# RECEPTOR-MEDIATED ADENYLATE CYCLASE ACTIVATION IN *DICTYOSTELIUM DISCOIDEUM*

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## 1. Introduction

Evidence has accumulated that signal transmission between aggregating cells of *Dictyostelium discoideum* is based on the propagation of cyclic-AMP pulses [1–4]. By binding to cell-surface receptors [5–7], cyclic AMP induces the release of cyclic AMP. This response implies a sharp rise of the net synthesis of cyclic AMP, beginning within the first 15 s after stimulation [8]. In a cell layer, pulses are propagated in the form of concentric or spiral-shaped waves [9–13]. The underlying mechanism is signal relay (i.e., amplification of the signal input by the responding cells) [9]. When expressed as the number of cyclic-AMP molecules used for stimulation as compared to the number released in response, amplification factors of one to two orders of magnitude are obtained [4].

Because of the crucial role of adenylate cyclase in signal generation, its regulatory properties are of particular interest [14–16]. Here we report on adenylate cyclase stimulation using the highly sensitive assay of Salomon, Londos and Rodbell [17].

## 2. Methods

*Organisms.* Either clone 206 or 214 of *D. discoideum* strain Ax-2 was cultivated on growth medium containing 1.8% maltose [18]. At cell densities between 3 and 8 X $10^6$/ml, the cells were washed and resuspended at 1 X $10^7$/ml in 17 mM phosphate buffer pH 6.0 [19]. Either 4 or 7 h later the cells were washed once again for use in the experiments.

*Homogenization.* Cells were homogenized for 5 s using a Branson B 12 Sonifier with a microtip. The output was between position 2 and 3; the medium contained 25 mM Tris–HCl pH 7.5, 0.25 mM ATP, 1 mM EDTA, 10 mM dithiothreitol and 250 mM saccharose. For the experiments shown in fig.1, washed cells were directly resuspended in the medium at a density of 2 X $10^8$/ml. For figs.2 and 3, suspensions of 2 X $10^8$ cells/ml in 17 mM phosphate pH 6.0

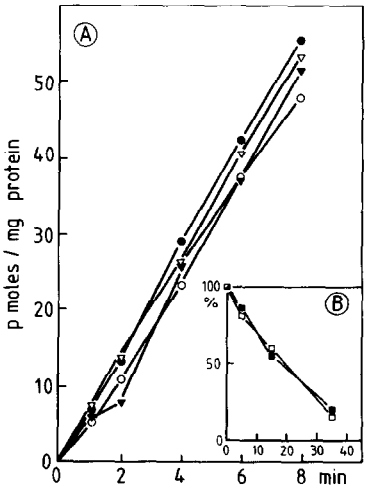


Fig.1. Cyclic-AMP synthesis in cell homogenates. Clone 214 cells were harvested 7 h after the end of growth and sonicated (A) Cyclic-AMP synthesis in assay mixtures containing 2 mM (●) or 1 mM cyclic AMP (○). To the latter, 1 mM 5′-AMP (▽) or 5 mM NaF was added (▼). (B) Degradation by beef-heart phosphodiesterase of the $^{32}P$ product (□) synthesized during 8 min in the presence of 1 mM cyclic AMP (corresponding to (○) in fig.1A). Authentic [$^3H$]cyclic AMP (■) was added to the same sample. Abscissa: Minutes of incubation.



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were mixed before sonication with an equal volume of double concentrated homogenization medium. 94 to 98% of the cells were homogenized.

*Adenylate cyclase assay.* If not stated otherwise, the assay was run at about 23°C with shaking in 25 mM Tris–HCl pH 7.5, 0.5 mM ATP, 2.5 mM phosphoenolpyruvate, 5 mM $MgCl_2$, 1 mM cyclic AMP, either 50 or 160 μg/ml pyruvate kinase, and 10 to 60 μCi/ml of α-[$^{32}$P]ATP (Amersham). The reaction was stopped by adding an equal volume of 1 mM cyclic AMP, 40 mM ATP and 2% SDS [17]. For purification, the method of Salomon et al. [17] was used. The recovery was about 80%. For fig.1, a boiled cell homogenate was used as a blank, and for figs.2 and 3 an unsonicated cell suspension. With both blanks, the background was < 0.0005% of the total radioactivity.

*Degradation by phosphodiesterase.* The adenylate cyclase assay was stopped by boiling for 5 minutes. After centrifugation, [$^{3}$H]cyclic AMP (Amersham) was added. The mixture was incubated at 37°C with 0.33 mg/ml beef heart cyclic-AMP phosphodiesterase (Boehringer Mannheim, 0.25 units/mg). The reaction was stopped and the cyclic AMP purified as described for the adenylate cyclase assay.

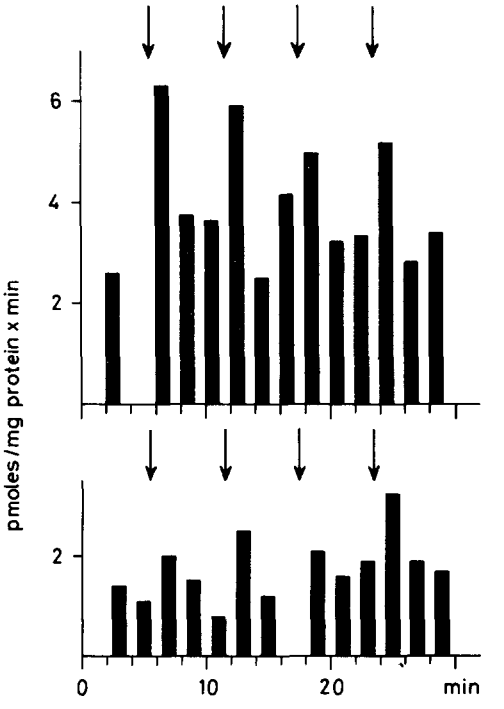


Fig.2. Cyclase activation by cyclic-AMP pulses. Clone 206 cells were harvested 4 hours after the end of growth, adjusted to 2 × $10^{8}$/ml and bubbled with oxygen at 23 to 24°C. At intervals of 2 min, samples were taken for sonication. As indicated by the arrows, pulses of 2 × $10^{-7}$ M cyclic AMP were applied either 30 s (top) or 60 s (bottom) before each third sample. Fifteen seconds after sampling, adenylate cyclase activity in the homogenate was assayed for 1 min.

## 3. Results and discussion

Low but significant adenylate-cyclase activities were obtained in cell homogenates (fig.1A). The phosphodiesterase sensitivity of the product indicates that the method of Salomon et al. [17] gives reliable data on cell-free synthesis of cyclic AMP in *D. discoideum* (fig.1B). This method was therefore used throughout. The cyclase was not activated by 5′-AMP [14] under our conditions, nor was it activated by NaF (fig.1A). The rate of [$^{32}$P]cyclic-AMP increase was similar in 1 mM and 2 mM cyclic AMP, indicating that the lower concentration was sufficient to prevent hydrolysis by phosphodiesterase. 1 mM cyclic AMP was used in all the other experiments.

We wish to show that the cyclase is activated during the generation of a cyclic-AMP signal, as is suggested from the increased net synthesis of cyclic AMP in intact cells. From in vivo measurements on periodically signalling cells it was concluded that strong activation of the cyclase during a pulse would persist not longer than about one minute, which is the time required for the steep increase of the cyclic-AMP concentration up to its sharp peak [8,20]. Spontaneous pulses are formed every six to nine minutes [19]. Therefore, by randomly sampling the cells, the cyclase will be mostly collected in its inactive state. In order to obtain a cell homogenate containing active cyclase, cells were sonicated shortly after stimulation by cyclic AMP, and the adenylate-cyclase activity was determined for 1 min.

Figure 2 shows that after cyclic-AMP pulses cyclase activity was about 2 times the basal level. These values are averages over the first minute after homogenization and not the full amplitudes of initial activation. According to fig.3 the cyclase has returned to near its basal activity by the end of the first minute. In this experiment the initial rate of cyclic-

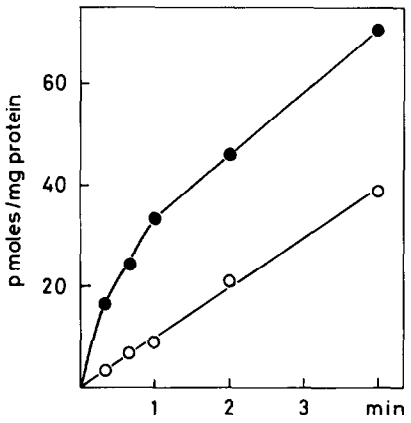


Fig.3. Persistence of the basal activity, and decay of the activated state of adenylate cyclase during the assay. Cells prepared as in fig.2 were sonicated 30 s after stimulation by a pulse of $2 \times 10^{-7}$ M cyclic AMP (•), and transferred within 15 s into the incubation mixture for the assay of adenylate cyclase. For basal activity, the cyclic-AMP pulse was replaced by $2 \times 10^{-7}$ M 5'-AMP (○). Ordinate: Synthesized cyclic AMP. Abscissa: Incubation time in the assay mixture.

AMP synthesis indicates a factor of 5 for cyclase activation.

When cells not previously stimulated by cyclic AMP were used, the initial burst of high-rate cyclic-AMP synthesis did not occur in the cell-free system (fig.3), although the incubation mixture contained unlabelled cyclic AMP for protection of the synthesized cyclic AMP against phosphodiesterase. This means that the absence of de novo activation after transfer of the homogenate into the incubation mixture was not simply due to the absence of the stimulating agent, cyclic AMP.

Together these results indicate that after stimulation of intact cells by a cyclic-AMP pulse, the adenylate cyclase is switched into an activated state which is short-lived but nevertheless still detectable after homogenization of the cells. The activation of adenylate cyclase is important not only for the receptor mediated signal relay, but also in the context of sustained oscillations of cyclic-AMP production. Spontaneous periodicity has been observed in aggregation centers as well as in cell suspensions of *Dictyostelium discoideum* [9,19,20].

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