cyclic adenosine monophosphate. *J. Cell Biol.* 98:2204-2214.

Welker, D. L., K. P. Hirth, and K. L. Williams. 1985. Inheritance of extrachromosomal ribosomal DNA during the asexual life cycle of *Dictyostelium discoideum*: examination by use of DNA polymorphisms. *Mol. Cell Biol.* 5:273-280.

Wilkinson, P. C. 1982. The measurement of leukocyte chemotaxis. *J. Immunol. Methods.* 51:133-148.

Williams, K. L., and P. C. Newell. 1976. A genetic study of aggregation in the cellular slime mould *Dictyostelium discoideum* using complementation analysis. *Genetics.* 82:287-307.

Zigmond, S. H. 1977. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *J. Cell Biol.* 75:606-616.