

# A Study on Sensing and Adaptation in *Dictyostelium discoideum*: Guanosine 3',5'-Phosphate Accumulation and Light-Scattering Responses

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**ABSTRACT** Cells of *Dictyostelium discoideum* respond to extracellular cyclic AMP with marked changes in intracellular cyclic GMP levels and light scattering. In this work, defined temporal increases in cyclic AMP were produced by the continuous addition of cyclic AMP to agitated suspensions of cells; concomitant hydrolysis of cyclic AMP by the cells subsequently established a constant, steady state concentration. The cells responded to the initial increase in extracellular cyclic AMP with a rapid increase in the intracellular cyclic GMP concentration and a rapid decrease in light scattering. At cyclic AMP input rates of  $0.5\text{--}5\text{ nM} \times \text{s}^{-1}$ , the fast reactions of cyclic GMP and light scattering had already relaxed while the cyclic AMP concentration in the cell suspension was still increasing. The cells responded to constant concentrations of cyclic AMP with constant elevated cyclic GMP concentrations and constant decreased levels of light scattering. Our results are consistent with the existence of two types of perception systems, one of which adapts to constant stimuli and one of which does not adapt.

Cells of *Dictyostelium discoideum* respond chemotactically to cyclic AMP (1, 2) as well as to folic acid (3, 4) and pterin (4). During differentiation from the growth phase to the aggregation-competent state, the chemotactic sensitivity of the cells toward cyclic AMP increases (2) and that toward folic acid and pterins decreases (4). The presence of receptors for cyclic AMP (5–7) and for folic acid (8, 9) at the cell surface has been demonstrated.

*Dictyostelium* cells inactivate their chemoattractants in enzyme-catalyzed reactions. Cyclic AMP is hydrolyzed to 5'-AMP by means of cyclic AMP phosphodiesterase (for references see reference 10), and folic acid and pterin most likely are deaminated hydrolytically to the corresponding lumazine forms by means of folic acid and pterin deaminases (for references see references 10, 11). Cyclic AMP phosphodiesterases as well as folic acid and pterin deaminases occur in extracellular and membrane-bound forms (10, 11).

The reaction to chemoattractants can be studied with suspensions of cells, using an optical technique (12). Addition of chemoattractants to cell suspensions causes decreases in light scattering (12, 13), which apparently reflect changes of cell shape (14). One intracellular mediator of the reaction to che-

moattractants may be cyclic GMP. In undifferentiated and differentiated cells, chemoattractants elicit rapid increases in the intracellular cyclic GMP concentration (15–17). These increases are due to activation of guanylate cyclase (18). In differentiated cells, chemoattractants also activate the synthesis of cyclic AMP (19–22). Cyclic AMP is released into the extracellular space (19–22) but cyclic GMP is not (16)

The responses of *Dictyostelium* cells often have been studied after one-step addition of chemoattractants. Under these conditions, the cells experience an instantaneous increase in attractant concentration and a subsequent negative temporal concentration gradient as the attractant is decomposed. Such a stimulus pattern causes transient changes in light scattering, cyclic GMP synthesis, and cyclic AMP synthesis.

To characterize a chemoreception system, one would like to know how it responds to defined concentration gradients and to constant concentrations of attractant. Employing an ingenious perfusion technique (23), Devreotes and Steck (22) and Dinauer et al. (24–26) obtained the result that stimulation of differentiated *D. discoideum* cells with constant cyclic AMP concentrations did not cause continuous activation of cyclic AMP synthesis. Within 3 min, cyclic AMP synthesis adapted to constant external stimuli (22). Gerisch et al. (27) and Rossier et al. (28) used either high concentrations of cyclic AMP or analogues of cyclic AMP that are hydrolyzed slowly as quasi-

This paper is dedicated to Professor Benno Hess on the occasion of his 60th birthday.

constant stimuli. They found that activation of cyclic AMP and cyclic GMP synthesis, as well as light-scattering responses, were transient.

The use of high attractant concentrations has two obvious disadvantages: First, the cellular reactions cannot be studied at physiological attractant concentrations, and second, the attractant concentration is not absolutely constant but slowly decreases with time. The technique employed in this study has neither of these drawbacks. We added the chemoattractant cyclic AMP continuously to cell suspensions and measured the resulting changes in intracellular cyclic GMP and light scattering. Continuous addition and decomposition of attractant resulted in a defined concentration increase with time and led to a constant, steady state concentration. Our data indicate that *D. discoideum* cells respond to constant attractant concentrations with constant changes of cyclic GMP concentration and light scattering.

## MATERIALS AND METHODS

**Chemicals:** Adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) were obtained from Boehringer, Mannheim GmbH, Federal Republic of Germany (FRG). [2,8-<sup>3</sup>H]Cyclic AMP (specific activity 38 Ci/mmol) was the product of Amersham Buchler, Braunschweig, FRG. Cyclic AMP-[<sup>125</sup>I]-tracer, cyclic GMP-[<sup>125</sup>I]-tracer, cyclic AMP antiserum, and cyclic GMP antiserum were purchased from New England Nuclear, Dreieich, FRG. Folic acid (pteroyl-monoglutamic acid) was obtained from Serva, Heidelberg, FRG.

**Culture Conditions:** *D. discoideum* strain Ax-2 and a morphogenetic mutant of strain Ax-2, agip 53 (29), were used. A clone of agip 53 was generously supplied by P. Brachet, Pasteur Institute, Paris. Cells were cultivated on nutrient medium supplemented with 1.8% maltose (30). Streptomycin (20 µg/ml) was added to cultures of agip 53. Cells were harvested at densities of 3–8 × 10<sup>6</sup> cells/ml, washed three times in the cold with 17 mM Sørensen phosphate buffer (pH 6.0), resuspended at 2 × 10<sup>7</sup> cells/ml, and shaken at 23°C. This buffer, cell density, and temperature were employed in all of the following experiments. Time (in hours) of starvation in phosphate buffer is designated as *t*<sub>n</sub>.

Experiments were performed with early preaggregation cells (between *t*<sub>1,6</sub> and *t*<sub>2</sub>) of strain Ax-2 and with differentiated cells of strain agip 53. These cell types were used because they do not synthesize and release cyclic AMP in response to external cyclic AMP (31–33), in contrast to differentiated cells of strain Ax-2 (19–22). Such release of cyclic AMP would produce a complicated time course of extracellular cyclic AMP concentration.

Differentiation of agip 53 cells was induced by applying cyclic AMP pulses (29, 34). Starting at *t*<sub>3</sub>, cell suspensions received a cyclic AMP pulse (2 × 10<sup>-8</sup> M final concentration) every 6 min for a period of 2 h. After this treatment, agip 53 cells showed the characteristics of differentiated cells; they formed EDTA-resistant aggregates (29, 35) and had an increased number of cyclic AMP binding sites (36). For further experiments, agip 53 cells were washed and resuspended in 17 mM Sørensen phosphate buffer (pH 6.0).

**Light-scattering Technique:** The technique described by Gerisch and Hess (12) was used. 2 ml of cell suspension were transferred into a cuvette and agitated by bubbling water-saturated oxygen through the suspension at a flow rate of 28 ml/min. The optical density was measured with a Zeiss PM6 spectrophotometer.

Cyclic AMP was added to cell suspension in the optical cuvette either as pulses of 2–10 µl volume or continuously at 0.07 µl × s<sup>-1</sup>. Agitation of the cell suspension caused a rapid uniform distribution of the chemoattractants. The mixing time was determined by injecting a quantity of folic acid that caused a measurable absorption increase at 345 nm. The optical density increased within 1 s to a maximal value.

Continuous supply of chemoattractants was achieved by means of a Perfusor V (Braun, Melsungen). This instrument accurately delivers a constant volume per time. As shown in Fig. 1 continuous addition of a folic acid solution to agitated buffer resulted in a linear increase of optical density with relatively small and short-lived fluctuations.

Parallel experiments for cyclic GMP determination were performed with 10-ml cell suspensions in a thermostated reaction vessel. Cell suspensions were stirred with a magnetic stirrer and supplied with water-saturated oxygen (28 ml/min). Cyclic AMP was continuously added at 0.167 µl × s<sup>-1</sup>. Input rates were calculated as mol × s<sup>-1</sup> per liter cell suspension and expressed as M × s<sup>-1</sup>.

**Assay of Cyclic AMP Hydrolysis:** A 2-µl pulse of 50 µM [<sup>3</sup>H]-cyclic AMP (50 nM, final concentration) was added to 2 ml cell suspensions, and

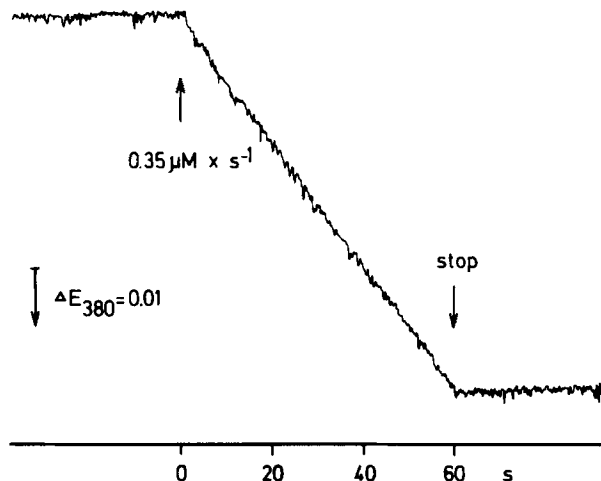


FIGURE 1 Constancy of the volume per time delivered by the Perfusor V. To 2 ml of buffer in the optical cuvette, folic acid was continuously added at a rate of 0.35 µM × s<sup>-1</sup>. The optical density was measured at 380 nm.

6-ml cell suspensions were continuously supplied with 0.167 µl × s<sup>-1</sup> of 18 µM [<sup>3</sup>H]-cyclic AMP. At various times, 50-µl samples were withdrawn from the suspensions, combined with 50 µl of 2 N HClO<sub>4</sub>, and neutralized with 3 M K<sub>2</sub>CO<sub>3</sub>. Cyclic AMP, adenosine 5'-monophosphate, and adenosine were separated by chromatography and their relative amounts determined as previously described (37).

**Assay of Cyclic AMP and Cyclic GMP:** Samples of 50 µl were withdrawn from cell suspensions and quenched with 1 N HClO<sub>4</sub>. Cyclic AMP and cyclic GMP contents were determined by sensitive radioimmune assays (38). Preparation of samples, calibration curves, and controls were as described previously (15). Extra- and intracellular cyclic nucleotides were not distinguished. Previous work showed that cyclic GMP only occurs intracellularly (16, 33).

## RESULTS

### Light-Scattering Responses to Chemoattractants

Addition of a pulse of cyclic AMP to early preaggregation cells of strain Ax-2 elicited a transient light-scattering response (Fig. 2A), as was reported earlier (12). The added cyclic AMP was rapidly hydrolyzed (Fig. 2A). Hydrolysis proceeded approximately as a first-order reaction. The rate constant varied from 0.017 s<sup>-1</sup> to 0.042 s<sup>-1</sup> in different experiments.

Continuous addition of cyclic AMP to a cell suspension resulted in a temporal increase of the cyclic AMP concentration up to a steady state value (Fig. 2B). Cyclic AMP concentration as a function of time can be approximated by the equation

$$[\text{cAMP}] = \frac{V_i}{k_h} (1 - e^{-k_h t}) \quad (1)$$

where  $V_i$  is the input rate and  $k_h$  the rate constant of hydrolysis. It follows from this equation that the time required to approach the steady state depends on the rate constant of hydrolysis. The steady state (ss) concentration of cyclic AMP

$$[\text{cAMP}]_{\text{ss}} = \frac{V_i}{k_h} \quad (2)$$

depends on the input rate of cyclic AMP and on the rate constant of hydrolysis.

In the experiment of Fig. 2B cyclic AMP was added at a rate of 0.5 nM × s<sup>-1</sup>. The increase of the cyclic AMP concentration elicited a fast decrease in optical density. Subsequently, the optical density proceeded to an intermediate level while the cyclic AMP concentration was still increasing. The optical density remained at an intermediate level as long as the cyclic

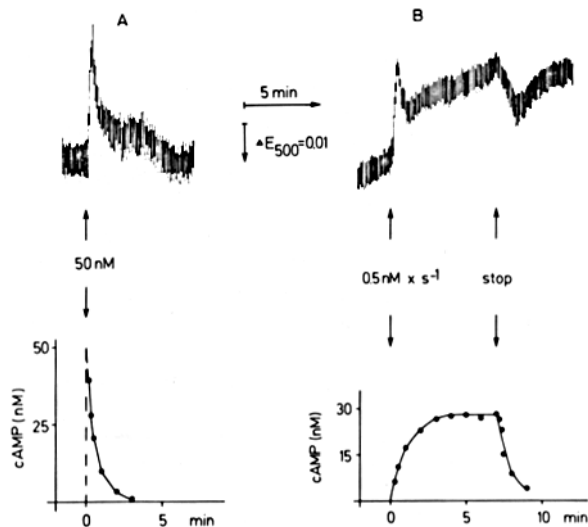


FIGURE 2 Light-scattering responses and hydrolysis of cyclic AMP. Early preaggregation cells of strain Ax-2 received pulses of 50 nM cyclic AMP (A) or were continuously supplied with cyclic AMP at a rate of  $0.5 \text{ nM} \times \text{s}^{-1}$  (B). Light scattering was recorded at 500 nm. Light-scattering experiments were repeated seven times with similar results. For determination of the time course of the cyclic AMP concentration,  $[^3\text{H}]$ -cyclic AMP was added to parallel cell suspensions. At appropriate time intervals,  $50 \mu\text{l}$  samples were withdrawn and their  $[^3\text{H}]$ -cyclic AMP content analyzed. Similar changes in cyclic AMP concentration with time were obtained in a second experiment.

AMP concentration stayed constant at the steady state value. After the cyclic AMP supply was stopped the optical density returned to the basal level. In most experiments the basal optical density was not constant, but slightly decreased with time. Also, dilution of the cell suspension by the added cyclic AMP produced a small decrease in optical density.

The light-scattering pattern depended on the rate of cyclic AMP addition (Fig. 3). The cells hardly responded to a cyclic AMP input of  $0.01 \text{ nM} \times \text{s}^{-1}$ . At the input rate of  $0.05 \text{ nM} \times \text{s}^{-1}$ , the extent of the initial decrease in optical density was smaller than at the input rate of  $0.5 \text{ nM} \times \text{s}^{-1}$ . At the input rate of  $0.05 \text{ nM} \times \text{s}^{-1}$ , after the initial decrease the optical density further decreased to a quasi-constant new level. The light-scattering pattern elicited by a cyclic AMP input of  $5 \text{ nM} \times \text{s}^{-1}$  was very similar to that obtained at  $0.5 \text{ nM} \times \text{s}^{-1}$ , indicating saturation of the responses.

### Changes in Intracellular Cyclic GMP in Response to Cyclic AMP

We measured cyclic GMP concentrations during continuous addition of cyclic AMP. In these experiments, cyclic GMP and cyclic AMP were determined by radioimmuno assays, which always had an error of  $\pm 20\%$ . The data of Fig. 4 were obtained with early preaggregation cells of strain Ax-2 supplied with cyclic AMP at a rate of  $5 \text{ nM} \times \text{s}^{-1}$ . This input rate resulted in a steady state concentration of 200 nM cyclic AMP. The change in cyclic GMP concentration with time resembled the time course of the light-scattering response to this input rate. The cyclic GMP concentration increased to a maximal value within 20 s and subsequently decreased to an intermediate value. The decrease occurred while the cyclic AMP concentration was still increasing. The cyclic GMP concentration remained approximately constant while cyclic AMP was maintained at the steady

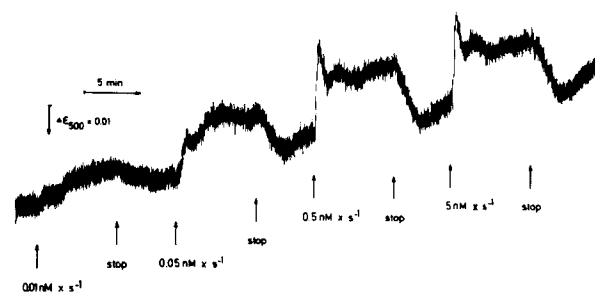


FIGURE 3 Light-scattering responses to varying input rates of cyclic AMP. Early preaggregation cells of strain Ax-2 were continuously supplied with cyclic AMP at rates varying from  $0.01 \text{ nM} \times \text{s}^{-1}$  to  $5 \text{ nM} \times \text{s}^{-1}$ . Light scattering was recorded at 500 nm. Experiments were repeated three times with similar results.

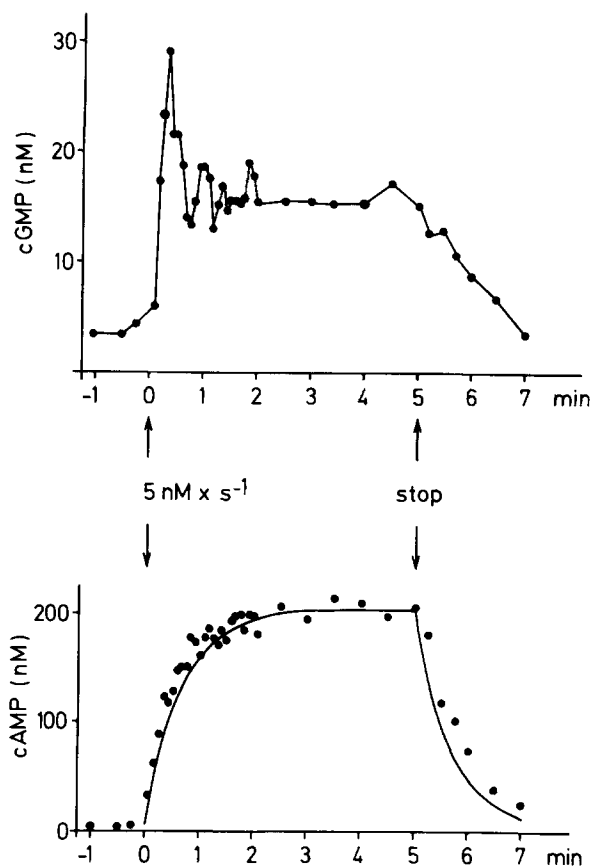


FIGURE 4 Cyclic GMP concentrations in response to continuous addition of cyclic AMP. Suspensions of early preaggregation cells of Ax-2 were supplied with cyclic AMP at a rate of  $5 \text{ nM} \times \text{s}^{-1}$ . During the course of the experiments samples of  $50 \mu\text{l}$  volume were withdrawn for determination of cyclic GMP and cyclic AMP contents by means of radioimmunoassays. Data represent means of quadruplicate assays and are expressed as concentrations (nmol per liter of cell suspension). The volume of the cell sediment was 1.5% of the total volume. Cellular contents of cyclic GMP (mol per liter densely packed cells) are therefore obtained by multiplication of the plotted values by a factor of 67. The solid line of the cyclic AMP concentration was computed by means of equation 1 (reaction to the steady state) and  $[\text{cAMP}] = [\text{cAMP}]_{\text{ss}} \cdot e^{-k_{\text{h}} t}$  (hydrolysis of cyclic AMP after termination of cyclic AMP supply). The value of  $k_{\text{h}}$  was obtained from the measured steady state concentration of cyclic AMP according to Eq. 2. In the calculations data were corrected for the basal (prestimulation) cyclic AMP concentration. Similar results were obtained in a total of three experiments.

state level. When the addition of cyclic AMP was stopped, the cyclic GMP concentration returned to the prestimulation value. Cyclic AMP input at  $0.5 \text{ nM} \times \text{s}^{-1}$ , which led to a steady state concentration of  $26 \text{ nM}$  cyclic AMP, elicited changes in cyclic GMP concentration qualitatively and quantitatively comparable to those shown in Fig. 4 (data not shown).

Differentiated cells of strain agip 53 responded similarly (Fig. 5). The increase of the cyclic AMP concentration elicited a fast 40-fold increase of the cyclic GMP concentration. The cyclic GMP concentration subsequently declined to a quasi-constant value that was about four times higher than the basal concentration. Cyclic GMP remained at this concentration while cyclic AMP was maintained at the steady state level, and decreased to the basal concentration after the addition of cyclic AMP was stopped.

The maximal cyclic GMP concentration elicited in differentiated agip 53 cells by a cyclic AMP input rate of  $4 \text{ nM} \times \text{s}^{-1}$  (Fig. 5) was sevenfold higher than that induced in early preaggregation cells of strain Ax-2 by an input rate of  $5 \text{ nM} \times \text{s}^{-1}$  (Fig. 4). Previous experiments had shown that cyclic AMP pulses gave rise to higher maximal cyclic GMP concentrations in differentiated cells of agip 53 and Ax-2 than in undifferentiated cells of either strain (33; B. Wurster, unpublished observation).

## DISCUSSION

Continuous addition of cyclic AMP to cell suspensions of *D.*

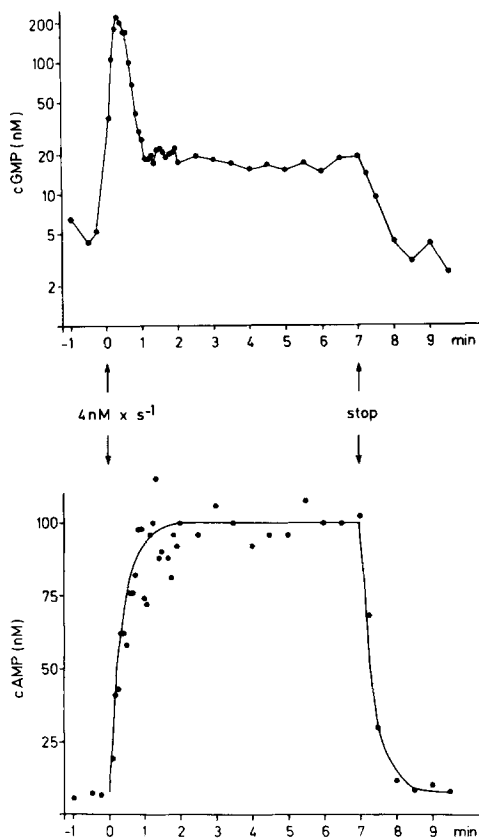


FIGURE 5 Cyclic GMP responses of differentiated agip 53 cells. The cell suspension was continuously supplied with cyclic AMP at a rate of  $4 \text{ nM} \times \text{s}^{-1}$ . At appropriate time intervals, samples were withdrawn for determination of cyclic GMP and cyclic AMP concentrations. Details were as described in the legend to Fig. 4. The volume of the cell sediment was 1% of the total volume. Similar results were obtained in a second experiment.

*discoideum* produced a defined temporal increase of the extracellular cyclic AMP concentration up to a constant steady state value. The cells responded to the initial increase of the extracellular cyclic AMP concentration with a rapid decrease in light scattering and a rapid increase in the intracellular cyclic GMP concentration. These fast reactions to continuous supply of cyclic AMP are similar to those elicited by cyclic AMP pulses (12).

At cyclic AMP input rates of  $0.5\text{--}5 \text{ nM} \times \text{s}^{-1}$  the fast reactions of light scattering and cyclic GMP had already relaxed while the cyclic AMP concentration in the cell suspension was still increasing (Figs. 2B, 4, and 5). This result poses the question: To which aspect of the cyclic AMP signal do the cells respond in the fast initial reaction? Obviously, it is not to the concentration *per se*. Neither do the cells simply react to an increase in cyclic AMP concentration with time, for  $d(\text{cAMP})/dt$  can be positive when the fast initial responses have already relaxed. The possibility that the cells will respond whenever  $d(\text{cAMP})/dt$  exceeds some threshold value is not substantiated by the results described here: In the experiment of Fig. 4, the cyclic GMP concentration increased for 20 s and thereafter decreased. Cyclic AMP was added at  $5 \text{ nM} \times \text{s}^{-1}$  and  $d(\text{cAMP})/dt$  was  $2.8 \text{ nM} \times \text{s}^{-1}$  after 20 s. The cells did not respond to this temporal change, but they did respond to the much smaller change of  $0.5 \text{ nM} \times \text{s}^{-1}$  at the beginning of the experiment with cyclic AMP input of  $0.5 \text{ nM} \times \text{s}^{-1}$ . The data suggest that the response may depend on  $d(\text{cAMP})/dt$  as well as on the background concentration of cyclic AMP. A dependence on both  $d[\text{attractant}]/dt$  ( $dC/dt$ ) and  $C$  has been reported for chemotactic response of bacteria (39).

After the initial fast reaction, the cyclic GMP concentration and light scattering did not return to prestimulation values, but declined to new excited levels and remained at these levels as long as the cyclic AMP concentration stayed constant at the steady state value. Our data indicate that *D. discoideum* cells respond to constant cyclic AMP concentrations with constant elevated cyclic GMP concentrations and constant decreased levels of light scattering. We also observed constant, elevated cyclic GMP concentrations and constant decreased levels of light scattering in response to a high ( $5 \times 10^{-5} \text{ M}$ ) cyclic AMP concentration (Wurster, B., and U. Butz, unpublished observations). Very recently Lappano and Coukell (40) reported that the cells responded to high cyclic AMP concentrations with prolonged, elevated cyclic GMP concentrations. Our data and those of Lappano and Coukell disagree with the conclusion drawn by Gerisch et al. (27) and Rossier et al. (28). In addition, in at least one experiment of Rossier et al. (41) stimulation with a slowly hydrolyzable, cyclic AMP analogue elicited a prolonged light-scattering response. Unfortunately, neither in this experiment nor in the experiment that served as argument for quasi-complete adaptation at the level of cyclic GMP did Rossier et al. (28, 41) measure the hydrolysis rate of their cyclic AMP analogue. We suspect that different phosphodiesterase activities, and hence different lifetimes of attractants, are the main reason for the different results obtained.

The complete response pattern obtained at cyclic AMP rates of  $0.5\text{--}5 \text{ nM} \times \text{s}^{-1}$  can be described phenomenologically as partial adaptation. It is known that some human senses only partially adapt to constant stimuli (e.g. sight) (42) while other senses show quasi-complete adaptation (e.g. taste) (42). Quasi-complete adaptation to constant stimuli has been reported for chemotaxis in bacteria (43) and for cyclic AMP relay in *D. discoideum* (22). The mechanism of adaptation can be desensitization of cell surface receptors or a time-dependent change

of a component of the signal processing pathway. Molecular mechanisms that can account for partial as well as quasi-complete adaptation have been reported (43–45). These mechanisms consider only one type of perception system.

Partial adaptation also can be interpreted in terms of two types of perception systems, one that adapts to constant stimuli and one that does not adapt. The possible presence of two types of cyclic AMP receptors (6) is compatible with two types of perception systems. Recent results obtained in this laboratory indicate that the cyclic-AMP-induced flux of  $Ca^{2+}$  into cells (46) does not adapt to constant cyclic AMP concentrations (Bumann, J., B. Wurster, and D. Malchow, Manuscript in preparation). The occurrence of nonadapting cellular reactions is expected if a nonadapting perception system exists.

Chemotaxis (47, 48), cyclic AMP relay (22, 27), and cell differentiation (29, 49) apparently are triggered by increases of attractant concentration. The question remains: What is the biological function of the cellular reaction to constant attractant concentrations? Constant attractant concentrations regulate the synthesis of cyclic AMP phosphodiesterase and its inhibitor (28, 50–52) as well as folic acid deaminase (53). Constant attractant concentrations may also regulate locomotion. Constant concentrations obviously cannot affect the direction of locomotion, but they could control the speed of locomotion. Chemokinetic responses, which have been observed with leukocytes (54), have so far not been reported in *D. discoideum* or other cellular slime molds. However, Varnum and Soll (55) recently described the existence, in *D. discoideum*, of a "spreading" response that appears to be distinct from the chemotactic response. The "spreading" response may be related to chemokinesis.

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