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CYCLIC AMP INDUCES THE SYNTHESIS OF DEVELOPMENTALLY REGULATED PLASMA MEMBRANE PROTEINS IN *DICTYOSTELIUM*

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Summary

Dictyostelium discoideum slugs (pseudoplasmodia) were disaggregated and the resynthesis of developmentally regulated plasma membrane proteins examined. The synthesis of the majority of these proteins was inhibited when cells were overlaid with Cellophane and maintained as a monolayer. However, cell contact and movement did occur under the Cellophane. The inhibition of differentiation may result from the inability of the cells to organise specifically into multicellular aggregates. The addition of cyclic AMP (1—5 mM) induced the synthesis of certain developmentally regulated plasma membrane proteins in cells overlaid with Cellophane. Hence, this confirms other work showing that cyclic AMP is required for at least some post-aggregative gene expression. Specific cell organisation and interactions are apparently required for an increase in or maintenance of intracellular cyclic AMP levels.

Signalling between cells in multicellular systems is believed to be important in the regulation and synchronization of activities such as metabolism, growth and differentiation [1—5]. Small molecules, in particular cyclic AMP, are the postulated mediators of intercellular communication [6—8]. The aggregation phase of *Dictyostelium* offers a tractable system with which to study cellular communication and the central role of cyclic AMP has been established [9,10]. Continuous cell contact is required for the subsequent morphogenetic development. Disaggregation of cells halts the programmed increase of certain enzymes [11,12] and the synthesis of specific membrane proteins [13—15,27]. During reaggregation a new round of protein accumulation and synthesis is induced, resynthesis occurring in the same se-

quence and at the same morphological stages as in normally developing cultures [11—15, 27]. Cyclic AMP has also been implicated in the morphogenetic development of *Dictyostelium* [16—22] and we show it is capable of mimicking cell reaggregation in inducing the synthesis of developmentally regulated plasma membrane proteins.

Morphogenetic development of *Dictyostelium* may be prevented by either rapid shaking of cell suspensions [21] or covering a monolayer of cells with Cellophane [19—20]. Cell clumping occurs during fast shaking so we preferred to use Cellophane.

We disaggregated slugs and replated them onto millipore filters over filter pads. Many of the major developmentally regulated proteins of the plasma membrane were then resynthesized (Fig. 1a, b). When cells were overlaid with Cellophane only one of the developmentally regulated proteins was synthesized (Fig. 1c, d). The synthesis of this 110 000 dalton glycoprotein normally begins during aggregation and ceases at the finger stage [13—15]. Long term isotopic labelling suggests it is subsequently lost from the plasma membrane [15]. Hence, disaggregation of slugs induces resynthesis of the glycoprotein.

The presence of 1—5 mM AMP in the filter pads had no effect on the pattern of protein synthesis in cells overlaid with Cellophane (Fig. 1e, f). However, high concentrations of cyclic AMP (1—5 mM) induced the synthesis of three additional developmentally regulated proteins (Fig. 1g, h) and at times (3—6 h after disaggregation) when their synthesis is occurring in reaggregating controls (Fig. 1b). Two are glycoproteins (95 000 and 90 000 daltons) (Fig. 1i) and their synthesis normally commences during early aggregation, reaches a maximum at tip formation and continues throughout the remainder of development [13, 15]. The 55 000 dalton protein is first synthesized at the finger stage in normally developing aggregates [15]. The function of these proteins is unknown, although the 95 000 dalton glycoprotein is the major surface antigen of post-aggregative cells and may replace contact sites A in facilitating cell adhesion [13].

Cyclic AMP did not induce the formation of either spore or stalk cells under the Cellophane. In some *Dictyostelium* strains cyclic AMP has been shown to initiate both stalk and spore cell development, but additional factors are required to produce mature spores and stalks [19, 20]. High cyclic AMP concentrations also mimic the effect of cell contact on the activity of certain developmentally regulated enzymes [21, 22].

How might Cellophane and cyclic AMP be functioning in the system? We found a wide range of cell densities (10^3—$2 \cdot 10^5$ cells$\cdot$cm^{-2}) under Cellophane gave similar results. At the higher densities cell contact certainly occurs, although the surface area involved is obviously less than when multilayer aggregates can form. The simplest explanation for the results is that the reduced cell contact interferes with the release and detection of cyclic AMP by cells. However, this is unlikely as amoebae become aggregation competent under Cellophane [20], implying that cyclic AMP signalling is occurring. Kay et al. [20] reported that starved amoebae plated on agar will aggregate as a monolayer under Cellophane. We also observed some cell movement under the Cellophane.

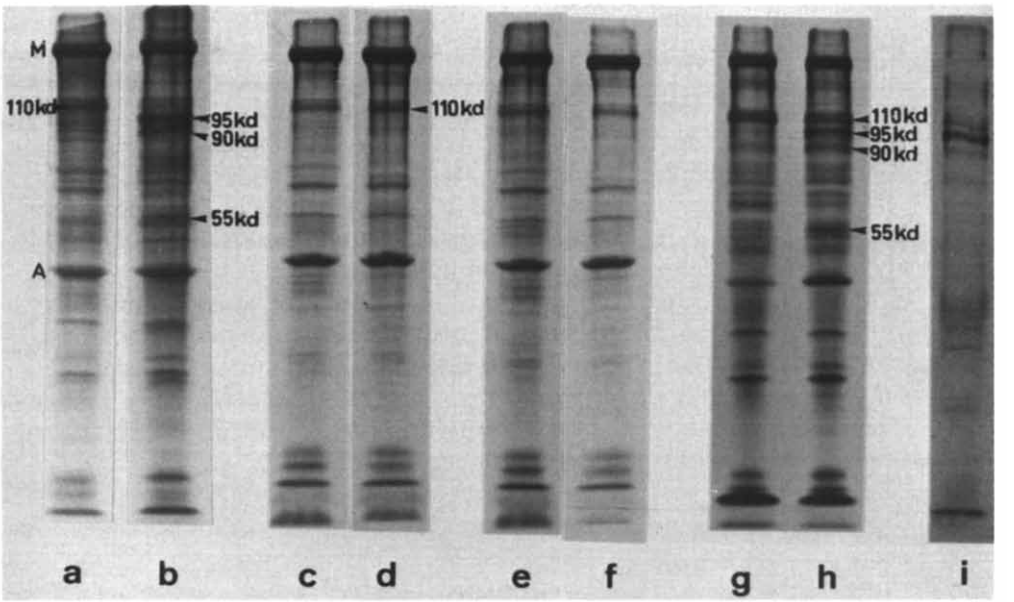

Fig. 1. Autoradiographs of SDS-gels of plasma membranes. *Dictyostelium discoideum* NC-4 (wild type) cells were allowed to develop until the slug (pseudoplasmodial) stage and then disaggregated by passing a number of times through a 10 ml syringe (ONCE, A-Sik, Denmark) in PDF* solution [23]. Cells were plated onto millipore filters ($2 \cdot 10^5$ cells$\cdot$cm^{-2}) over filter pads soaked with PDF solution [23]. Morphological differentiation was prevented by overlaying the cells with a moistened sheet of Cellophane [19]. [1-^{14}C]Acetate (20 μCi) or D-[1-^{14}C]glucosamine hydrochloride (5 μCi) (both 60 mCi/mmol, Amersham) were added to the top of the filters in 20 μl of distilled water via a Hamilton syringe [12—14]. Cells were harvested in PDF buffer and plasma membrane isolated [24, 25]. SDS-gel electrophoresis in 10% acrylamide gels and autoradiography were carried out as previously described [13, 15]. (a)—(h): labelled with [^{14}C]acetate. (a) Control (no Cellophane) labelled from 0—3 h, harvested at tip stage. (b) Control (no Cellophane), labelled from 3—6 h, harvested at early culmination stage. Cellophane, labelled 0—3 h (c) and 3—6 h (d). Cellophane, 1 mM 5′-AMP, labelled 0—3 h (e) and 3—6 h (f). Cellophane, 1 mM cyclic AMP, labelled 0—3 h (g) and 3—6 h (h). (i) Normally aggregating cells labelled with [^{14}C]glucosamine for 2.5 h between the tip and finger stages. Apparent molecular weights of developmentally regulated proteins are indicated. A = actin; M = myosin heavy chains.

Although the reduced number of cell contacts under Cellophane is apparently adequate for aggregation competence and cyclic AMP signalling, many more contacts may be required for the expression of certain post-aggregative genes. On the other hand, Cellophane could be inhibiting the formation of specific cell contacts. Such contacts would presumably only arise when cells are free to form multilayers and cell mixing/sorting out can readily occur. Hence, Cellophane may function by physically preventing the specific organisation of cells in aggregates rather than by inhibiting cell contact per se. This specific organisation may lead to an increase in intracellular cyclic AMP (e.g. via activation of adenylyl cyclase [26]) which in turn induces synthesis of the plasma membrane proteins. The high concentrations of cyclic AMP required in the experiments may be due to inefficient transport into cells and phosphodiesterase activity. Different mechanisms of cyclic AMP signalling might occur between aggregation-phase and post-aggregative cells.

*PDF: 20 mM KCl, 25 mM $MgSO_4$, 0.5 g/l streptomycin sulfate in 0.05 M Sørensens phosphate buffer, pH 6.5.

Specific cell contacts may facilitate the transport of cyclic AMP between aggregated cells. Such contacts would be important, for example, if only a small number of cells in the aggregate synthesize cyclic AMP autonomously. These cells would then provide the cyclic AMP necessary to induce cyclic AMP synthesis in the remaining cells. Adenylyl cyclase of aggregating cells is activated by cyclic AMP pulses but not by cyclic AMP added continuously [26]. It is not known whether cyclic AMP pulsing occurs in *Dictyostelium* aggregates, although induction of the three developmentally regulated proteins (Fig. 1h) was not dependent on pulsing.

The results confirm that cyclic AMP is required for at least some post-aggregative gene expression. Inhibition of specific cell interactions prevents this gene expression, perhaps by preventing the increase in or maintenance of intracellular cyclic AMP levels.

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