University of Groningen

Cyclic AMP relay and cyclic AMP-induced cyclic GMP accumulation during development of *Dictyostelium discoideum*

Kesbeke, Fanja; Haastert, Peter J.M. van; Schaap, Pauline

Published in:
FEMS Microbiology Letters

DOI:
[10.1111/j.1574-6968.1986.tb01354.x](https://doi.org/10.1111/j.1574-6968.1986.tb01354.x)

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:
1986

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Kesbeke, F., Haastert, P. J. M. V., & Schaap, P. (1986). Cyclic AMP relay and cyclic AMP-induced cyclic GMP accumulation during development of *Dictyostelium discoideum*. *FEMS Microbiology Letters*, 34(1). <https://doi.org/10.1111/j.1574-6968.1986.tb01354.x>

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.

FEM 02372

Cyclic AMP relay and cyclic AMP-induced cyclic GMP accumulation during development of *Dictyostelium discoideum*

(*Dictyostelium* development; cAMP signalling; chemotaxis)

Fanja Kesbeke, Peter J.M. van Haastert and Pauline Schaap

Cell Biology and Morphogenesis Unit, Zoological Laboratory, University of Leiden, Kaiserstraat, 63, 2311 GP, Leiden, The Netherlands

Received 25 October 1985

Revision received and accepted 19 November 1985

1. SUMMARY

Cyclic AMP-induced cAMP and cGMP responses during development of *Dictyostelium discoideum* were investigated. The cAMP-induced cGMP response is maximal when aggregation is in full progress, and then decreases to about 10% of the maximal level during further multicellular development. The cAMP response increases upon starvation, reaches its maximum at the onset of aggregation, and then decreases to about 8% of the maximum level. The dynamics of the post-aggregative cAMP response are in qualitative agreement with the dynamics of the cAMP relay response in aggregation-competent cells.

2. INTRODUCTION

Starving cells of the cellular slime molds aggregate by means of chemotaxis, to form a multicellular structure. This structure performs a series of shape changes, which ultimately lead to formation of a fruiting body. Cell movement in multicellular structures is guided by a small group of cells, which is situated at the apex and is generally called a tip [1]. Considerable evidence has been collected which indicates that the tip functions by

autonomously secreting pulses of the chemoattractant cAMP. cAMP oscillations control the aggregation process of some species, such as *D. discoideum* [2]. The cAMP signal is transmitted by a relay process, a pulse of cAMP induces a pulse of cAMP synthesis and secretion in responsive cells [3]. In other species, such as *Dictyostelium minutum*, this system is first evident after aggregation [4].

The evidence for tip control by cAMP oscillations involves: the ability of tips to attract aggregation competent cells [5]; the observation of absorbance waves, which emanate from the tip [6], the concomitant appearance of absorbance waves, cell surface cAMP receptors and cAMP phosphodiesterase (cAMP-PDE) in *D. minutum* aggregates [4], the partial maintenance of cell surface receptors and PDE after aggregation of *D. discoideum* [7], disorientation of cell movement in slugs after exposure to cAMP, or to caffeine, a cAMP relay inhibitor [8–10], and a transient cAMP-induced cGMP increase in *D. minutum* tip-forming aggregates [11]; the latter response is considered to be involved in chemotaxis [12–15]. The most direct evidence so far is provided by the observation that a cAMP pulse induces transient synthesis and secretion of cAMP in tip-forming *D. minutum* aggregates [16]. The dynamics of the

cAMP relay response have been extensively studied in *D. discoideum* aggregative cells [2], but cAMP relay after aggregation of *D. discoideum* has not yet been demonstrated. It is also not known whether cAMP can induce a transient increase in cGMP after aggregation of this species.

Since the control of morphogenesis and gene expression in the cellular slime molds is primarily studied in the species *D. discoideum* [17], it seems essential to provide direct evidence for the continuation of cAMP relay and the chemotaxis response in this species. We therefore studied the expression of the cAMP relay response and the cAMP-induced cGMP response in *D. discoideum* during development from vegetative amoeba to fruiting body.

3. MATERIALS AND METHODS

3.1. Materials

cAMP, 2'-deoxy-adenosine 3'5'-monophosphate (dcAMP) and dithiothreitol (DTT) were from Sigma. The cAMP assay kit and the cGMP RIA assay kit were obtained from Amersham.

3.2. Organisms and culture method

D. discoideum NC-4 was cultured in association with *Escherichia coli* 281 on glucose peptone agar [7]. Vegetative cells were freed from bacteria by repeated washing with 10 mM Na/K phosphate buffer, pH 6.5 (PB) and distributed on non-nutrient agar (1.5% agar in PB) at a density of 2.5×10^6 cells/cm². Cells were incubated at 21°C in the dark until the required developmental stage was reached. Up to the stage of tip formation, cells were harvested from the plates by aspiration with PB, which caused the dissociation of aggregates into small clumps and single cells. Slugs and culminating structures were carefully rinsed from the plates and sieved through a nylon sieve (pore diameter 20 µm) to remove unaggregated cells.

3.3. Measurement of the cAMP relay response

Suspensions of cells (10^8 /ml) or intact multicellular structures ($2-3 \times 10^8$ cells/ml) were aerated for 10 min. Just before starting the experiment, intact structures were pipetted 3 times through a

pasteur pipette, to break up large clusters into single slugs. Aliquots of 50 µl were stimulated at $t = 0$ min with 5 µM dcAMP and 5 mM DTT in PB (final concentrations), while being shaken continuously [18]. At various intervals, the reaction was stopped by adding 50 µl 3.5% (w/v) perchloric acid (PCA). Samples were neutralized with 25 µl of a 50% saturated KHCO₃ solution and centrifuged for 2 min at $8000 \times g$. The cAMP content of 20 µl of supernatant was assayed with a commercial cAMP isotope dilution assay kit [16,18].

3.4. Measurement of cAMP-induced cGMP accumulation

Intact slugs and culminating structures (2×10^8 cells/ml) were dissociated into small cell clumps and single cells by vigorous aspiration through a Pasteur pipette. Prior to the experiment, the cells were aerated for 10 min. Aliquots of 50 µl cell suspension were stimulated at $t = 0$ s with 0.1 µM cAMP, and after 10 s the reaction was terminated by adding 50 µl PCA. $t = 0$ samples were obtained by adding cAMP and PCA simultaneously.

Alternatively, 1 ml cell suspension was stimulated with 0.1 µM cAMP, and after various intervals, 50 µl of suspension was pipetted into 50 µl PCA. Samples were neutralized as described above, and cGMP was determined in 25 µl of supernatant with a commercial cGMP RIA assay kit.

4. RESULTS AND DISCUSSION

4.1. cGMP response in migrating slugs

Cyclic AMP (1 µM) induced an increase of 2.0 pmol cGMP/mg protein above the basal level of 3.0 pmol cGMP/mg protein (Fig. 1). The cGMP level peaked at 6–9 s, and returned to its basal value within 60 s. The maximum of the response seemed to be reached somewhat faster than was observed in aggregation-competent cells [12,13]. The dynamics of the cGMP response result from the relative activities of guanylate cyclase and cGMP phosphodiesterase (cGMP-PDE). Guanylate cyclase is activated by cAMP [19] and deactivated by an adaptation process [20]; cGMP-PDE is activated by low cGMP concentra-

tions [21]. A shift in the maximum of the cGMP response may occur if the relative activities or the regulation of these enzymes change during development. One of the enzymes involved in the cyclic GMP response, cyclic GMP-PDE, was found to increase considerably after aggregation [22].

4.2. cAMP relay in migrating slugs

Intact slugs were stimulated in suspension with 5 μ M dcAMP and 5 mM DTT. A small increase (10 pmol/mg protein) of the basal cAMP level (5 pmol/mg protein) was observed (Fig. 2). Half maximal accumulation of cAMP was reached after about 1 min and the accumulation stopped after 3 min. This may indicate that the cAMP response has become adapted. Adaptation to the ambient cAMP concentration is a characteristic property of the cAMP relay response in aggregation-competent cells [2,23].

DTT (5 mM) also induced an accumulation of cAMP, which seemed to reach its maximum somewhat slower. A DTT-induced cAMP accumulation was also observed in aggregation-competent cells [24]. This response is probably due to autocatalytic activation of adenylate cyclase by minor amounts of cAMP, which are secreted by the cells and no longer degraded by cAMP-PDE. It could be argued that when DTT is added to the cells, the

Fig. 1. cGMP response in slugs. A suspension of early migrating slugs (18 h of development, 2×10^8 cells in 1 ml PB), was stimulated at $t = 0$ s with 1 μ M cAMP. At 0, 3, 6, 9, 12, 15, 20, 30, 45 and 60 s, 50- μ l aliquots were transferred to 50- μ l PCA. The cGMP content of the samples was measured. Means and SEM of 4 experiments are presented.

Fig. 2. cAMP response in slugs. Aliquots (50 μ l) of a suspension of intact slugs (18 h of development, 3×10^8 cells/ml) were stimulated at $t = 0$ min with 5 μ M dcAMP and 5 mM DTT ■—■, or 5 mM DTT □—□. At the indicated time intervals, 50 μ l PCA was added. The cAMP content of the samples was measured. Means and SEM of 6 or 2 (DTT stimulation) experiments performed in duplicate are presented.

accumulation of cAMP is not due to transient activation of adenylate cyclase, but to basal adenylate cyclase activity and basal cAMP secretion. However, since both the DTT- and dcAMP/DTT-induced cAMP accumulation are terminated after a few minutes, this is not very likely.

Intact slugs are not a very good system for measuring cAMP relay, since not all the cells are reached by the stimulus at the same time. However, we could not obtain reliable results with dissociated slugs. When slugs were dissociated into single cells by BAL/pronase treatment [25], no cAMP accumulation could be induced at all. After mechanical dissociation of slugs into smaller cell clumps and single cells, the cAMP accumulation was often less than in intact slugs or was sometimes completely absent. We therefore continued to use intact slugs and culminating structures.

4.3. cAMP relay and cGMP response during development

Both the cAMP-induced cGMP peak and the dcAMP-induced cAMP accumulation were much lower in migrating slugs than previously reported values for aggregation-competent cells [12,13,18]. The developmental expression of the cAMP and cGMP response is shown in Fig. 3. During starvation, the cAMP-induced cGMP accumulation

Fig. 3. cAMP and cGMP response during development. The different developmental stages were harvested and prepared for measurement of the cAMP and cGMP response as indicated in MATERIALS AND METHODS. (A) cGMP levels after 0 (●—●) and 10 s (○—○) of stimulation with 1 μ M cAMP. (B) cAMP levels after 0 (●—●) and 4 min (○—○) of stimulation with 5 μ M dcAMP and 5 mM DTT. Means and SEM of two experiments performed in quadruplicate are presented.

gradually increased to reach its maximum when aggregation was in full progress ($t = 8$ h). The cGMP response then decreased to 10% of its maximum during tight aggregate formation, and remained approximately constant during further development (Fig. 3A).

The dcAMP-induced cAMP response increased faster than the cGMP response during starvation and reached its maximum 2 h earlier, when aggregation had just started (Fig. 3B). The different developmental profiles of the two responses were found in two separate experiments and were not an artefact due to averaging of the data. Differences in developmental regulation of the cAMP and cGMP response could be a consequence of the

proposed mediation of these responses by different subclasses of cell-surface cAMP receptors. The cAMP response is mediated by rapidly dissociating sites of low affinity, and the cGMP response by slowly dissociating sites of high affinity [26,27]. Green and Newell observed that the complexity of cAMP binding changes during pre-aggregative development: the maximum of low-affinity binding is reached 2 h earlier than the maximum of high-affinity binding [28].

During aggregation a decrease of the cAMP response takes place, and when tight aggregates are formed only 9% of the maximal response is left. During further development the cAMP response does not change significantly. Both the cAMP and cGMP response decrease considerably after aggregation. The decrease in the cGMP response may at least partly be due to the increased activity of cGMP-PDE [22]. Cell surface receptors also decrease after aggregation [7,28,29], which may account for a general decline in responsiveness to cAMP.

We do not know how cAMP levels, measured during stimulation of intact slugs, relate to cAMP concentrations which would result from autonomous cAMP oscillations in the slug. As we argued before in discussing cAMP relay in *D. minutum* aggregates [16], secretion of small amounts of cAMP may yield relatively high cAMP concentrations in the narrow intercellular spaces of the slug. The present and previous report [11,16] demonstrate that the key biochemical components of oscillatory signaling and chemotaxis are present during multicellular development of *Dictyostelium* and may function in the control of morphogenesis.

REFERENCES

- [1] Raper, K.B. (1940) *J. Elisha Mitchell Sci. Soc.* 56, 241–271.
- [2] Devreotes, P.N. (1983) *Adv. Cycl. Nucl. Res.* 15, 55–96.
- [3] Shaffer, B.M. (1975) *Nature* 255, 549–552.
- [4] Schaap, P., Konijn, T.M. and van Haastert, P.J.M. (1984) *Proc. Natl. Acad. Sci. USA* 81, 2122–2126.
- [5] Bonner, J.T. (1949) *J. Exp. Zool.* 110, 259–271.
- [6] Durston, A.J., Cohen, M.H., Drage, D.J., Potel, M.J., Robertson, A. and Wonio, D. (1976) *Dev. Biol.* 52, 173–180.
- [7] Schaap, P. and Spek, W. (1984) *Differentiation* 27, 83–97.
- [8] George, R.P. (1977) *Cell. Differ.* 293–300.

- [9] Brenner, M. and Thoms, S.D. (1984) *Dev. Biol.* 101, 136–146.
- [10] Wang, M. and Schaap, P. (1985) *Differentiation*, in press.
- [11] Schaap, P. and Wang, M. (1985) *Cell. Differ.* 16, 29–33.
- [12] Mato, J.M., Krens, F.A., van Haastert, P.J.M. and Konijn, T.M. (1977) *Proc. Natl. Acad. Sci. USA* 74, 2348–2351.
- [13] Wurster, B., Schubiger, K., Wick, U. and Gerisch, G. (1977) *FEBS Lett.* 76, 141–144.
- [14] Ross, F.M. and Newell, P.C. (1981) *J. Gen. Microbiol.* 127, 339–350.
- [15] van Haastert, P.J.M., van Lookeren Campagne, M.M. and Ross, F.M. (1982) *FEBS Lett.* 147, 149–152.
- [16] Schaap, P. (1985) *Differentiation* 28, 205–208.
- [17] Loomis, W.F. (1982) *The Development of Dictyostelium discoideum*. Academic Press, New York.
- [18] van Haastert, P.J.M. (1984) *J. Gen. Microbiol.* 130, 2559–2564.
- [19] Mato, J.M. and Malchow, D. (1978) *FEBS Lett.* 90, 119–122.
- [20] van Haastert, P.J.M. and van der Heyden, P.R. (1983) *J. Cell Biol.* 98, 347–353.
- [21] van Haastert, P.J.M. and van Lookeren Campagne, M.M. (1984) *J. Cell. Biol.* 96, 709–716.
- [22] Coukell, M.B., Cameron, A.M., Pitre, C.M. and Mee, J.D. (1984) *Dev. Biol.* 103, 246–256.
- [23] Devreotes, P.N. and Steck, T.L. (1979) *J. Cell. Biol.* 80, 300–309.
- [24] Devreotes, P.N., Derstine, P.L. and Steck, T.L. (1979) *J. Cell Biol.* 291–299.
- [25] Takeuchi, I. and Yabuno, K. (1970) *Exp. Cell Res.* 61, 183–190.
- [26] van Haastert, P.J.M. and de Wit, R.J.W. (1984) *J. Biol. Chem.* 259, 13321–13328.
- [27] Kesbeke, F. and van Haastert, P.J.M. (1985) *Biochim. Biophys. Acta*, in press.
- [28] Green, A.A. and Newell, P.C. (1975) *Cell* 6, 129–136.
- [29] Henderson, E.J. (1975) *J. Biol. Chem.* 250, 4730–4736.