A developmentally regulated glycoprotein implicated in adhesion of *Dictyostelium* slugs is predominantly in prespore cells

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Prestalk and prespore cells were isolated from *Dictyostelium discoideum* slugs after [1-^{14}C]acetate labelling and the distribution of developmentally regulated plasma membrane proteins between the two cell types determined. A glycoprotein (M_r 95000) involved in slug cell adhesion, and all the developmentally regulated plasma membrane proteins synthesized during the latter half of development, were predominantly associated with prespore cells. Sorting-out patterns in slugs indicated that cell adhesion is greater between prespore than prestalk cells. The M_r 95000 glycoprotein may be part of the cell recognition and sorting mechanisms involved in fruiting body formation. Actin and myosin synthesis ceased in prespore but not prestalk cells. When slugs were disaggregated, synthesis of these two proteins was reactivated in prespore cells, but synthesis of developmentally regulated plasma membrane proteins was not induced in prestalk cells. Actin and myosin synthesis was activated in prespore cells following disaggregation.

Differentiation *Cell adhesion* *Cell sorting out* Dictyostelium discoideum *Glycoprotein synthesis* *Plasma membrane*

1. INTRODUCTION

A plasma membrane glycoprotein, M_r ~80000, is the target site of adhesion-blocking Fab in aggregating *Dictyostelium discoideum* cells [1–3]. This protein is no longer synthesized after aggregation [4–5] and is substantially degraded [4,6]. A second glycoprotein (M_r 95000) is synthesized from the tip stage until completion of development [5] and appears to be involved in cell adhesion during these later stages [7,8].

We have suggested that the replacement of one adhesion-related protein by a second may be related to sorting out of the two major cell types [7]. Following aggregation, prestalk and prespore cells begin to appear and sorting out is believed to occur so that the prestalk cells are in the anterior 1/3 and the prespore cells in the posterior 2/3 of the slug [9–13]. We postulated that all cells possess the M_r 80000 contact protein during aggregation. It is subsequently replaced by the M_r 95000 glycoprotein and unequal concentrations of this 'contact site' are present on the two cell types. This would result in differential adhesiveness of the prestalk and prespore cells and could assist their sorting out. We have examined this hypothesis by isolating the two cell types and determining the distribution of the M_r 95000 glycoprotein between them.

2. MATERIALS AND METHODS

Growth and differentiation of *D. discoideum* NC-4 (wild type) cells, labelling of cultures with [1-^{14}C]acetate, plasma membrane isolation, SDS–polyacrylamide gel electrophoresis and autoradiography were performed as in [4,5]. Non-aggregated cells were removed from slugs by washing through a Nylon filter. Slugs were dissociated using 0.1% protease (type VI, Sigma), 2,3-dimercaptopropanol in 50 mM Tris–HCl (pH 7.0) and syringing. Prestalk and prespore cells

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were separated using Percoll gradients (20 mM K–K-phosphate, 0.65% NaCl, 2 mM EDTA, pH 6.0; Percoll starting density 1.123 g/ml) centrifuged at 15000 rev./min for 30 min (Beckman JA-20 rotor) [14]; $\leqslant 10^8$ cells could be satisfactorily separated per gradient. Prespore cells were detected using FITC-labelled antiserum against *D. mucuroides* spores. The most dense cells (~1.12 g/ml) were consistently >85% prespore cells whereas the lighter cells (~1.09 g/ml) were 75–80% prestalk cells. The fractions enriched in either of the two cell types were collected, plasma membranes isolated, separated on SDS–PAGE and autoradiographed.

Studies on sorting out of prestalk and prespore cells in shaken liquid cultures involved firstly disaggregating slugs and separating the two cell types as described above. Percoll was removed by washing the cells and the prestalk cells labelled with tetramethylrhodamine isothiocyanate. The two cell types were mixed and allowed to reaggregate in a roller culture (40 rev./min, Multipurpose Rotator, Scientific Industries) in 20 mM KCl, 24 mM $MgCl_2$ and 10 mM EDTA in 0.05 M Sørensen's phosphate buffer (pH 6.5, 10^7 cells/ml). The aggregates were examined using a Zeiss microscope equipped with epifluorescent illumination and filter combination to selectively detect the red fluorescence of rhodamine.

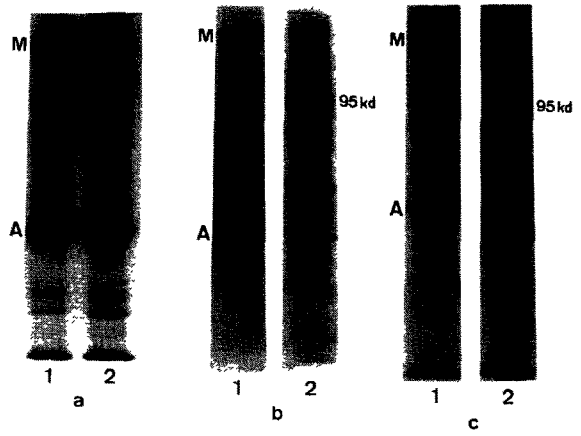

Fig.1. The plasma membranes of prespore and prestalk cells isolated from slugs: proteins incorporating [1-^{14}C]acetate following labelling at different developmental stages. Autoradiographs of SDS gels are shown. Identical protein concentrations of plasma membranes from both cell types were loaded: (1) prestalk cells; (2) prespore cells; (A) actin; (M) myosin heavy chains. (a) Cells labelled from the time of plating-out until late aggregate formation; pulse-chased with unlabelled acetate until slug stage; (b) Cells labelled from tip until slug stage; (c) Slugs disaggregated and labelled during reaggregation; labelled slugs again disaggregated and the two cell types separated.

3. RESULTS AND DISCUSSION

Slug cells were separated into prestalk and prespore cells using Percoll gradients [14]. The slugs were initially dissociated using a protease. However, we were unable to detect hydrolysis of developmentally regulated plasma membrane proteins during dissociation. The cell distribution in gradients which gave good separation of the two cell types was similar to that shown in [14]. At the top of the gradient clumped and partly damaged cells, a mixture of both cell types, were present.

Pulse–chase experiments using radioactive and unlabelled acetate indicated that identical plasma membrane proteins were synthesized in cells destined to become prestalk or prespore cells up to the late aggregation-tip stages (fig.1a). Subsequently, almost no plasma membrane proteins were synthesized in prestalk cells (fig.1b) and the weakly labelled proteins that were detected most probably result from contamination by prespore cells. All of the 'late' developmentally regulated plasma membrane proteins (i.e., tip to culmination stages) [5] were found in prespore cells (fig.1b). The major labelled component was the M_r 95000 glycoprotein believed to be involved in cell adhesion [7,8]. Synthesis of plasma membrane-associated actin and myosin was still active in prestalk cells, but was greatly reduced in prespore cells (fig.1b). We also examined cells from all fractions of the gradient. There was a steady increase in the percentage of prestalk cells with decreasing density and this was reflected in a decrease in the concentration of the M_r 95000 glycoprotein (not shown).

Unlabelled slugs were disaggregated, and allowed to reaggregate in the presence of [1-^{14}C]acetate. Disaggregation switches off the synthesis of slug-specific plasma membrane proteins [15]. Reaggregation results in the resynthesis of most of the developmentally regulated proteins in the same sequence and at about the same morphological

stages as found in normally developing cultures [15]. When the reaggregated cells reached the slug stage they were again disaggregated and the two cell types separated. Once again the developmentally regulated plasma membrane proteins were present in prespore cells, while prestalk cells contained only weakly labelled plasma membrane proteins (fig.1c). During reaggregation, actin and myosin synthesis was switched on in the prespore cells (fig.1c). When cells were labelled prior to disaggregation but not during reaggregation, the labelled plasma membrane proteins were also found associated with prespore cells (not shown).

Hence, during reaggregation of disaggregated slug cells, the two cell types retain their identity; i.e., there is no significant conversion. This was confirmed by labelling prestalk cells with rhodamine before mixing them with prespore cells. Following reaggregation all the fluorescent prestalk cells were present in the anterior region of the slug (not shown). Similar results are obtained when slugs are treated with vital dyes (which darkly stain prestalk cells), disaggregated and allowed to reaggregate on agar [16].

Since the prespore cells contain the majority (and possibly all) of the M_r 95000 glycoprotein, they may cohere more strongly than prestalk cells. When rhodamine-labelled prestalk cells were mixed with prespore cells and shaken in buffer, mixed aggregates were rapidly formed [7]. After 3 h the prestalk cells had sorted out to the periphery of the aggregate (fig.2). According to Steinberg's differential adhesiveness hypothesis [17], this indicates the prespore cells do indeed cohere more strongly than prestalk cells. Moreover, cell–cell adhesion among prespore cells is much more resistant to EDTA dissociation than 10-h cells and prestalk cells [18].

The developmentally regulated differences in the plasma membrane proteins of prestalk and prespore cells may be involved in self-recognition and sorting out of the two cell types. Nevertheless, the latter probably also involves differential chemotaxis. In [19], sorting out of the two cell types was induced by exposing them to an external cAMP source. This reflects a stronger chemotactic response of prestalk cells to cAMP. When disaggregated, slug cells are distributed submerged as a monolayer on agar and not shaken, chemotaxis is important in aggregation. Hence, the first cells to

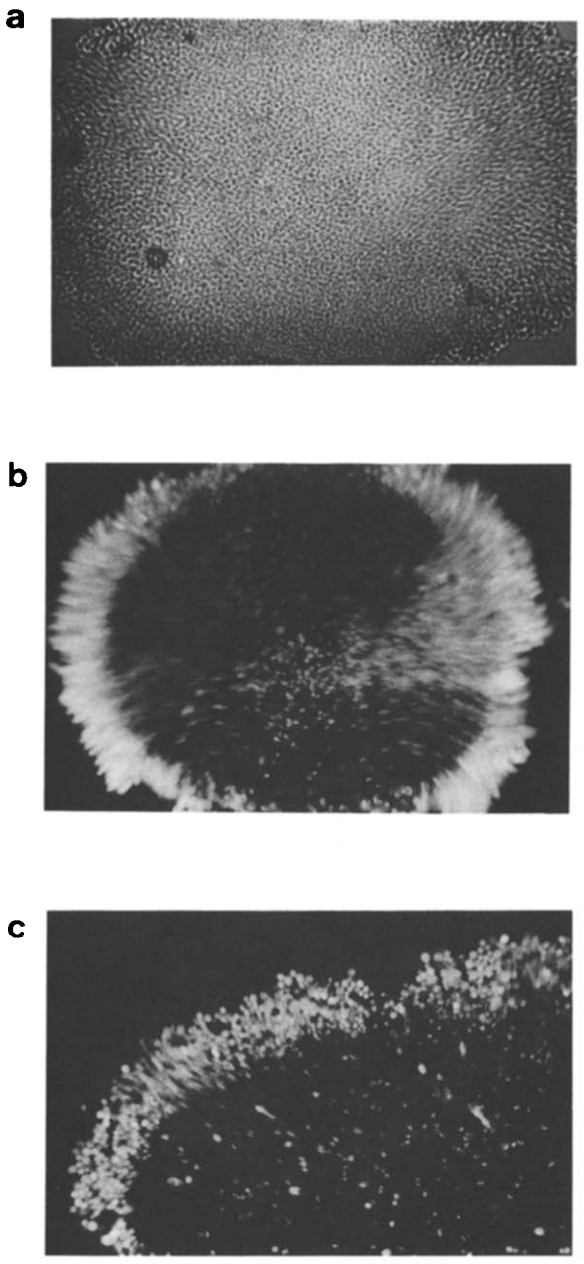

Fig.2. Sorting out of prestalk and prespore cells in shaken liquid culture. Prestalk cells were labelled with rhodamine, mixed with unlabelled prespore cells and allowed to reaggregate in roller culture. Typical 3-h aggregates are shown. Aggregates were partly squashed under the coverslip, and many cells are not in focus: (a) phase-contrast micrograph; (b,c) fluorescence micrographs showing rhodamine-treated prestalk cells.

aggregate are prestalk cells [16]. The synthesis of actin and myosin in prestalk cells may be related to their chemotactic activity in slugs. The adhesive system of prestalk cells apparently does not utilize developmentally regulated plasma membrane proteins and soluble, extracellular molecules may be involved.

In [20] slugs were dissected and 4 glycoproteins found non-uniformly distributed along the slug axis. Two glycoproteins (M_r 110000 and 100000) were associated with the prestalk region, the other two (M_r 80000 and 21000) with the prespore region. They all bound wheat germ agglutinin, a property of the M_r 95000 antigen [8,21]. The discrepancy with our results is difficult to explain, although we were looking at proteins synthesized (i.e., incorporating acetate, amino acids and sugars) [5,7,15] during development. The prestalk proteins in [20] may fail to incorporate these isotopes.

The signals directing cells into the stalk or spore pathway, controlling conversion between prespore and prestalk cells and maintaining correct ratios must either activate or repress the developmental programme leading to the synthesis of 'late' plasma membrane proteins. Hence, the low-M_r, differentiation-inducing factor (DIF) implicated in stalk formation [22,23] would be expected to repress this programme.

Disaggregating prespore cells switched off the synthesis of 'late' plasma membrane proteins, and this programme was reactivated in the prespore cells during reaggregation. However, disaggregation did not activate the programme in prestalk cells, so cell contact is not involved in repression. Actin and myosin synthesis was, however, activated in prespore cells following disaggregation.

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REFERENCES

[1] Beug, H., Katz, F. and Gerisch, G. (1973) J. Cell Biol. 56, 647–658.
[2] Müller, K. and Gerisch, G. (1978) Nature 274, 445–449.
[3] Müller, K., Gerisch, G., Fromme, I., Mayer, M. and Tsugita, A. (1979) Eur. J. Biochem. 99, 419–426.
[4] Parish, R.W., Schmidlin, S. and Parish, C.R. (1978) FEBS Lett. 95, 366–370.
[5] Parish, R.W. and Schmidlin, S. (1979) FEBS Lett. 98, 251–256.
[6] Ochiai, H., Schwarz, H., Merkl, R., Wagle, G. and Gerisch, G. (1982) Cell Diff. 11, 1–13.
[7] Steinemann, C. and Parish, R.W. (1980) Nature 286, 621–623.
[8] Saxe, C.L. and Sussman, M. (1982) Cell 29, 755–759.
[9] Takeuchi, J. (1973) Devl. Biol. 8, 1–26.
[10] Francis, D. and O'Day, D. (1971) J. Exp. Zool. 176, 265–272.
[11] Müller, U. and Hohl, H.R. (1973) Differentiation 1, 267–276.
[12] Forman, D. and Garrod, D.R. (1977) J. Embryol. Exp. Morphol. 40, 229–243.
[13] Durston, A.J. and Vork, F. (1979) J. Cell. Sci. 36, 261–279.
[14] Tsang, A. and Bradbury, J.M. (1980) Exp. Cell Res. 132, 433–441.
[15] Parish, R.W. and Schmidlin, S. (1979) FEBS Lett. 99, 270–274.
[16] Sternfeld, J. and David, C.N. (1981) Differentiation 20, 10–21.
[17] Steinberg, M.S. (1963) Science 141, 401–408.
[18] Lam, T.Y., Pickering, G., Geltosky, J. and Siu, C.H. (1981) Differentiation 20, 22–28.
[19] Matsukuma, S. and Durston, A.J. (1979) J. Embryol. Exp. Morphol. 50, 243–251.
[20] West, C.M. and McMahon, D. (1979) Exp. Cell Res. 124, 393–401.
[21] Keller, T. (1981) Diplomarbeit, University of Zürich.
[22] Kay, R.R. (1982) Proc. Natl. Acad. Sci. USA 79, 3228–3231.
[23] Brookman, J.J., Town, C.D., Jermyn, K.A. and Kay, R.R. (1982) Dev. Biol. 91, 191–196.