Localization of Chemoattractant Receptors on Dictyostelium discoideum Cells during Aggregation and Down-regulation

Wang, Mei; Haastert, Peter J.M. van; Devreotes, Peter N.; Schaap, Pauline

Published in:
Developmental Biology

DOI:
10.1016/0012-1606(88)90268-0

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1988

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Localization of Chemoattractant Receptors on *Dictyostelium discoideum* Cells during Aggregation and Down-regulation

Mei Wang, Peter J. M. Van Haastert, Peter N. Devreotes, and Pauline Schaap

*Cell Biology and Genetics Unit, Zoological Laboratory, University of Leiden, Kaiserstraat 63, NL 2311 GP Leiden, The Netherlands; and Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Accepted March 3, 1988

CAMP chemoattractant receptors on the surface of *Dictyostelium discoideum* cells are visualized by means of immuno-cytochemistry. Receptor antigen is virtually absent from growing cells and begins to accumulate after about 6 hr of starvation, concomitant with the increase in surface CAMP binding activity. In aggregating cells, the antigen is uniformly distributed over the cell surface. Persistent CAMP stimulation, which leads to down-regulation of CAMP binding activity, induces a striking rearrangement of receptor antigen into patches or internal vesicles. A similar patching of receptor antigen is observed during tight aggregate formation, when surface CAMP binding activity decreases. These observations indicate that receptor down-regulation involves receptor agglomeration and suggest that receptor down-regulation takes place in vivo, when tight aggregates are being formed.

**INTRODUCTION**

Upon starvation *Dictyostelium discoideum* cells form multicellular aggregates by means of chemotaxis. The chemotactic signal is extracellular CAMP, which is secreted by the cells in a pulsatile manner and is detected by cell surface CAMP receptors. The receptor has been purified to homogeneity, cloned, and sequenced. Its structure appears to fall into a family of receptors which interact with G-proteins and which bear seven transmembrane domains (P. Klein et al., in preparation). Evidence has been presented that *D. discoideum* chemoattractant receptors interact directly with one or more G-proteins (Van Haastert, 1984; Janssens et al., 1985; Theibert and Devreotes, 1986; Van Haastert et al., 1986).

The chemoattractant-receptor interaction evokes a number of transient intracellular responses, such as actin polymerization (McRobbie and Newell, 1983), and the activation of adenylate and guanylate cyclases. These responses are terminated in spite of persistent stimulation by desensitization mechanisms: adaptation and down-regulation (Devreotes and Steck, 1979; Van Haastert and Van der Heijden, 1983; Klein and Juliani, 1977). It has been proposed that adaptation of adenylate cyclase involves a reversible chemoattractant-induced phosphorylation of the receptor, which causes a transition in electrophoretic mobility from $M_r 40,000$ to $M_r 43,000$. (Klein et al., 1985; 1987b; Devreotes and Sherrington, 1985). During down-regulation, a second mode of desensitization, constant CAMP stimuli induce a rapid loss of CAMP binding activity (Klein and Juliani, 1977; Kesbeke and Van Haastert, 1985). The mechanism of receptor down-regulation is not yet known, but the kinetics and dose dependence of this process differ from adaptation and CAMP-induced phosphorylation of the CAMP receptor (Van Haastert, 1987).

A polyclonal antisera was prepared against the *D. discoideum* surface CAMP receptor, purified from membrane preparations by hydroxyapatite chromatography and two-dimensional SDS-PAGE electrophoresis (Klein et al., 1987a, b). In Western blots of *Dictyostelium* membrane proteins these antisera specifically recognize the two bands of $M_r 40,000$ and $M_r 43,000$. In the present study, the antisera was used to study the localization of the CAMP receptor on intact cells during development and during receptor down-regulation by CAMP. In aggregation stage cells, receptors appear to be uniformly distributed on the cell surface; however, ligand-induced receptor down-regulation is accompanied by an apparent agglomeration of CAMP receptors.

**MATERIALS AND METHODS**

*Materials*

Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (CARPITC) was obtained from Nordic Laboratories (Tilburg, The Netherlands), peroxidase-conjugated swine anti-rabbit IgG was from DAKO (Denmark), and $[2,8-^3$H]cAMP (40 Ci/mmol) was from Amersham (UK).
Culture and Incubation Conditions

*D. discoideum* NC-4 cells were grown in association with *Escherichia coli* 281 on glucose-peptone agar (Schaap and Spek, 1984). Cells were freed from bacteria by repeated washing with 10 mM phosphate buffer, pH 6.5 (PB). The cells were either incubated at 21°C on nonnutrient agar (1.5% agar in PB) at a density of 2.5 × 10^6 cells/cm^2 or shaken at 150 rpm and 22°C in PB at a density of 10^7 cells/ml. Shaken cells were stimulated with 2 × 10^{-8} M cAMP pulses at 6-min intervals for 6 hr.

Immunocytochemical Methods

Cells were collected and resuspended to 5 × 10^6 cells/ml in PB. Five microliter droplets of cell suspension were placed on a glass slide and allowed to adhere for 5 min at room temperature. Excess PB was removed and the cells were fixed for 20 min in methanol at 4°C. In some experiments, the cells were covered with a 50- to 100-μm thick layer of 1.5% agarose in PB, before methanol fixation. This technique causes the cells to spread to a very thin layer and strongly enhances the visualization of cellular structures (Yunura and Fukui, 1985). The agarose layer is rinsed off during fixation. After fixation, the slides were washed three times in 0.7% NaCl in 10 mM phosphate buffer, pH 7.4 (PBS), and incubated overnight at 4°C with an antiserum which was evoked in rabbits against the purified *D. discoideum* cAMP receptor (Klein et al., 1987). The serum was either affinity-purified or preadsorbed to growing *D. discoideum* cells (see above). Control antiserum was incubated in 0.03% SDS in the absence of cAMP receptor.

Adsorption of Antiserum to Purified cAMP Receptor

The cAMP receptor was purified to homogeneity by hydroxyapatite chromatography and two-dimensional SDS-PAGE (Klein et al., 1987). One microliter of 200 ng/ml purified receptor in SDS (final concentration 0.03%) was incubated for 16 hr at 4°C with 25 μl of receptor antiserum, which had been preadsorbed to vegetative cells (see above). Control antiserum was incubated in 0.03% SDS in the absence of cAMP receptor.

Assay for Cell Surface cAMP Binding Activity

Cells from different stages of development were resuspended in PB at 10^6 cells/ml. Aliquots (45 μl) of cell suspension were incubated for 45 sec at 0°C with 5 μl of 10^{-7} M [3H]cAMP (20,000 cpm) in 50 mM dithiothreitol. The cells were subsequently separated from the incubation mixture by centrifugation through silicone oil, and the radioactivity of the pellet was measured (Van Haastert and DeWit, 1984). Assay blanks, obtained by including 10^{-4} M cAMP in the incubation mixture, were subtracted.

RESULTS AND DISCUSSION

Localization of Receptors in Aggregation Competent Cells

We first investigated the localization of the chemotactic cAMP receptor in cells which had been stimulated for 6 hr at 6-min intervals with 20 nM cAMP to induce high levels of cAMP receptors. Figure 1A demonstrates that these cells exhibit a diffuse distribution of fluorescence over the entire cell with a relatively high fluorescence intensity at the cell periphery. Such a staining pattern would be expected if the antigen were equally distributed over the cell surface; the tangential view of the plasma membrane at the edge of the cell results in apparently intense fluorescence.

As a crucial control experiment, we preadsorbed the antiserum to cAMP receptor, which had been purified to homogeneity. Cells with high levels of cAMP receptors do not react to this antiserum anymore (Fig. 1B). This indicates that the antiserum, which was not preadsorbed to cAMP receptor (Fig. 1A), reacts very...
FIG. 1. Localization of CAMP receptors in aggregation competent cells. Growing cells were incubated for 6 hr at 10^5 cells/ml in PB; pulses of 2 \times 10^{-8} M cAMP were added at 6-min intervals. The cells were collected and resuspended to 5 \times 10^6 cells/ml; 5 \mu l droplets were placed on glass slides, covered with agarose, and fixed in 100% cold methanol. The slides were subsequently incubated with rabbit antiserum against the CAMP receptor, which was adsorbed to vegetative cells (A) or similarly treated receptor antiserum additionally adsorbed to purified CAMP receptor (B). Cell-associated anti-receptor IgG was visualized by incubation with FITC-conjugated goat anti-rabbit IgG (GARFITC). (A) 600×. (B) 600×.

specifically with the cAMP receptor in methanol-fixed cells.

Developmental Regulation of Receptor Antigen and Surface CAMP Binding Activity

As has been previously demonstrated, cell surface CAMP binding activity is developmentally regulated. It is low in growing cells, increases after a few hours of starvation to reach a maximum during early aggregation, and then decreases as the aggregates are forming tips (Fig. 2) (Malchow and Gerisch, 1974; Henderson, 1975; Green and Newell, 1975; Schaap and Spek, 1984).

This time course was compared to the expression of CAMP receptor antigen (Fig. 3). The intensity of fluorescent staining is low from 0 to 6 hr of starvation but increases strikingly during the next 4 hr. During aggregation (8 to 10 hr of starvation) fluorescent staining is specifically associated with the cell periphery. At 12 hr, when tight aggregates have been formed, the peripheral fluorescence seems to decrease, while some of the antigenic activity becomes localized in patches.

FIG. 2. Developmental regulation of cAMP binding activity. Growing cells were freed from bacteria and distributed over nonnutrient agar plates at a density of 2.5 \times 10^6 cells/cm^2. Every 2 hr, cells were harvested for the assay of cAMP binding activity and prepared for immunocytochemistry. Binding data were expressed as percentages of the maximal level reached during 12 hr of development.
FIG. 3. Developmental regulation of receptor antigen. Cells, developing as described in the legend to Fig. 2, were harvested at 0, 2, 4, 6, 8, 10, and 12 hr of starvation, fixed in methanol, and stained with affinity-purified cAMP receptor antiserum and GARFITC. 600X.

Distribution of cAMP-Receptor Antigen during Receptor Down-Regulation

It was previously shown that the continued presence of cAMP induces a loss of surface cAMP binding activity (Klein and Juliani, 1977; Kesbeke and Van Haastert, 1985). At saturating cAMP concentrations (10 μM) surface cAMP binding activity decreases to 30% of the original activity after about 5 min of incubation. Within the next 20 min no further decrease takes place (Van Haastert, 1987). Little loss of receptor proteins occurs during down-regulation, since down-regulated receptors can be exposed with ammonium sulfate (Van Haastert, 1987), and receptor antigen, detected in Western blots, is not lost (Snaar-Jagalska et al., 1988).

To determine whether cAMP-induced receptor down-regulation affects the distribution of receptors, the localization of the antigen was investigated after treatment of cells with 3 μM cAMP in the presence of 10 mM DTT. After 15 min of cAMP treatment, the antigen was dramatically rearranged. As shown in Figs. 4A and 4B, it appears that the antigen has accumulated into...
patches, while at the same time the amount of antigen at the periphery seems to be reduced. In control cells, which were not stimulated with cAMP, the antigen remained uniformly distributed over the cell periphery (Figs. 4C and 4D).

The concomitant decrease in cAMP binding activity and agglomeration of antigen, which occurs during stimulation of cells with constant cAMP stimuli, also seem to occur during tight aggregate formation in vivo (Figs. 2 and 3). As is the case during receptor down-regulation, a pronounced decrease in cAMP binding activity occurs during tight aggregate formation, which is accompanied by only a moderate loss of receptor antigen (compare Figs. 2 and 3, t = 12 hr). These observations suggest that during tight aggregate formation sufficiently high cAMP levels accumulate to induce receptor down-regulation.

In many systems, the down-regulation of surface receptors is accompanied by internalization of the receptor in clathrin-coated pits (Pastan and Willingham, 1981). This occurs for instance during down-regulation of the EGF receptor in KB cells and fibroblasts (Beguinot et al., 1984; Stoscheck and Carpenter, 1984), during down-regulation of the α-factor pheromone receptor by its ligand in yeast (Jenness and Spatrick, 1986), and during down-regulation of β-adrenergic receptors in frog erythrocytes (De-Maw et al., 1980).

In this study we demonstrate that the chemotactic cAMP receptor is uniformly distributed over the cell surface during the period of chemotactic cell movement and that a dramatic agglomeration of receptors occurs during ligand-induced receptor down-regulation. Further immunoelectronmicroscopic studies will be performed to identify the structural components which are involved in receptor patching and to follow the fate of the internalized receptor.

REFERENCES


McRobbie, S. J., and Newell, P. C. (1983). Changes in actin association with the cytoskeleton following chemotactic stimulation of


