RESYNTHESIS OF DEVELOPMENTALLY REGULATED PLASMA MEMBRANE PROTEINS FOLLOWING DISAGGREGATION OF *DICTYOSTELIUM* PSEUDOPLASMODIA

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1. Introduction

Changes in the cell surface properties are considered important in the differentiation of *Dictyostelium discoideum* amoebae to form fruiting bodies [1–7]. We are examining changes in the plasma membrane [8–11] and have found 13 major new proteins, 11 of them glycoproteins, are synthesized at specific developmental stages [12–14].

When developing *D. discoideum* cultures are disaggregated the cells are capable of recapitulating the same sequence of previous morphological changes and in a fraction of the original time [4,15–17]. We show here that when slugs (pseudoplasmodia) are disaggregated, replated and allowed to redifferentiate the majority of developmentally regulated proteins are again synthesised. Synthesis occurs in the same sequence and at approximately the same morphological stages as found in normally developing cultures. A notable exception is the glycoprotein corresponding to contact sites A which is not synthesised during redifferentiation. Hence, 'contact sites A' may only be required for initial cell adhesion and replaced later by other adhesive surface proteins.

2. Materials and methods

Dictyostelium discoideum NC-4 (wild-type) amoebae were grown in liquid medium with *Escherichia coli* according to [18]. Amoebae were used from cultures still containing bacteria by centrifugation and washing in PDF solution [18]. Amoebae were then plated out on millipore filters over filter pads containing PDF solution [19]. Slugs were disaggregated by passing a number of times through a 10 ml syringe (ONCE, A-Sik, Denmark) in PDF solution. The cells were finally syringed once with PDF containing 10 mM EDTA, washed with PDF and again pipetted onto millipore filters.

Labelling of cells was carried out by adding [1-^{14}C]acetate (20 μCi, 60 mCi/mmol; Amersham) in 20 μl dist. water via a Hamilton syringe to the top of the millipore filter.

Plasma membrane isolation, SDS–gel electrophoresis, staining for proteins with amido black and glycoproteins with concanavalin A and peroxidase were carried out as in [10,11,20]. Plasma membranes isolated with digitonin and two-phase separation [7,21,22] gave similar results. Gels were stained, dried and autoradiographed using Fuji R_X medical film. When labelling was weak exposure times were shortened by impregnating gels with PPO in dimethylsulfoxide according to [23].

3. Results

Slugs were disaggregated, placed on millipore filters and labelled at various times with [^{14}C]acetate. The results of two such experiments are shown in fig.1,2.

The myosin heavy chains and, in particular, actin associated with the plasma membrane were again synthesised. In the first experiment (fig.1a) considerable actin synthesis occurred up to the pre-culmination stage, while in the second experiment (fig.2a) synthesis was reduced after tip formation.

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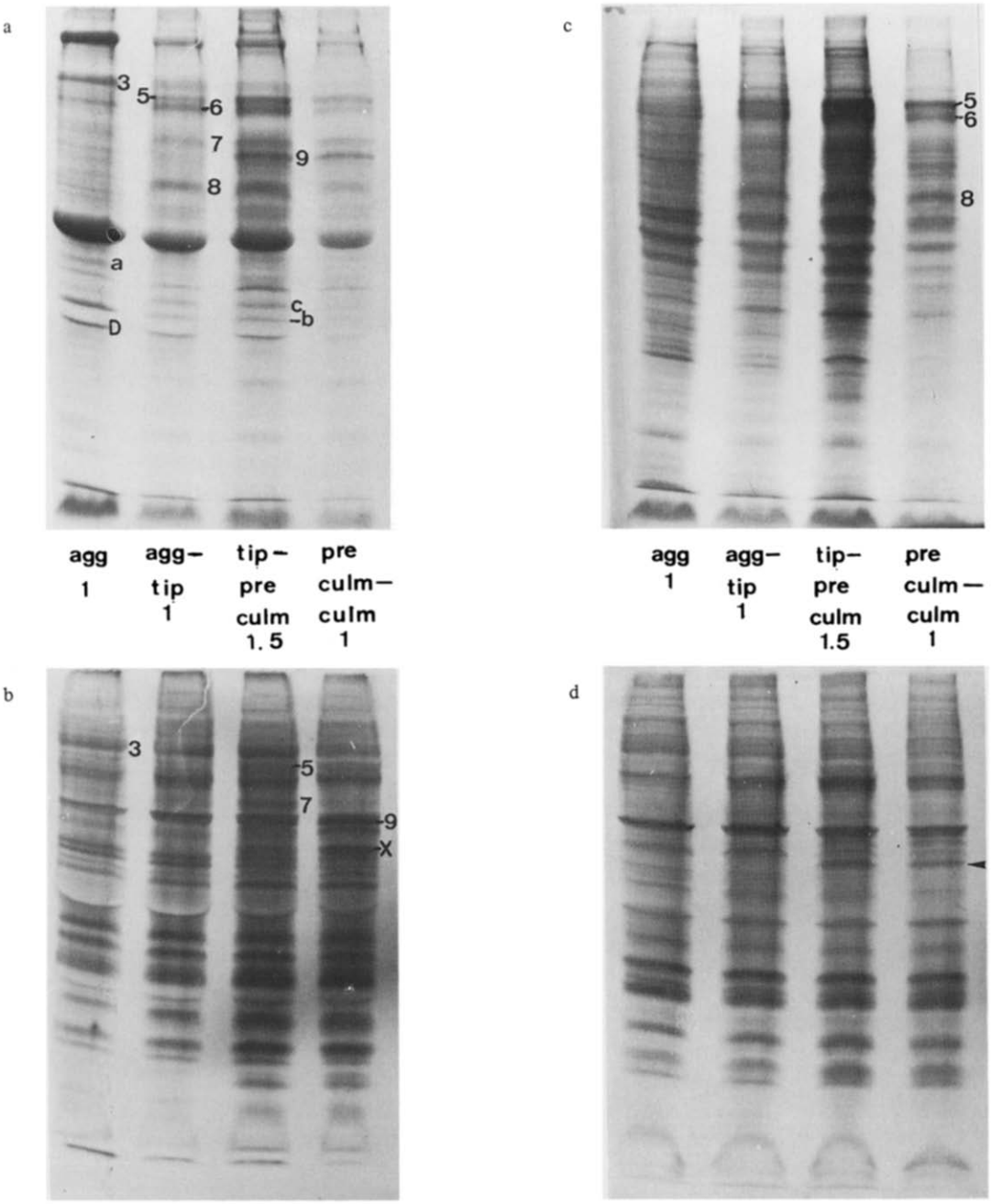


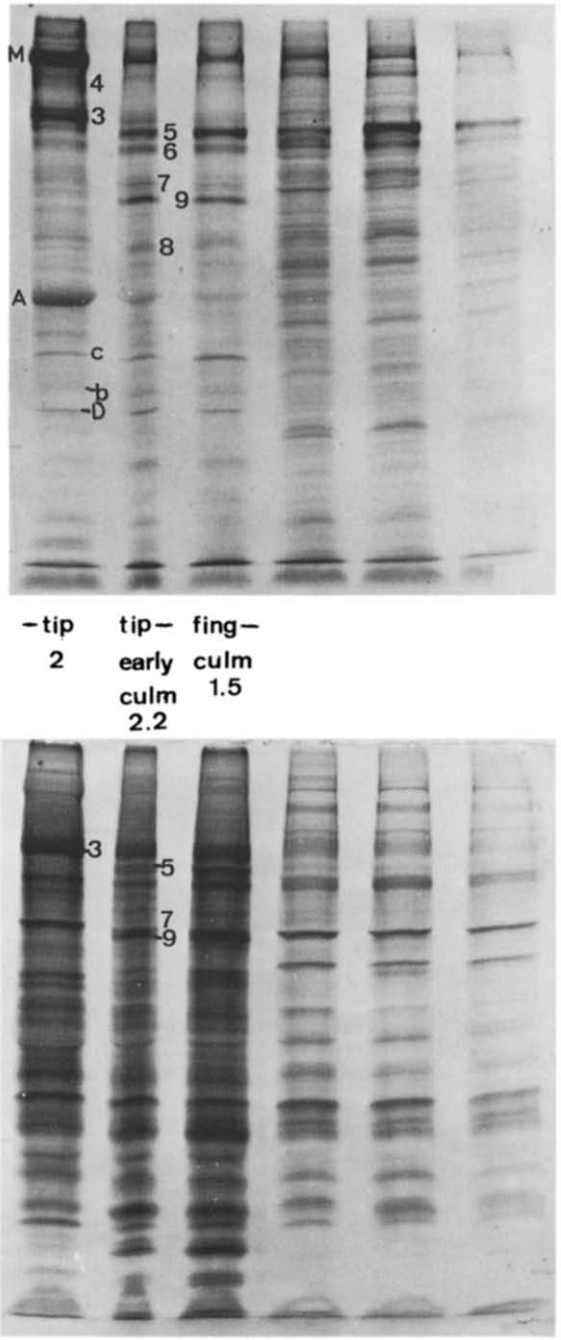
Fig.1. Dodecylsulfate gels of plasma membranes and 'supernatants' isolated from 4 developmental stages following disaggregation and replating of slugs. (a) Autoradiograph of plasma membrane proteins; cells were labelled with [^{14}C]acetate for times (hours) indicated beneath the morphological descriptions. (b) The same plasma membrane samples shown in fig.1a stained for glycoproteins. 'X' indicates a glycoprotein which appears during development but fails to incorporate radioactivity. Glycoproteins readily identifiable with radioactive bands in fig.1a are accordingly numbered. (c) Autoradiograph of 'supernatant' fractions obtained following removal of plasma membranes; labelling as in fig.1a. Plasma membrane bands 5, 6 and 8 are also identifiable in the 'supernatant' fractions. (d) 'Supernatant' fractions stained for glycoprotein; the arrow indicates a glycoprotein which appears during development but fails to incorporate activity (probably identical to protein 'X' found in the plasma membrane). A, actin; M, myosin heavy chains; D, 'discoidin' (tentative identification based on molecular weight); agg, aggregation stage; culm, culmination stage.

Synthesis of myosin heavy chains was maximal during the early stages of reaggregation.

Eleven of the 13 developmentally regulated plasma membrane proteins were labelled during reaggregation (fig.1,2). The appearance of the radioactive bands occurred in the same order (expect band 6 appeared slightly before band 5) and approximately at the same morphological stages as found in normally developing cultures (bands 5–8 seemed to appear a little earlier, before tip formation). The interphase period was more or less non-existent, aggregation beginning immediately after the cells were replated.

The glycoprotein patterns are shown for both experiments (fig.1b,2b), and changes corresponding to radioactive bands 5, 7 and 9 were readily detectable. (Bands 3 and 5 were accompanied by weaker bands immediately above and below them, respectively, on some gels.) Between the tip and pre-culmination stages a new plasma membrane glycoprotein appeared (denoted 'X') which did not correspond to any of the labelled bands (fig.1b).

The patterns of glycoproteins and ^{14}C-labelled bands in the 'supernatants' are shown in fig.1c,d and fig.2a,b. Plasma membrane bands 5, 6 and possibly 8 were detectable in supernatant fractions. Relatively high levels of band 5 were detected before much activity was associated with the plasma membrane (fig.2a).



4. Discussion

The membrane proteins synthesised in normally developing and reaggregating cultures are depicted in fig.3. The speed of reaggregation makes estimation of the precise initiation and cessation times of protein synthesis difficult.

Nevertheless, it can be seen that most of the developmentally regulated plasma membrane proteins are resynthesized following disaggregation of slugs. This

Fig.2. Dodecylsulfate gels of plasma membranes and 'supernatants' isolated from 3 developmental stages following disaggregation and replating of slugs. (a) Autoradiograph of plasma membranes (3 left-hand samples) and 'supernatants' (3 right-hand samples loaded in the same order as the plasma membrane fractions) from cells labelled with [^{14}C]-acetate (times in hours indicated beneath the morphological descriptions). fing, finger stage; (b) The same samples as shown in fig.1a stained for glycoproteins; glycoproteins corresponding to labelled bands are accordingly numbered.

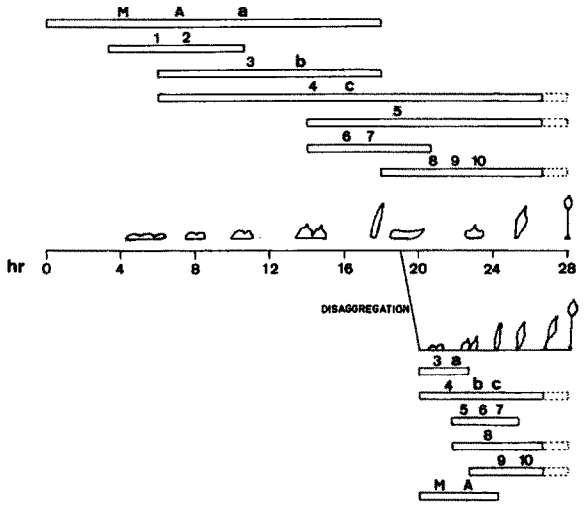


Fig.3. Scheme relating times of maximum synthesis of developmentally regulated membrane proteins to morphological changes in normally developing cultures and reaggregating cultures (following disaggregation). M, myosin heavy chains; A, actin; protein 2 represents 'contact sites A' [12–14,22]. Synthesis at the latter stages of differentiation were not determined (dashed lines) since clean plasma membranes could not be isolated. Except for proteins 8, a, M and A, all proteins shown are glycoproteins [14]. The apparent molecular weights (kd) of the proteins in 10% acrylamide gels were: (1) 132; (2) 82; (3) 110; (4) 155; (5) 95; (6) 90; (7) 76; (8) 55; (9) 69; (10) 210; (a) 37; (b) 28.5; (c) 31; (M) 210; (A) 42.

resynthesis is reminiscent of the behavior of certain enzymes whose accumulation also correlates with specific development stages [4,16,24]. Irrespective of the amount of these enzymes present when cells are disaggregated, they make the full quantum of enzyme associated with differentiation. The disaggregated cells are apparently unable to utilize stored transcripts so that reaggregation switches on again the programmed transcription of the enzyme mRNA which is rapidly translated. The plasma membrane protein changes, like those of the enzymes, are maintained 'in step' with the morphogenetic changes associated with fruiting [4,24].

Reactivation of all parts of the genome initiated during the first round of development does not necessarily occur during recapitulation of the developmental sequence [24]. Synthesis of plasma membrane bands 1 and 2, for example was not detectable during reaggregation. Long-term labelling experiments [14] and immunological methods [12] indicate that these two proteins are lost from the plasma membrane when their synthesis has ceased. We have identified band 2 as 'contact sites A' based on sugar incorporation, molecular weight, time of appearance, high antigenicity and butanol extraction. The loss of 'contact sites A' from the plasma membrane plus their failure to reappear during reaggregation suggests another glycoprotein(s) takes over their role in mediating cell adhesion during the later stages of differentiation. A candidate is band 5, also a highly antigenic glycoprotein [12].

Band 3 also disappears from the plasma membrane at the finger stage of development. However, its synthesis recommences when slug cells are disaggregated. On the other hand, disaggregation inhibits further synthesis of other plasma membrane proteins. The subsequent resynthesis of these proteins appears to depend on specific cell interactions within the aggregate. If the formation of such interactions is prevented by maintaining the disaggregated cells as a monolayer under Cellophane (which does not, however, inhibit cell movement and contact) only band 3, myosin heavy chains and actin are resynthesized [25]. Since cyclic AMP can induce synthesis of the other developmentally regulated plasma membrane proteins when added to the Cellophane-inhibited system, we have suggested specific cell interactions may be important in increasing or maintaining intracellular cyclic AMP levels [25].

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