Antigens reactive with prestalk/prespore specific monoclonal antibodies in *Dictyostelium discoideum*

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We have obtained specific monoclonal antibodies against prestalk and prespore cells of *Dictyostelium discoideum* [(1983) Proc. Natl. Acad. Sci. USA 80, 5340]. The proteins recognized by these antibodies were studied by immunoblotting. Immunoblots with prespore-specific antibodies (SB5,B6) revealed antigen proteins of 105 and 76 kDa for SB5 and of 180, 80 and 73 kDa for B6. Prestalk-specific antibody (C1) reacted against an 84-kDa protein. The developmental kinetics and cell-type specificity of all the antigens agree well with the results of previous immunocytochemical studies.

Dictyostelium discoideum *Monoclonal antibody* *Immunoblotting* *Prestalk* *Prespore* *Cell differentiation*

1. INTRODUCTION

After the vegetative stage, *Dictyostelium discoideum* cells initiate a developmental program which eventually leads to formation of a fruiting body consisting of stalk and spore cells. During this process, cells aggregate to form a slug-shaped cell mass, in which prestalk and prespore cells differentiate.

To study the processes of prestalk and prespore differentiation, we have obtained monoclonal antibodies specifically reactive against either cell type. Immunofluorescent staining with these antibodies showed that cells stained with two prespore specific antibodies (SB5, B6) first appear within a tipped cell aggregate prior to slug formation, confirming the previous results with polyclonal antispore serum [1–4]. By contrast, cells stained with a prestalk specific antibody (C1) appear in the majority of cells at early aggregation and then decrease in number to a level of ~20% by the slug stage [1]. The timing of appearance of these cell-type specific antigens coincides well with that detected by the syntheses of prestalk and prespore specific proteins [5,6] and mRNAs [7,8].

Here, we have tried to identify the proteins reactive with the prestalk and prespore specific monoclonal antibodies using an immunoblotting method.

2. MATERIALS AND METHODS

2.1. *Sample preparation*

D. discoideum, wild type NC4, cells were grown with *Escherichia coli* in a 5LP medium and washed cells allowed to develop on Millipore filters, as previously described [1]. Prestalk and prespore cells were separated from migrating slugs after 18 h of starvation, by a Percoll (80%) gradient centrifugation [9]. Cells were solubilized in SDS sample buffer containing 2% SDS, 20% glycerol, 0.1 M dithiothreitol (DTT), 80 mM Tris-HCl (pH 6.8), at a cell density of 1×10^8 cells/ml, heated to 100°C for 5 min and stored at −70°C.

2.2. *Immunoblotting*

20 μl samples, equivalent to 2×10^6 cells, were electrophoresed on 7.5 or 10% polyacrylamide

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SDS gels [10] and transferred to nitrocellulose filters (Toyo TM2, 0.45 μm) [11]. The filters were then soaked for 30 min in Tris-NaCl-Tween buffer (0.9% NaCl, 0.02% NaN_3, 0.05% Tween 20, 0.1% BSA, 10 mM Tris-HCl, pH 7.4) containing in addition 3% BSA and incubated overnight with the supernatant of hybridoma cultures, at 4°C. After washing 3 times (each for 1 h) with the Tris-NaCl-Tween buffer, they were incubated for 2 h in a moist chamber with 10 ml solution of ^{125}I-conjugated rabbit anti-mouse-IgG (New England Nuclear) which had been diluted to 1–2 μCi/ml with 10 mM phosphate buffered saline (pH 7.2) containing 0.02% NaN_3 and 5% (v/v) normal rabbit serum, washed 4 times (each for more than 1 h) with the Tris-NaCl-Tween buffer and air-dried. Autoradiography was done with an intensifying screen (Fuji Hi-screen) at −70°C.

3. RESULTS AND DISCUSSION

3.1. *Prespore-specific antibodies*

As shown in fig.1, a prespore specific monoclonal, SB5 reacted against proteins of apparent molecular masses of 105 and 76 kDa. They were present in cells collected after 15 h of development, but not before. This is consistent with the results of the previous immunocytochemical studies [1], which also showed that SB5 antigens are mainly localized in the extracellular matrix of prespore cells. It is known that proteins of similar molecular mass on the cell surface (95, 75 kDa) [12] or in the slime sheath (106, 78 kDa) [13] appear at about the same stage as the SB5 antigens.

Antigens which react with another prespore-specific monoclonal, B6 showed some unique characteristics. When the antigens were prepared with SDS sample buffer, no reactant was detected (fig.2, lane 1). By excluding DTT from the buffer, however, three main bands of 80, 73 and 68 kDa, and a faint band of 180 kDa were recognized by B6 (lane 2). They did not appear until the formation of slugs, where they are present in prespores, but not in prestalks (lanes 3–7). Weak bands observed in the prestalk sample may be attributed to unavoidable contamination of 10–20% prespores in the fraction. Disappearance of the antigenic reactivity by DTT suggests that the reactivity of the

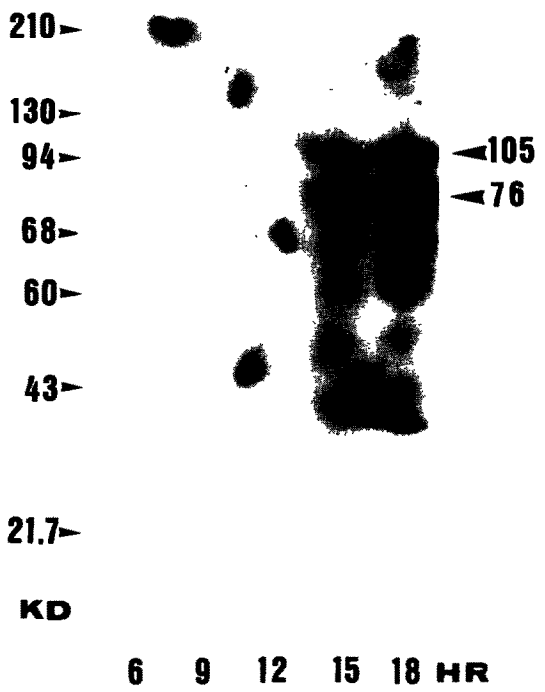

Fig.1. Immunoblotting of developing cells with prespore-specific SB5 antibody. Cells were collected at various stages (after 6–18 h of development) and solubilized in SDS sample buffer. The cell extracts were run on 10% gels, transferred to filters and labeled with SB5 antibody and ^{125}I-antimouse IgG (2 days exposure).

antigens requires the presence of disulfate bonds.

When the antigens were prepared with SDS buffer containing a proteinase inhibitor, phenylmethylsulphonyl fluoride (PMSF) without DTT, the reactivity of 180 kDa was markedly strengthened while that of 68 kDa weakened (fig.2, lane 8,9). Such an effect of PMSF was not observed in the case of the SB5 antigens (not shown). It is most likely that the proteins recognized by B6 are naturally 180, 80 and 73 kDa, while 68 kDa is the product of partial proteolysis of 180 kDa. A previous immunocytochemical study [1] showed that the B6 antigens are localized in cytoplasmic granules which behave identically with prespore-specific vacuoles (PSVs). They seem to be different from spore coat proteins included in the PSVs [14], for they do not constitute spore coat after excretion [1].

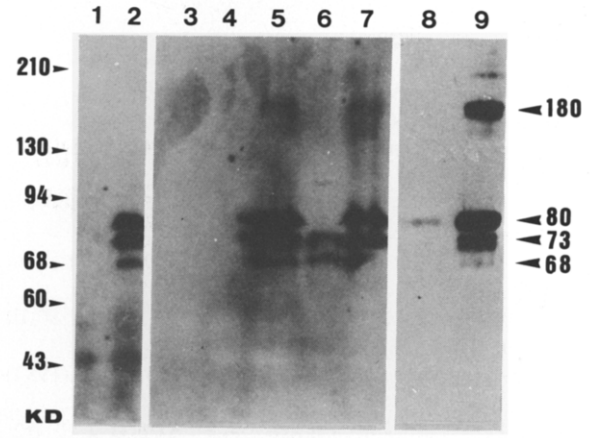

Fig.2. Immunoblotting of developing cells with prespore-specific B6 antibody. Slug cell extracts were prepared with SDS sample buffer with DTT (lane 1) or without DTT (lane 2). The other samples (lane 3–9) were prepared without DTT: vegetative cells (lane 3); aggregative cells, 8 h starvation (lane 4); slug cells, 18 h (lane 5); prestalk (lane 6,8) and prespore cells (lane 7,9) separated from slug. Cells (lane 8,9) were prepared with 1 mM PMSF. Contamination of prespore cells in the prestalk fractions was 22% for lane 6 and 12% for lane 8. 7.5% gels were used (2 days exposure).

3.2. *Prestalk-specific antibody*

Immunofluorescent staining with C1 antibody of aggregative cells appears different from that of slug cells. As shown in fig.3, the former were

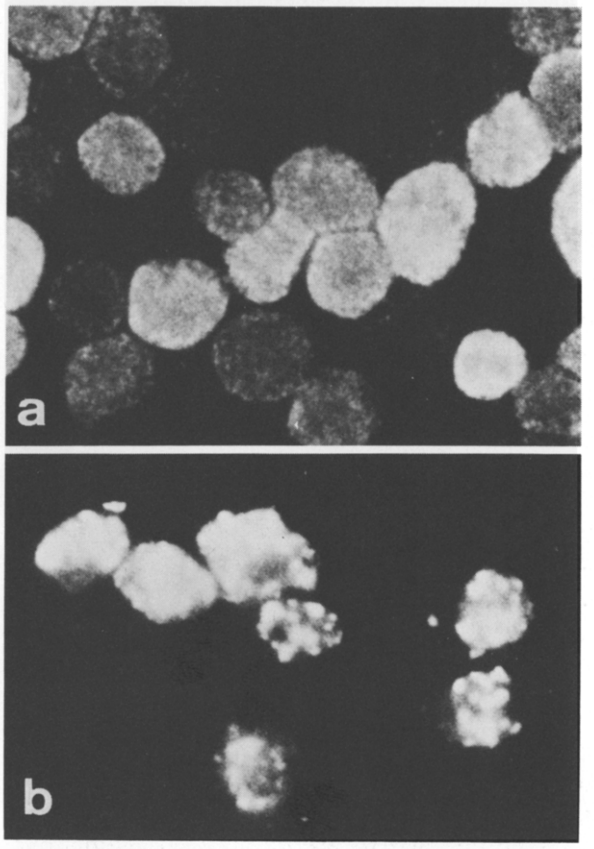

Fig.3. Photomicrographs of immunofluorescent staining with prestalk-specific C1 antibody. (a) Aggregative (8 h) and (b) dissociated slug cells were stained with C1 antibody by the use of FITC-anti-mouse IgG, as previously described [1]. (a, × 1100; b, × 1400).

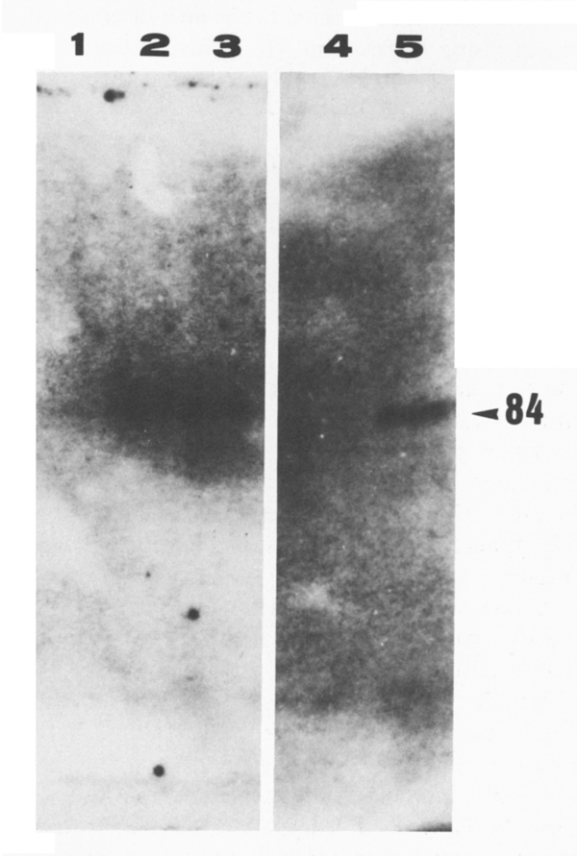

Fig.4. Immunoblotting of developing cells with C1 antibody. Samples were prepared with SDS sample buffer containing 1 mM PMSF and run on 7.5% gels. Vegetative cells (lane 1); aggregative cells, 8 h (lane 2); slug cells (lane 3); prespore (lane 4) and prestalk cells (lane 5) (4–7 days exposure).

stained in numerous very fine cytoplasmic granules while the latter were stained in much larger granules. It was thus questioned whether or not the same antigens react with C1 at both stages.

The reactivity of C1 in immunoblotting was very weak as compared with other antibodies, and occasionally no reaction was observed at all. When the positive reaction was obtained, however, immunoblots constantly showed a protein of 84 kDa. Omission of DTT and PMSF had no effect (not shown). The antigen was present in aggregative and slug cells but not in vegetative cells. At the slug stage, it was found in prestalks, but not in prespores (fig.4). This indicates that the same antigen is reactive in both aggregative and slug cells. Besides, the intensities of the immunoblots for both cells appeared the same, in spite of the fact that the number of stained cells decreased to ~1/5 during slug formation [1]. This indicates that prestalk cells considerably increase the antigen content within a slug.

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