EFFECT OF CYCLIC AMP PULSES ON THE SYNTHESIS OF PLASMA MEMBRANE PROTEINS IN AGGREGATELESS MUTANTS OF *DICTYOSTELIUM DISCOIDEUM*

Roger W. PARISH, Sylvia SCHMIDLIN and Monica WEIBEL
Cytology, Plant Biology Institute, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

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1. Introduction

After an initial period of starvation *Dictyostelium discoideum* amoebae migrate towards attracting centres and form aggregates [1]. These aggregates further differentiate into fruiting bodies [1]. The migration of cells is induced by cAMP pulses emitted by the attracting centres [2–6]. Cyclic AMP pulses also regulate the differentiation of cells to aggregation competence in addition to acting as the chemotactic signal [7,8].

A number of biochemical changes associated with aggregation competence (increases in adenylyl cyclase and cellular cAMP phosphodiesterase activities, increase in the concentration cAMP-binding sites, the appearance of EDTA-resistant cell contacts) are thought to occur predominantly in the plasma membrane [9–12]. Experiments with cycloheximide indicated protein synthesis is required for all these changes [13–15].

We are studying the synthesis and disappearance of plasma membrane proteins during differentiation [16–18] and wished to examine which proteins are synthesized in response to cAMP pulses. We have used two aggregateless mutants which do not autonomously produce cAMP pulses [19–21]. However, they are capable of differentiation when cAMP pulses are provided exogenously [19–21].

Although the 4 biochemical changes mentioned above are induced in the mutants by applied cAMP pulses, the synthesis of only one new plasma membrane protein could be detected. This was the glycoprotein involved in the EDTA-resistant cell contacts (contact sites A) [10,22].

2. Materials and methods

Experiments were performed with NC-4 (wild-type) cells grown in liquid medium with *Escherichia coli* [23] and two non-aggregating, 'aggregateless' mutants, Agip 45 and Agip 53 [19–21], grown in axenic medium [24]. When cAMP pulses are applied to Agip 45 cells (a 'group 2' mutant) they induce the formation of aggregates, but these aggregates do not mature and produce fruiting bodies [19–21]. Following a limited-pulse treatment, the cells of Agip 53 (a 'group 3' mutant) are able to form normal fruiting bodies when deposited on solid supports [19–21]. Wild-type cells were washed and plated onto millipore filters over filter pads containing PDF solution [25]. Developing cultures were labelled at various times by adding isotopes (Amersham) to the top of the filter in 20 μl dist. water with a Hamilton syringe [17,18]. The amounts per filter were: 15 μCi [1-^{14}C]acetate and 4 μCi D-[1-^{14}C]glucosamine hydrochloride (both spec. act. 60 mCi/mmol). The mutants were shaken in 5 ml PDF (10^7 cells/ml) and 30 μCi [1-^{14}C]acetate was added at various times. An electronically-controlled peristaltic pump was used to pulse cell suspensions every 7 min with cAMP, giving 10^{-7} M final conc. Controls were not pulsed.

Plasma membrane isolation, SDS–gel electrophoresis, staining for glycoproteins with concanavalin A / peroxidase and gel autoradiography were carried out as in [17,26–28,30].

3. Results

The plasma membrane proteins that incorporated

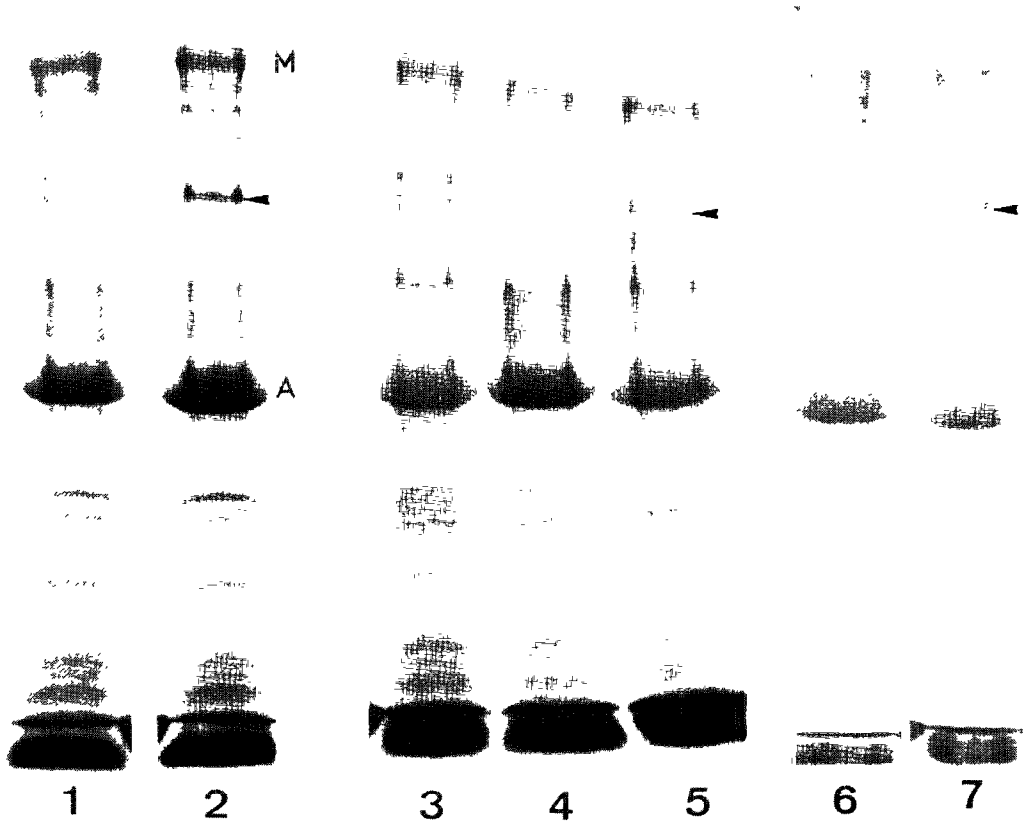

Fig.1. Autoradiographs of SDS–gels of plasma membranes isolated from [^{14}C]acetate labelled suspensions of starved mutants. (1) Agip 45, non-pulsed control, labelled 3–5 h after nutrient removal. (2) Agip 45, cAMP-pulsed and labelled 3–5 h after nutrient removal. (3) Agip 53, non-pulsed control, labelled 3–4 h after nutrient removal. (4) Agip 53, cAMP-pulsed, labelled 1–3 h after nutrient removal. (5) Agip 53, cAMP-pulsed, labelled 3–4 h after nutrient removal. (6) Agip 53, non-pulsed control, labelled 4–6 h after nutrient removal. (7) Agip 53, cAMP-pulsed, labelled 4–6 h after nutrient removal. The arrows indicate newly synthesised contact sites A. (M) Myosin heavy chains. (A) Actin.

[^{14}C]acetate after different shaking times were identified by autoradiography of SDS–gels (fig.1). During the first 3 h no differences were observed between mutants pulsed with cAMP and controls. However, between 3 and 5 h after pulsing had begun a new protein appeared in the plasma membrane of both mutants (fig.1). Otherwise no significant differences between controls and pulsed cells were observed, even when pulsing was continued up to 10 h.

The protein induced by pulsing was detectable on concanavalin A / peroxidase-stained gels and therefore was a glycoprotein (fig.2).

The new glycoprotein had app. mol. wt 82 000–84 000 in 10% gels and corresponded to the 'contact sites A' of wild-type cells [10,17,22]. Labelling experiments using NC-4 wild-type cells show the synthesis of contact sites A commences as cells become aggregation competent (fig.3) and ceases after mid-aggregation [17]. [^{14}C]Glucosamine (fig.3) and [^{14}C]fucose [17] are incorporated into contact sites A.

4. Discussion

The labelling experiments described were performed

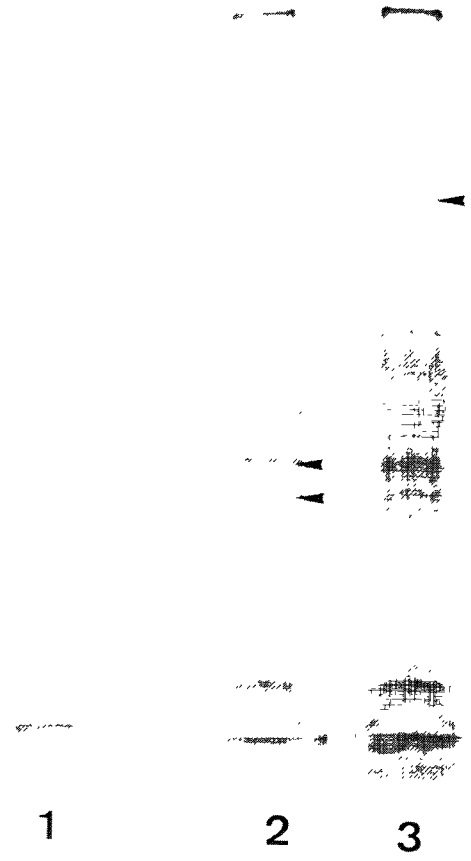

Fig.2. SDS–gels of plasma membranes isolated from suspensions of starved Agip 45 cells and stained for glycoproteins. (1) Non-pulsed control, 3 h after nutrient removal. (Pulsed cells gave the same result.) (2) Non-pulsed control, 5 h after nutrient removal. Arrows indicate two new glycoproteins (which do not, however, incorporate [^{14}C]acetate). (3) Cyclic AMP-pulsed cells, 5 h after nutrient removal. Arrows indicate glycoprotein corresponding to contact sites A.

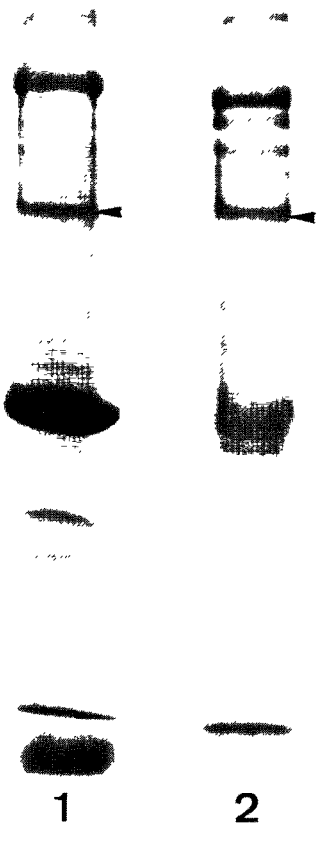

Fig.3. Autoradiographs of SDS–gels of plasma membranes isolated from [^{14}C]acetate and [^{14}C]glucosamine labelled cultures of NC-4 (wild-type) cells. (1) Cultures labelled with [^{14}C]acetate for 2.5 h between acquisition of aggregation competence and early aggregation. (2) Cultures labelled with [^{14}C]glucosamine for 2.5 h between acquisition of aggregation competence and mid-aggregation. Arrows indicate contact sites A. (M) Myosin heavy chains. (A) Actin.

when all 4 biochemical changes related to aggregation competence and associated with the plasma membrane were occurring. Since only the synthesis of contact sites A was detected during pulsing the other 3 changes may reflect recruitment of active molecules from an inactive pool.

In both mutants cAMP pulses induce dramatic increases in the level of cAMP-binding sites and cellular phosphodiesterase activity [19–21], and these two proteins are present on the surface of aggregation competent cells [9,11,29]. The labelling experiments suggest that the synthesis of both proteins occurs either in vegetative amoebae or in starved, non-pulsed mutants, cAMP pulses being required for activation. (Two new low molecular weight glycoproteins did appear in the plasma membrane of control and pulsed mutants after 3–4 h starvation. These glycoproteins did not incorporate radioactive acetate or glucosamine.) Changes in plasma membrane structure [21] may regulate the number of surface cAMP receptors.

In vitro adenylyl cyclase activity increases in non-pulsed Agip 45 cells, although endogenous cAMP

levels are only increased when cells are pulsed [21]. The in vitro adenylyl cyclase activity shows little or no increase in non-pulsed Agip 53 cells, but does increase similarly to wild type when cells are pulsed [21]. The labelling experiments indicate, therefore, that even the increase in in vitro activity (e.g., in non-pulsed Agip 45 [21]) does not involve enzyme synthesis. However, discussion of this point is premature since the postulated plasma membrane location of the adenylyl cyclase is open to doubt (Hintermann and R.W.P., in preparation).

The induction of contact sites A by pulsing has been described in aggregateless mutants (monitored with EDTA) [8,19] and in stationary axenic strains (monitored with EDTA and univalent antibody fragments) [7]. Previous work did not, however, differentiate between activation and synthesis of the glycoprotein.

We have found that the synthesis of contact sites A ceases at mid-aggregation and they are subsequently lost from the plasma membrane of wild-type cells [17,18]. The synthesis of contact sites A appears to be dependent on cAMP pulsing and so the termination of synthesis may reflect the cessation of pulsing in aggregates.

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