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Published in:
The Journal of Cell Biology

DOI:
10.1083/jcb.96.6.1559

Publication date:
1983

Sensory Adaptation of *Dictyostelium discoideum* Cells to Chemotactic Signals

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ABSTRACT Postvegetative *Dictyostelium discoideum* cells react chemotactically to gradients of cAMP, folic acid, and pterin. In the presence of a constant concentration of 10^{-5}M cAMP cells move at random. They still are able to respond to superimposed gradients of cAMP, although the response is less efficient than without the high background level of cAMP. Cells which are accommodated to 10^{-5}M cAMP do not react to a gradient of cAMP if the mean cAMP concentration is decreasing with time. This indicates the involvement of adaptation in the detection of chemotactic gradients: cells adapt to the mean concentration of chemoattractant and respond to positive deviations from the mean concentration. Cells adapted to high cAMP concentrations react normally to gradients of folic acid or pterin. Adaptation to one of these compounds does not affect the response to the other attractants. This suggests that cAMP, folic acid, and pterin are detected by different receptors, and that adaptation is localized at a step in the transduction process before the signals from these receptors coincide into one pathway. I discuss the implications of adaptation for chemotaxis and cell aggregation.

Chemotaxis is very important during the life cycle of the cellular slime molds. In the vegetative stage the amebae have to find their bacterial food in the soil which they inhabit. At this time the amebae are chemotactic to folic acid (FA) and pterin (Pte) (26, 27),[1] both of which are excreted by bacteria, and therefore it seems probable that this mechanism is used to find food. When the food source is exhausted the amebae aggregate to form a multicellular slug which then differentiates into a fruiting body. Cell aggregation is mediated by chemotaxis (1). The best studied species, *Dictyostelium discoideum*, uses pulsatile signals of the chemoattractant cAMP (14).

During the last decade evidence has been accumulating that cAMP, FA, and Pte's are detected by different receptors (35) localized on the cell surface (6, 12, 17, 19). Even if separate receptors exist, it seems likely that these chemoattractants share a common transduction pathway to directed locomotion. This might be evident from the observation that all chemoattractants induce a similar transient cGMP accumulation in sensitive cells (20, 21, 24, 39, 40).

The detection and particularly the analysis of chemotactic signals in the cellular slime molds are far from understood. Mato et al. (23) presented evidence that the primary input signal for chemotaxis is a spatial gradient of cAMP. This is comparable to the input signal for chemotaxis in leukocytes (41) but in contrast to bacteria where the input signal is a temporal gradient of attractant (16). The threshold spatial gradient of cAMP or FA between the ends of a cell is ~1% of the mean concentration around the cell (23, 25). Chemotaxis in leukocytes shows a comparable sensitivity to chemotactic signals (42). Thus, a cell is able to discriminate between a relative chemoattractant concentration of 100 at its front and 99 at its back.

During cell aggregation, cAMP is released in a pulsatile manner by the aggregation center. Cells in the neighborhood of the center detect cAMP, react chemotactically, and excrete cAMP themselves by which the cAMP signal is relayed (29, 32). Due to the pulsatile release of cAMP and the relay mechanism, waves of cAMP pass through a population of sensitive cells (34). As the cAMP wave approaches a cell, the cell senses a gradient of cAMP and moves to the highest concentration, which guides it to the aggregation center. As the gradient passes the cell, the gradient must reverse; however, the cell does not reverse but terminates its directed locomotion (34). A refractory period has been proposed (31) to explain this "back of the wave" problem. Using microcapillaries filled with cAMP, Gerisch et al. (11) and, more recently, Swanson and Taylor (33) and Futrelle et al. (10) have shown that cells can react chemotactically to different gradients of cAMP placed

[1] cAMP, adenosine 3',5'-monophosphate. cGMP, guanosine 3',5'-monophosphate. FA, folic acid. Pte, pterin. DTT, dithiothreitol. SSS, standard salt solution.

shortly after each other. This rules out an absolute refractory period. In chemotactic experiments with microcapillaries the concentration of cAMP at the cell is always rising with time even if the gradient of chemoattractant is reversed (10). This is in contrast to the gradient reversal in a propagating wave during cell aggregation where the concentration of cAMP at the cell always decreases after gradient reversal, which may suggest that both temporal and spatial properties of the gradients are involved in the analysis of chemotactic signals.

I describe experiments on the chemotactic response of *D. discoideum cells* to different gradients of cAMP, FA, and Pte. The spatial and temporal properties of these gradients were qualitatively known. The results show that the chemotactic response of *D. discoideum* cells to cAMP, FA, and Pte is controlled by an adaptation process, which means that a cell accommodates to the mean concentration of chemoattractant. The implications of adaptation for the "back of the wave" problem and for the detection and analysis of chemotactic signals will be discussed.

MATERIALS AND METHODS

Culture Conditions: *D. discoideum*, NC-4(H), was grown in association with *Escherichia coli* B/r on a medium containing 3.3 g of peptone, 3.3 g of glucose, 4.5 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 2H_2O$, and 15 g of agar per liter. Cells were harvested in the mid-log phase with 1% standard salt solution (SSS) (1), and freed from bacteria by repeated washing and centrifugation at 100 *g* for 4 min. Cells were suspended in 1% SSS at a density of 5×10^6 cells/ml.

Chemotactic Assay: Chemotaxis was tested with the small population assay (13). ~80 g of agar (Difco Laboratories Inc., Detroit, MI) was extensively washed with 60 l of deionized water on a Buchner funnel (diameter 25 cm). The agar slurry was lyophilized. Lyophylized powder (7.5 g) was solved in 1 l of SSS by boiling for ~10 min. After cooling till 50–60°C, 25 ml of the boiled agar was poured into each petri dish (diameter, 9.4 cm). Additives, if required, were included in the agar by placing 250 μl of a concentrated solution of the additives in the petri dish before adding the agar solution. After the agar had set, the plates were stored at 4°C for at least 12 h, but not longer than 2 d.

Small droplets (0.1 μl) of the cell suspension were deposited on the agar surface with a microcapillary, giving a final radius of 0.3 mm. After 30 min at 22°C, test solutions (0.1 μl) were deposited close to the small populations of amebae. The liquid of these droplets has evaporated within 1 min after their deposition on the agar surface. At 5–10-min intervals, I observed the distribution of the amebae within the small population.

Amebae can freely move within the boundaries of the small population, but they cannot cross the boundary because of the hydrophobicity of the agar surface. A chemoattractant placed close to the small population will diffuse in the agar and change the random movement of the cells into directed movement by which cells accumulate at the edge of the population closest to the test solution. The chemotactic response was scored positive if at least twice as many amebae are pressed against the edge closest to the test solution as against the opposite edge. 20 populations were observed for each test solution. The distance between the centers of the amebal population and the test solution was ~1.0 mm. The distance between the two most nearby edges of amebal and test droplet was ~0.4 mm. In mathematics on diffusion of chemoattractants a point geometry of the chemoattractant source was assumed (23). In calculations the distance between the source of chemoattractant and responding amebae was taken as 0.7 mm.

Locomotion of amebae on hydrophobic agar, on hydrophobic agar containing 2.5 mM dithiothreitol (DTT), and on hydrophobic agar containing 2.5 mM DTT and 10^{-5}M cAMP was investigated using time-lapse cinematography at a rate of one frame per 12 s. The film was projected on a sheet of paper and the images of 25 cells were drawn for 25 successive frames. The distance between start and finish of the path (displacement), and the length of the path (trajectory) were recorded. These data were used to calculate the velocity of locomotion (trajectory/time) and the persistence of locomotion (displacement/trajectory).

Demonstration of cAMP Gradient: For this experiment a small tissue culture dish was used (Falcon 3010; radius, 10 mm) (Falcon Plastics, Oxnard, CA). Hydrophobic agar (2 ml) contained the following additives: 1 mM phosphate buffer, pH 7.0, 1 mM $MgSO_4$, 2.5 mM DTT, and 10^{-5}M [8-^{3}H]cAMP (3.7 M Bq/dish; 3.7 M Bq = 100 μCi = 7×10^7 cpm). At $t = 0$, a droplet containing 10 ng of beef heart phosphodiesterase was deposited on the agar surface, yielding a final radius of 0.3 mm. At t = 10, 20, 30, and 60 min, five small droplets a–e (0.1 μl) were deposited at different distances from the phosphodiesterase (see Fig. 2 for the geometry). The droplets a–e were taken back with a microcapillary at ~15 s after their deposition and added to 225 μl 10 mM phosphate buffer, pH 3.0 (10 Pb3). The radioactivity recovered was ~400–600 cpm. This solution (200 μl) was chromatographed on small reversed phase columns (6 ID × 11 mm; Bonda pak G8/Porasil B, 37–75 μm, Waters Associates Inc., Milford, MA). 5'AMP was eluted with 0.8 ml of 10 Pb3, 1% methanol, and cAMP was eluted subsequently with 1.0 ml of 10 Pb3, 15% methanol. The radioactivity in the fractions was determined by liquid scintillation counting. The local concentration of cAMP in the agar was calculated by using the fraction of cAMP degraded to 5'AMP.

Materials: cAMP and beef heart phosphodiesterase were obtained from Boehringer (Mannheim, Federal Republic of Germany), Pte and DTT were purchased from Sigma Chemical Co. (St. Louis, MO), and FA from Fluka (Buchs, Switzerland). [8-^{3}H]cAMP (0.95 TBq/mmol) was obtained from the Radiochemical Centre (Amersham, England).

RESULTS

Our study's objective was to investigate the chemotactic response of *D. discoideum* cells to gradients of cAMP with different spatial and temporal properties. One approach is to establish a constant extracellular cAMP concentration at a cell, followed by the addition or removal of cAMP at one side of the cell. This will generate spatial gradients of cAMP with respectively rising and falling cAMP concentrations. *D. discoideum* cells excrete cAMP in response to cAMP (relay [see references 4, 5, 7. 8, 29, 32]), and cells degrade cAMP by cell-surface and extracellular phosphodiesterases (18, 28). Relay and phosphodiesterase activity may modify the concentration around the cell. Since cells are sensitive to very shallow gradients of cAMP (23), these activities should be prevented. Phosphodiesterase activity is inhibited by DTT (28). I used postvegetative cells for the following reasons: (*a*) phosphodiesterase activity is low in postvegetative cells (28), (*b*) cAMP-relay is almost absent in this stage (4), (*c*) postvegetative cells excrete about 100-fold less cAMP than aggregative cells and are chemotactically about 100-fold less sensitive to cAMP than aggregative cells (2) (and, therefore, the possible excretion of small amounts of cAMP will be less effective than in aggregative cells), and (*d*) postvegetative cells are also chemotactic to FA (26) and to Pte (27). This allows us to investigate the effect of the background concentration of cAMP on the activity of chemoattractants that are not detected with cAMP receptors. Chemotaxis was measured with the small population assay on hydrophobic agar (13).

Postvegetative *D. discoideum* cells placed on plain hydrophobic agar are initially distributed at random. After ~1 h, small clumps of a few cells are formed (Fig. 1 *a*). Cell aggregation starts after ~10 h. In postvegetative cells, 10^{-7}M cAMP induced a chemotactic response in ~50% of the small populations. All populations reacted positively to 10^{-6}M cAMP (Fig. 1 *b*).

Cell clumps are not formed if 2.5 mM DTT is included in the agar. Cell locomotion is normal up till ~3 h. After 6 h, cells round up and become immobile; cell aggregation does not take place. If 10^{-5}M cAMP is included in the agar without DTT, cell clumps are not formed and the distribution of amebae is initially homogeneous. After 1.5–2 h, cells become located preferentially at the boundaries of the small population and often they crawl out of the population (Fig. 1 *c*). This situation is comparable to the cellophane square assay for chemotaxis (3). Supposedly, cAMP is degraded by phosphodiesterase in and below the small population. After the cells have been on the agar for 1.5–2 h, they start to move to higher cAMP concentrations which are located outside the small population.

The distribution of cells in the small population remains homogeneous if 10^{-5}M cAMP with DTT is included in the

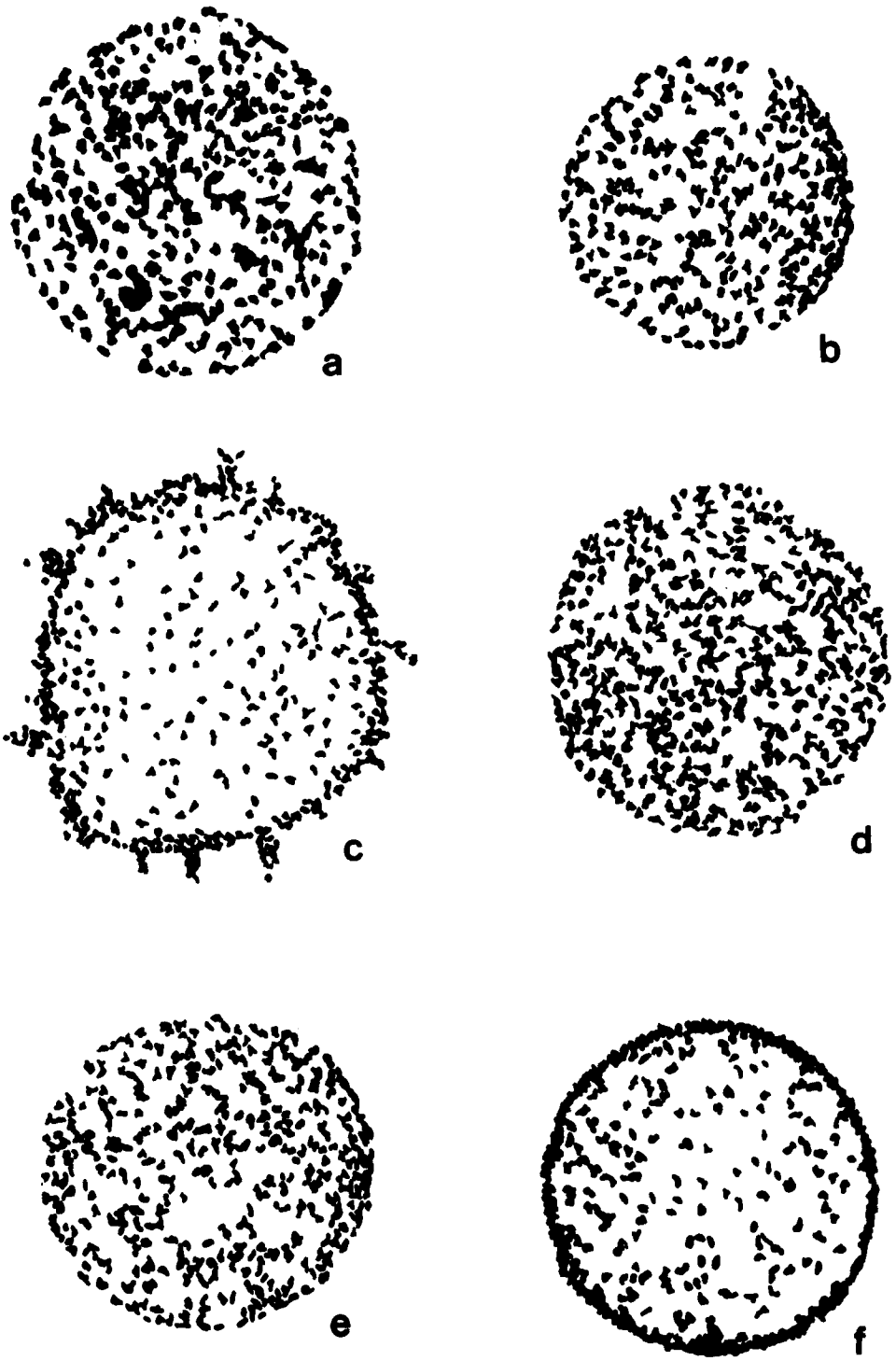

FIGURE 1 Responses of postvegetative cells to gradients of cAMP and to constant concentrations of cAMP. (*a*) Cells on plain hydrophobic agar after 1 h. (*b*) Cells were placed on plain hydrophobic agar. After 30 min, 10^{-6}M cAMP was deposited at the right. The picture was made at $t = 45$ min. (*c*) Cells were placed on hydrophobic agar containing 10^{-5}M cAMP. Response after 3 h. (*d*) Cells were placed on hydrophobic agar containing 10^{-5}M cAMP and 2.5 mM DTT. Response after 1 h; after 3 h the distribution is essentially the same. (*e*) Cells were placed on hydrophobic agar containing 10^{-5}M cAMP and 2.5 mM DTT. After 30 min, a droplet containing 10^{-6}M cAMP was placed at the right. The picture was made at $t = 45$ min. (*f*) Cells were placed on plain hydrophobic agar. After 30 min, a droplet containing 10^{-3}M cAMP was deposited at the right. The picture was made at $t = 90$ min. The agar on which the cells rest makes it difficult to obtain high-contrast photographs for reproduction. Drawings from representative photographs are shown.

agar (Fig. 1 *d*). Cell locomotion occurs as on plain agar. Apparently, cells do not affect the distribution of cAMP, and a constant concentration of cAMP does not affect the distribution of cells. A constant concentration of cAMP seems to be ignored by the cells. Are such cells responsive to a gradient of cAMP which is superimposed on a high background concentration of 10^{-5}M cAMP? The results of Table I show that cells with a background concentration of 10^{-5}M cAMP respond chemotactically to a test solution with 10^{-6}M cAMP. The 50% response is comparable to the response to 10^{-7}M cAMP on plain agar.

Are cells which are accommodated to high background concentrations of cAMP responsive to a gradient of cAMP which has everywhere a lower concentration than the original background concentration? To answer this question, a test solution was deposited containing beef heart phosphodiesterase, which is not inhibited by DTT. Due to the local addition of phosphodiesterase activity a spatial gradient of cAMP will arise in the small population. The cAMP concentration decreases in the direction of the test solution. Due to degradation by phosphodiesterase, the concentrations of cAMP are lower than the concentration which was around the cell originally. Table I shows that the cells do not move away from the phosphodiesterase activity. To show that a gradient of cAMP was built by the phosphodiesterase, we first placed the test solution with phosphodiesterase. After 30 min the cells were

placed close to the test solutions. These cells, which were not exposed to the high cAMP concentrations, moved away from the phosphodiesterase activity (Table I). Direct evidence that a cAMP gradient is formed by phosphodiesterase was obtained by loading the agar with radioactive cAMP and detecting the hydrolysis of cAMP at different distances from the phosphodiesterase (Fig. 2). This indicates that the cAMP gradient was present and that cells cannot react chemotactically to a spatial gradient of cAMP if the mean cAMP concentration decreases with time.

The aforementioned results point to the involvement of an adaptation process in the detection of chemotactic signals. Cells respond to gradients of cAMP but adapt to constant concentrations. If the background concentration lowers, cells should deadapt in order to detect gradients of cAMP.

Table I shows that cells adapted to 10^{-5}M cAMP responded to additional 10^{-6}M cAMP with an efficiency comparable to that of the response of nonadapted cells to 10^{-7}M cAMP. In Fig. 3, the chemotactic activities of different cAMP concentrations were measured with cells placed on agar containing different cAMP concentrations. Higher background concentra-

TABLE I

Demonstration of the Involvement of Adaptation in Chemotaxis

Test solution	Chemotactic response (%)	
	Cells placed first*	Test solutions placed first‡
—	0	0
10^{-6}M cAMP	+60	+50
PDE§	0	−75
Boiled PDE§	0	0
Boiled PDE§ with 10^{-6}M cAMP	+50	+60

Chemotactic activity was tested with the small population assay (13) on hydrophobic agar containing the following additives: 1 mM phosphate buffer, pH 7.0, 1 mM $MgSO_4$, 10^{-5}M cAMP, 2.5 mM DTT.

* Vegetative cells were deposited on the agar surface at $t = 0$ min, and the test solutions were deposited at $t = 30$ min. The chemotactic response was observed at 10-min intervals. The response at $t = 60$ min is presented.

‡ Test solutions were deposited on the agar surface at $t = 0$ min, and the cells at $t = 30$ min. The chemotactic response at $t = 60$ min is presented. (+) Attraction to test solutions. (−) Repulsion from test solutions.

§ The test solution (0.1 μl) contained 10 ng of beaf heart phosphodiesterase.

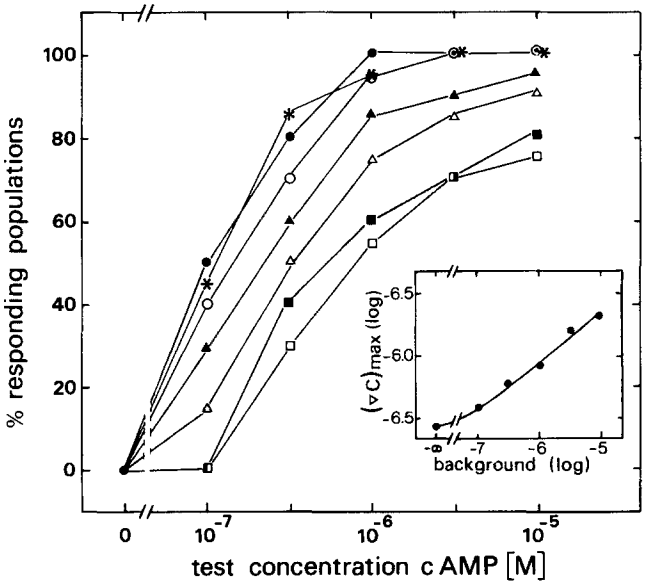

FIGURE 3 Chemotactic response of postvegetative *D. discoideum* cells to cAMP in the presence of various background concentrations of cAMP. Cells were deposited on hydrophobic agar containing different concentrations of cAMP with or without 2.5 mM DTT. After 30 min, droplets with various cAMP concentrations were deposited close to the small populations with amebae. The response was observed at 10-min intervals; the maximal response is shown. (●) Plain agar. (*) Agar with 2.5 mM DTT. (○) Agar with 10^{-7}M cAMP and 2.5 mM DTT. (▲) Agar with 3×10^{-7}M cAMP and 2.5 mM DTT. (△) Agar with 10^{-6}M cAMP and 2.5 mM DTT. (■) Agar with 3×10^{-6}M cAMP and 2.5 mM DTT. (□) Agar with 10^{-5}M cAMP and 2.5 mM DTT. (*Inset*) The logarithm of the threshold spatial gradient (∇C_{max} from Table II) is expressed against the logarithm of the background cAMP concentration in the agar. According to the Weber-Fechner Law of Adaptation (cited in reference 15), this should yield a straight line.

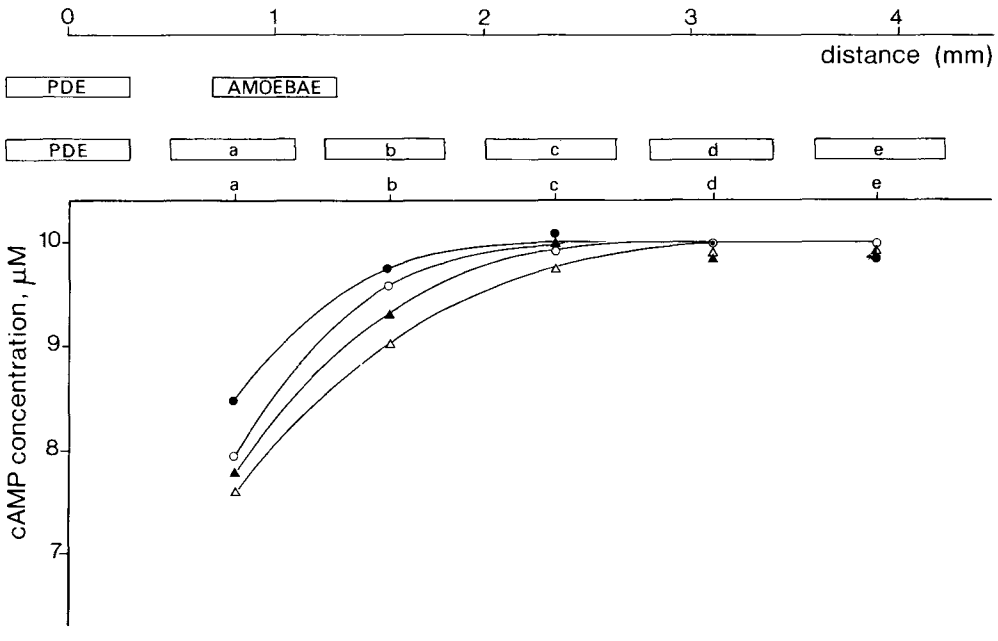

FIGURE 2 The formation of a spatial gradient of cAMP with the mean concentration of cAMP continually decreasing. Radioactive cAMP (7×10^7 cpm) was included in the hydrophobic agar (2 ml). At $t = 0$, a small droplet containing 10 ng of phosphodiesterase was deposited on the agar surface. At $t =$ 10, 20, 30, and 60 min, series of five droplets (*a–e*) were deposited on the agar surface at different distances from the phosphodiesterase as indicated (the geometrics of the chemotactic assay conditions has been shown for comparison). 15 s later, the droplets were taken back (containing ~400–600 cpm), and chromatographed (see Materials and Methods). The local cAMP concentration was calculated by using the percent of cAMP degraded to 5′AMP.

TABLE II

Threshold Spatial Gradients of cAMP for Various Background cAMP Concentrations

C^b	C^t	N^t	∇C_{max}
(*M*)	(*M*)	(*mol*)	(Mcm^{-1})
0	10^{-7}	10^{-14}	3×10^{-7}
10^{-7}	1.5×10^{-7}	1.5×10^{-14}	4×10^{-7}
3×10^{-7}	2.1×10^{-7}	2.1×10^{-14}	6×10^{-7}
10^{-6}	3×10^{-7}	3×10^{-14}	8×10^{-7}
3×10^{-6}	5.5×10^{-7}	5.5×10^{-14}	15×10^{-7}
10^{-5}	8×10^{-7}	8×10^{-14}	21×10^{-7}

Analysis of the data of figure 3. C^b is the background cAMP concentration in the agar; C^t is the cAMP concentration of the test droplet which would induce a 50% response; N^t is the amount of moles in this test droplet; ∇C_{max} is the maximal spatial gradient of cAMP in the amebal population, calculated from $\nabla C_{max} = 0.64\ N/d^4$ (see reference 23), in which d is the distance between amoebae and test droplet ($d = 0.07$ cm).

tions result in a reduction of the sensitivity to superimposed cAMP gradients. The Weber-Fechner Law related the stimulus concentration which induces a threshold response with the background level to which sensory systems are adapted (cited in reference 15). Mato et al. (23) have shown that the input signal for a chemotactic response of aggregative cells to cAMP is a spatial gradient of cAMP. Here, the assumption is made that postvegetative cells detect cAMP by the same mechanism. In Table II the results of Fig. 3 are expressed in a quantitative way, by calculating the maximal spatial gradient of cAMP (∇C_{max}) which is induced by a threshold test concentration. According to the Weber-Fechner Law a double logarithmic plot of threshold stimulus (∇C_{max}) versus background concentration should yield a straight line (inset Fig. 3). The small slope of this curve ($n = 0.35$) reveals the potency of the cells to maintain a high sensitivity to cAMP gradients if the background concentration of cAMP is increased; thus, the threshold gradient increases only sixfold if the background concentration increases 100-fold.

The quantitative data of Table II consolidate the conclusion drawn from the results in Table I and Fig. 2, that cells cannot orient in a gradient of cAMP if the mean cAMP concentration is decreasing with time. At 10 min after phosphodiesterase addition, a cAMP gradient is formed in the amebal population which is $\sim 2 \times 10^{-5}$M/cm steep with a mean cAMP concentration of $\sim 0.85 \times 10^{-5}$M. This spatial gradient is about 10-fold steeper than the threshold spatial gradient for increasing cAMP concentrations at this mean background cAMP concentration (Fig. 3, *inset*).

Postvegetative *D. discoideum* cells are simultaneously sensitive to cAMP, FA, and Pte. Although these chemoattractants are detected by different receptors (35), it is likely that their pathways will combine somewhere in the transduction network to pseudopod formation. Fig. 4 shows that cells maintain the same sensitivity to FA if various concentrations of cAMP are included in the agar. Clearly, adaptation to cAMP does not affect the response to FA.

A test solution with a low cAMP concentration (10^{-6}M) induces a chemotactic response with cells on plain agar within 10 min. A test solution with a very high cAMP concentration (10^{-3}M) induces a response only after 45 min. The response is a radially outward movement of the cells (Fig. 1*f*). Since cells should detect at least some of the 10^{-3}M cAMP after 10 min, the absence of a response indicates an adaptation process. The same observations have been made with FA and Pte: 10^{-6}M induces a fast response, and 10^{-3}M induces a slow response.

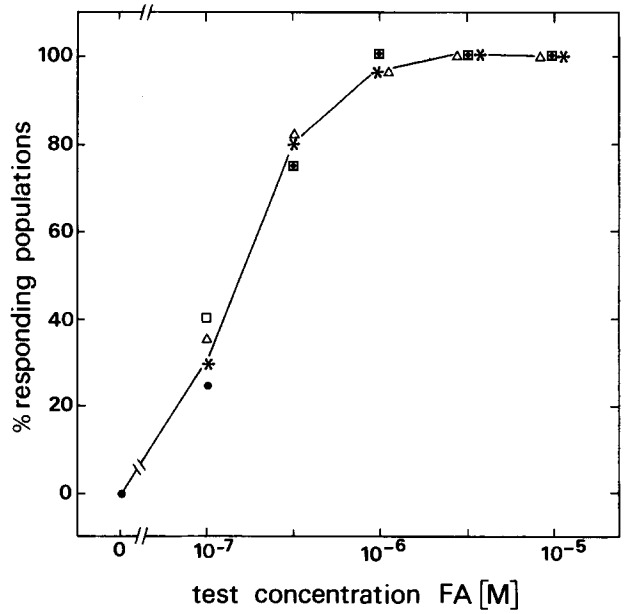

FIGURE 4 Chemotactic response of postvegetative *D. discoideum* cells to folic acid in the presence of various background concentrations of cAMP. Experiment and symbols as in Fig. 3, except that the test solutions contained folic acid instead of cAMP.

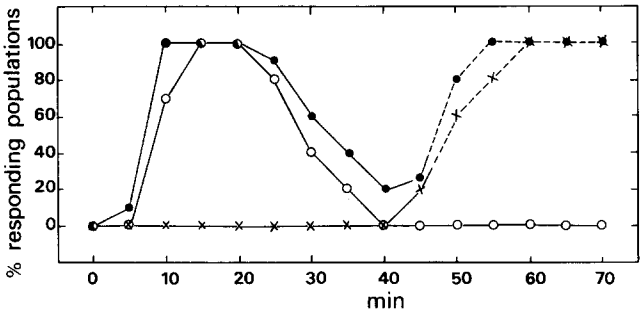

FIGURE 5 Postvegetative cells were placed on plain agar. After 30 min ($t = 0$ in figure), test solutions were deposited close to the amebal populations, and the response was observed at 5-min intervals. (○) 10^{-6}M cAMP. (×) 10^{-3}M folic acid. (●) 10^{-6}M cAMP and 10^{-3}M FA. (——) No response or positive response (c.f. Fig. 1 *b*). (- - -) Radial response (c.f. Fig. 1 *f*).

Fig. 5 shows that cells adapted to 10^{-3}M FA react normally to 10^{-6}M cAMP. All combinations between 10^{-6}M and 10^{-3}M of cAMP, FA, and Pte have been tested (Table III). In all situations, 10^{-6}M chemoattractant induces a response if cells are adapted to 10^{-3}M of another attractant. Thus, also FA and Pte are detected by different receptors. Furthermore, adaptation takes place before the signals of these three receptors combine to one pathway.

DISCUSSION

The results strongly suggest that: (*a*) *D. discoideum* amebae exhibit adaptation to chemotactic stimuli: exposure of cells to a uniform background concentration of 10^{-5}M cAMP results in reduced responsiveness to superimposed gradients of cAMP. (*b*) There is a correlation between the background concentration of cAMP and the magnitude of the superimposed gradient which will induce a threshold response, and this correlates with the Weber-Fechner Law concerning sensory adaptation. (*c*) While a cell can orient in a gradient of cAMP if the mean concentration around the cell is constant or increasing with

TABLE III

Chemotactic Reaction to Low Concentrations of Chemoattractants in the Presence of High Concentrations of Other Chemoattractants

	H_2O	cAMP 10^{-3} M	FA 10^{-3}M	PTe 10^{-3}M
H_2O	0	0	0	0
cAMP 10^{-6}M	+	*nd*	+	+
FA 10^{-6}M	+	+	*nd*	+
Pte 10^{-6}M	+	+	+	*nd*

The chemotactic activity of cAMP, FA, and Pte was tested with the small population assay. The compounds placed at the top were mixed with the compounds placed at the left (final concentrations are presented). Small droplets (0.1 μl) of the mixtures were deposited close to small populations of postvegetative *D. discoideum* cells. The distribution of cells within the population was monitored after 15 min. (*0*) No response. (+) Positive response in >50% of the populations. (*nd*) Not determined.

time, it will not orient to gradients in which the mean concentration is decreasing. (*d*) Adaptation to cAMP has no discernible effects on the ability of postvegetative amebae to sense or orient in gradients of the other attractant FA or Pte.

The observation that cells cannot orient in a spatial gradient if the mean concentration is decreasing with time solves the "back of the wave" problem during cell aggregation. As a wave of cAMP passes a cell, the cell can only react chemotactically on the rising flank of the wave, and not on the falling flank of the wave. This observation is in conflict with the conclusion made by Futrelle (9) from an experiment in which a passing wave was mimicked by using a micropipette source of cAMP moving through a field of aggregative amebae. In the experiment the mean concentration increased as the pipette approached the cell. After the pipette passed the cell, the gradient reversed and the concentration decreased with time. The chemotactic index (CI) reached a maximum of about +0.5 on the rising flank of the wave and about −0.15 on the falling flank of the wave. Although the CI = −0.15 is significantly different from zero, it is not significantly different from the CI of the cells long before or after the gradient has passed. (The CI of the most left or most right determination in Fig. 3 of reference 9 are, respectively, −0.10 and −0.17). A possible explanation for the apparently negative CI without chemotactic stimulation might be the asymmetric cell chamber used (Fig. 1 in reference 9), resulting in liquid streams or light and temperature gradients, which may cause deviations from at random movement.

The primary input signal for chemotaxis in aggregative *D. discoideum* cells seems to be a spatial gradient of cAMP (23), comparable to the detection of chemotactic signals in leukocytes (41). In this report it is shown that a spatial gradient is not sufficient to induce a chemotactic response. There are at least two conditions which should be fulfilled. (*a*) The temporal gradient of chemoattractant should not be negative. (*b*) The spatial gradient should be above a threshold level; this threshold is determined by the background concentration of chemoattractant.

Adaptation is also involved in the detection of chemotactic signals by leukocytes (43). It can be a powerful tool in a cell for the detection of gradients of chemoattractant (see Fig. 6). Leukocytes and *D. discoideum* cells can detect a 1% difference of chemoattractant over their cell length (23, 25, 42). Thus, they are able to measure the difference between 100 and 99. The signal produced intracellularly will be comparable to the extracellular signal if cells do not possess an adaptation mechanism (Fig. 6*a*). Using adaptation, by which cells ignore the mean concentration (or just above the mean concentration),

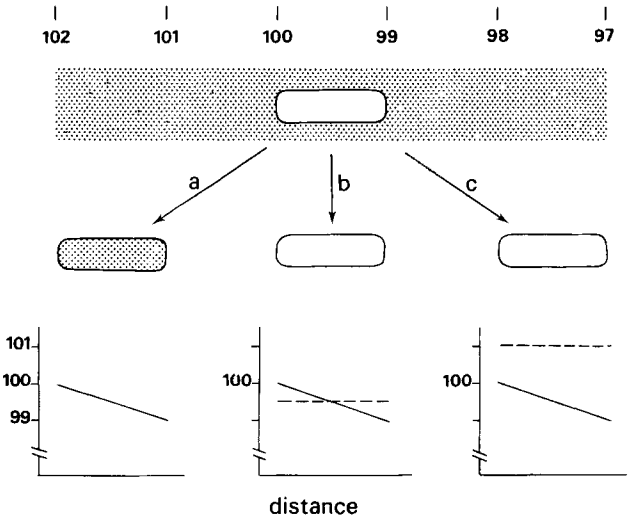

FIGURE 6 Representation of the involvement of adaptation in the detection of chemotactic signals. *Top:* An amoeba is located in a threshold gradient of chemoattractant. Figures above represent the dot density. *Middle and bottom:* (*a*) The amoeba may detect the spatial gradient without using adaptation. The signal produced intracellularly is comparable to the extracellular signal. (*b*) Detection of a stable gradient of chemoattractant using adaptation (the level of adaptation is 99.5). (*c*) The amoeba does not respond to a gradient of chemoattractant of which the mean concentration is decreasing with time, because the ameba is adapted to the earlier mean concentration (arbitrary value 101). (——) Excitation. (- - -) Adaptation.

the difference between 100 and 99 is simplified to the difference between 0.5 and 0 (Fig. 6*b*). When cells have been located in a relatively high cAMP concentration and the concentration decreases with time, the level of adaptation will be higher than the level of excitation. Cells will not react chemotactically, even if a spatial gradient of cAMP is present (Fig. 6*c*).

As a wave of cAMP passes a cell, the concentration of cAMP increases from below 10^{-8}M to about 10^{-6}M within 1.5 min (34). To use the advantage of adaptation effectively, cells should rapidly adapt to this fast increasing cAMP concentration. After the maximal concentration of cAMP has passed the cells, the concentration declines to below 10^{-8}M during ~1.5 min (34). Deadaptation should be slower than the rate of decline of the cAMP concentration, because cells would otherwise reverse. ~5 min after a wave of cAMP has passed a cell, a new wave of cAMP arrives (34). Deadaptation should have proceeded far enough to make detection of this new wave possible. This suggests that adaptation occurs within a fraction of a minute and that deadaptation occurs within a few minutes. Is there a biochemical network in *D. discoideum* that shows these properties?

Dinauer et al. (7, 8) have extensively investigated the signal for the relay response; they revealed that adaptation is involved in this process. However, the rate of adaptation is relatively slow (4–10 min), probably too slow to be involved in the chemotactic response. Furthermore, many slime mold species such as *Dictyostelium lacteum* and *Dictyostelium minutum* do not possess a relay mechanism, although they react chemotactically. Evidence is accumulating that intracellular cGMP is involved in the chemotactic reaction (20–22, 24, 30, 37, 39, 40). Recently, we have investigated the input signal for the cAMP-mediated cGMP accumulation in aggregative *D. discoideum* cells (36). Adaptation is also involved in this process. The signal for adaptation has entered the cell after 1–2 s, and

adaptation is completed within 10 s. Deadaptation of the cAMP-mediated cGMP response shows a half-life of 1.5 min (36). The kinetic properties of adaptation and deadaptation of the cGMP response are feasible for the involvement in the chemotactic response.

Mato et al. (23) have shown that in aggregative *D. discoideum* cells the threshold cAMP signal is 1% with a mean cAMP concentration of $\sim 3 \times 10^{-9}$M. Due to adaptation, the effective signal at the front of the cell is only $\sim 3 \times 10^{-11}$M cAMP. In a cell suspension, such a cAMP signal will generate only 2,000 molecules of cGMP intracellularly (21). Nonequilibrium kinetics of an intracellular cGMP-binding protein (38) revealed that such minute increases of cGMP levels are detectable; addition of 3×10^{-11}M cAMP to a cell suspension will induce about a 30% transient occupancy of this intracellular cGMP-binding protein (38).

An intriguing question is where to localize the adaptation process physically. Intuitively, the most effective place would be at the plasma membrane before the signal is liberated intracellularly. This view is supported by the observation that chemotactic signals detected by different receptors do not show cross-adaptation.

I enthusiastically acknowledge Theo M. Konijn for valuable discussions and advice.

This work was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by The Netherlands Organization for the Advancement of Pure Research.

Received for publication 3 August 1982, and in revised form 8 December 1982.

REFERENCES

1. Bonner, J. T. 1947. Evidence for the formation of cell aggregates by chemotaxis in the cellular slime mold *Dictyostelium discoideum*. *J. Exp. Zool.* 106:1–26.
2. Bonner, J. T., D. S. Barkley, E. M. Hall, T. M. Konijn, J. W. Mason, G. O'Keefe, and P. B. Wolfe. 1969. Acrasin, acrasinase, and the sensitivity of acrasin in *Dictyostelium discoideum*. *Dev. Biol.* 20:72–87.
3. Bonner, J. T., A. P. Kelso, and R. G. Gillmor. 1966. A new appraoch to the problem of aggregation in the cellular slime molds. *Biol. Bull.* 130:28–42.
4. Devreotes, P. N., P. L. Derstine, and T. L. Steck. 1979. Cyclic 3',5'-AMP relay in *Dictyostelium discoideum*. I. A technique to monitor responses to controlled stimuli. *J. Cell Biol.* 80: 291–299.
5. 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.
6. De Wit, R. J. W. 1982. Two distinct types of cell surface folic acid binding proteins in *Dictyostelium discoideum*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 150:445–448.
7. Dinauer, M. C., T. L. Steck, and P. N. Devreotes. 1980. Cyclic 3',5'-AMP relay in *Dictyostelium discoideum*. IV. Recovery of the cAMP signalling response after adaptation to cAMP. *J. Cell Biol.* 86:545–553.
8. Dinauer, M. C., T. L. Steck, and P. N. Devreotes. 1980. Cyclic 3',5'-AMP relay in *Dictyostelium discoideum*. V. Adaptation of the cAMP signaling response during cAMP stimulation. *J. Cell Biol.* 86:554–561.
9. 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.
10. Futrelle, R. P., J. Traut, and W. G. McKee. 1982. Cell behavior in *Dictyostelium discoideum*: preaggregative response to localized cyclic AMP pulses. *J. Cell Biol.* 92:807–821.
11. Gerisch, G., D. Hülser, D. Malchow, and U. Wick. 1975. Cell communication by periodic cyclic AMP pulses. *Philos. Trans. R. Soc. Lond. Biol. Sci. B.* 272:181–192.
12. Green, A. A., and P. C. Newell. 1975. Evidence for the existence of two types of cAMP binding sites in aggregative cells of *Dictyostelium discoideum*. *Cell.* 6:129–136.
13. Konijn, T. M. 1970. Microbiological assay of cyclic 3',5'-AMP. *Experientia (Basil).* 26:367–369.
14. Konijn, T. M., J. G. C. Van de Meene, J. T. Bonner, and D. S. Barkley. 1967. The acrasin activity of adenosine-3',5'-cyclic phosphate. *Proc. Natl. Acad. Sci. USA.* 58:1152–1154.
15. Koshland, D. E. 1980. Bacterial chemotaxis as a model behavioral system. Raven Press, New York. 107–125.
16. Macnab, R. M., and D. E. Koshland. 1972. The gradient sensing mechanism in bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA.* 69:2509–2512.
17. Malchow, D., and G. Gerisch. 1974. Short-term binding and hydrolysis of cyclic 3':5'-adenosine monophosphate by aggregating *Dictyostelium* cells. *Proc. Natl. Acad. Sci. USA.* 71:2423–2427.
18. Malchow, C., B. Nägele, H. Schwarz, and G. Gerisch. 1972. Membrane-bound cyclic AMP phosphodiesterase in chemotactically responding cells of *Dictyostelium discoideum*. *Eur. J. Biochem.* 28:136–142.
19. Mato, J. M., and T. M. Konijn. 1975. Chemotaxis and binding of cyclic AMP in cellular slime molds. *Biochim. Biophys. Acta.* 385:173–179.
20. Mato, J. M., and T. M. Konijn. 1977. Chemotactic signals and cyclic GMP accumulation in *Dictyostelium*. *In* Development and Differentiation in the Cellular Slime Moulds. P. Cappuccinelli and J. M. Ashworth, editors. Elsevier North Holland, Amsterdam. 93–103.
21. Mato, J. M., F. A. Krens, P. J. M. Van Haastert, and T. M. Konijn. 1977. 3':5'-cyclic AMP-dependent 3':5'-cyclic GMP accumulation in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA* 74:2348–2351.
22. Mato, J. M., F. A. Krens, P. J. M. Van Haastert, and T. M. Konijn. 1977. Unified control of chemotaxis and cAMP mediated cGMP accumulation by cAMP in *Dictyostelium discoideum*. *Biochem. Biophys. Res. Commun.* 77: 399–402.
23. 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.
24. Mato, J. M., P. J. M. Van Haastert, F. A. Krens, E. H. Rhijnsburger, F. C. P. M. Dobbe, and T. M. Konijn. 1977. Cyclic AMP and folic acid mediated cyclic GMP accumulation in *Dictyostelium discoideum*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 79:331–336.
25. Nandini-Kishore, S. G., and W. A. Frazier. 1981. (^{3}H)-Methotrexate as a ligand for the folate receptor of *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA.* 78:7299–7303.
26. Pan, P., E. M. Hall, and J. T. Bonner. 1972. Folic acid as second chemotactic substance in the cellular slime moulds. *Nat. New Biol.* 237:181–182.
27. Pan, P., E. M. Hall, and J. T. Bonner. 1975. Determination of the active portion of the folic acid molecule in cellular slime mold chemotaxis. *J. Bacteriol.* 122:185–191.
28. Pannbacker, R. G., and L. J. Bravard. 1972. Phosphodiesterase in *Dictyostelium discoideum* and the chemotactic response to cyclic adenosine monophosphate. *Science (Wash. DC).* 175:1014–1015.
29. Roos, W., V. Nanjundiah, D. Malchow, and G. Gerisch. 1975. Amplification of cyclic AMP signals in aggregation cells of *Dictyostelium discoideum*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 53:139–142.
30. 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.
31. Shaffer, B. M. 1957. Aspects of aggregation in cellular slime molds. I. Orientation and chemotaxis. *Am. Nat.* 91:19–35.
32. Schaffer, B. M. 1975. Secretion of cyclic AMP induced by cyclic AMP in the cellular slime mould *Dictyostelium discoideum*. *Nature (Lond.).* 255:549–552.
33. Swanson, J. A., and D. L. Taylor. 1982. Local and spatially coordinated movements in *Dictyostelium discoideum* amebae during chemotaxis. *Cell.* 28:225–232.
34. Tomchik, K. J., and P. N. Devreotes. 1981. Adenosine 3',5'-monophosphate waves in *Dictyostelium discoideum*: a demonstration by isotope dilution fluorography. *Science (Wash. DC).* 212:443–446.
35. Van Haastert, P. J. M., R. J. W. De Wit, and T. M. Konijn. 1982. Antagonists for chemoattractants reveal separate receptors for cAMP, folic acid and pterin in *Dictyostelium*. *Exp. Cell Res.* 140:453–456.
36. Van Haastert, P. J. M., and P. R. Van der Heijden. 1983. Excitation, adaptation and deadaptation of the cAMP mediated cGMP response in *Dictyostelium discoideum*. *J. Cell Biol.* 96:347–353.
37. Van Haastert, P. J. 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.
38. Van Haastert, P. J. M., J. Van Walsum, and F. A. Pasveer. 1982. Nonequilibrium kinetics of a cGMP binding protein from *Dictyostelium discoideum*. *J. Cell Biol.* 94:271–278.
39. Wurster, B., S. Bozzaro, and G. Gerisch. 1978. Cyclic GMP regualtion and responses of *Polysphondylium violaceum* to chemoattractants. *Cell Biol. Int. Rep.* 2:61–69.
40. Wurster, B., K. Schubiger, U. Wick, and G. Gerisch. 1977. Cyclic GMP in *Dictyostelium*: oscillations and pulses in response to folic acid and cyclic AMP signals. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 76:141–144.
41. Zigmond, S. H. 1974. Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes. *Nature (Lond.).* 249:450–452.
42. Zigmond, S. H. 1977. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *J. Cell Biol.* 75:606–616.
43. Zigmond, S. H., and S. J. Sullivan. 1979. Sensory adaptation of leukocytes to chemotactic peptides. *J. Cell Biol.* 82:517–527.