Probing an adhesion mutant of *Dictyostelium discoideum* with cDNA clones and monoclonal antibodies indicates a specific defect in the contact site A glycoprotein

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Communicated by G.Gerisch

Expression of developmentally regulated membrane proteins of aggregating cells of *Dictyostelium discoideum* is subject to several control mechanisms. One of them involves periodic cyclic-AMP pulses as signals for gene expression. To increase the probability of selecting mutants specifically defective in the contact site A (csA) glycoprotein, one of the characteristic proteins of aggregating cells, we have bypassed the requirement for both cyclic-AMP pulses and another control element by two runs of mutagenesis. A 'double bypass' mutant, HG592, was obtained which aggregated in nutrient medium where wild-type did not develop. Mutants defective in expression of the csA-glycoprotein were selected from HG592 by fluorescence-activated cell sorting and colony immunoblotting using a monoclonal antibody specific for that protein. One among 51 csA-negative mutants, HG693, specifically lacked the capability of forming EDTA-stable intercellular contacts. It acquired chemotactic responsiveness and developed into fruiting bodies. Expression of the transcripts for eight developmentally regulated proteins was determined in HG693. Seven of the RNA species were normally expressed; they were recognized by cDNA clones which had been produced from poly(A)$^+$ RNA isolated from membrane-bound polysomes. The single RNA species which was not substantially expressed in HG693 was recognized by a cDNA clone that was obtained by screening a λgt11 library with an antibody specific for the csA-glycoprotein. When probing RNA from wild-type cells, this clone hybridized with a single developmentally regulated RNA species of 1.9 kb whose expression was strongly enhanced by cyclic-AMP pulses. Appearance of this RNA coincided with the expression of the csA-glycoprotein.

Key words: cell adhesion/contact sites A/bypass mutants/cyclic-AMP signals/*Dictyostelium discoideum*

Introduction

During early development of *Dictyostelium discoideum*, cells become capable of producing cyclic-AMP signals and of responding to them by chemotactic orientation (Bonner *et al.*, 1969). In addition, aggregating cells form EDTA-stable intercellular contacts and assemble into streams of elongated cells during their movement to aggregation centers (Beug *et al.*, 1973). The onset of chemotaxis and the acquisition of EDTA-stable adhesiveness are accompanied by the increased expression of a number of membrane proteins such as adenylate cyclase, cyclic-AMP phosphodiesterase, cyclic-AMP receptors and the contact site A (csA) glycoprotein. With the exception of adenylate cyclase, these are plasma membrane proteins exposed on the outer surface. The csA-glycoprotein is an integral membrane protein with an apparent mol. wt. of 80 kd which is expressed at the same time as the cells acquire the capability of forming EDTA-stable contacts (Müller and Gerisch, 1978). Cyclic-AMP pulses stimulate the expression of the above-mentioned proteins. Cells of the AX2 strain of *D. discoideum* produce pulses of cyclic AMP autonomously by periodic activation of adenylate cyclase (Roos *et al.*, 1977) and respond to cyclic AMP via cell surface receptors. In another strain, AX3, the production of cyclic-AMP signals is insufficient to carry on normal development in suspension cultures. In such cultures AX3 cells become fully aggregation competent only if they are stimulated by applied pulses of cyclic AMP (Chisholm *et al.*, 1984; Gerisch *et al.*, 1984). Pulsatile stimuli are required for enhancement of development because the response system adapts to steady concentrations of cyclic AMP, a process which is accompanied by phosphorylation of the cyclic-AMP receptors (C.Klein *et al.*, 1985; P.Klein *et al.*, 1985; Devreotes and Sherring, 1985). Phosphodiesterase is an essential component of the signal system since it degrades cyclic-AMP between the pulses and thus allows the response system to de-adapt. Early development is inhibited in a phosphodiesterase-negative mutant (Brachet *et al.*, 1979) and in wild-type cells which are kept in the adapted state by exposure to a low steady-state concentration of cyclic AMP (Gerisch *et al.*, 1984).

To analyse the system that controls early development up to the aggregation stage, and to investigate the function of proteins whose expression is coupled to that system, we have isolated mutants in which some of the controls are bypassed. Mutants which do not require pulsatile cyclic-AMP signals for their development were isolated by screening mutagenized cells of the AX2 strain on agar containing 3′,5′-cyclic adenosine phosphorothioate (cAMPS) (Rossier *et al.*, 1980). Because cAMPS is a phosphodiesterase-resistant agonist of the cyclic-AMP receptors it inhibits wild-type development, as does a low steady-state concentration of cyclic AMP (Rossier *et al.*, 1978). Mutants which aggregate in the presence of cAMPS were collected and one of them, HG302, was chosen for further analysis (Wallraff *et al.*, 1984). In that mutant the csA-glycoprotein is still under stringent developmental control. This finding suggests that a second control system exists which suppresses the csA-glycoprotein during growth and enables its expression in starved cells. HG302, in turn, was mutagenized and a 'double-bypass' mutant, HG592, was isolated which aggregated in nutrient medium (Gerisch *et al.*, 1985b). Wild-type cells need to be transferred to non-nutrient buffer in order to develop. They do not express the csA-glycoprotein as long as they remain in nutrient medium, even after entering the stationary phase. HG592 expresses the csA-glycoprotein in nutrient medium but not before the end of growth, indicating that there is at least one control step not bypassed in this mutant, and that this step prevents the csA-glycoprotein from being constitutively expressed. We mutagenized HG592 cells for the selection of mutants defective in expression of the csA-glycoprotein by cell-surface labeling with a csA-specific monoclonal antibody and fluorescence-activated cell sorting.

Use of mutagenized HG592 cells for the selection of mutants

defective in certain developmentally regulated proteins prevents part of the pleiotropic mutants from being selected which do not develop because of a defect in some of the regulatory genes. Thus the probability is increased of selecting a mutant which is specifically defective in the csA-glycoprotein. In this paper we describe a mutant obtained from HG592, HG693, in which a defect in EDTA-stable cell adhesion is associated with a defect in csA expression. We have probed the transcripts of eight developmentally regulated genes using cDNA clones and show that HG693 is not generally blocked in development.

Results

Selection of mutant HG693 defective in the csA-glycoprotein

Mutagenized cells of the double bypass mutant HG592 were grown for about four generations in nutrient medium and kept in that medium until non-mutated cells had expressed the csA-glycoprotein on their surfaces. The cells were then incubated with mAb 71, an antibody recognizing an external portion of the csA-glycoprotein, and subsequently labeled with FITC-conjugated anti-mouse IgG. Cells showing weak or no fluorescence were selected by a cell sorter and grown for ~10 generations. After development to aggregation competence the cells were labeled again with antibody and sorted, and were cloned into a lawn of bacteria on nutrient agar plates. Colonies were blotted onto nitrocellulose filters and labeled with [^{125}I]mAb 71. Out of 803 selected clones, 51 colonies, i.e., 6.3%, showed no detectable labeling with the antibody. Except for one clone, these mutant clones showed no aggregation or only loose assemblies of cells which did not develop further. Because development was generally blocked in these mutants we assume that they were defective in regulatory genes whose activities were still essential for development of the progenitor strain, HG592. The only csA-defective mutant selected which showed aggregation and proceeded with development into fruiting bodies was HG693 (Figure 1).

The defect in csA-glycoprotein expression is paralleled by a defect in EDTA-stable cell adhesion

To prove that the entire csA-glycoprotein and not only an epitope on it is lacking in mutant HG693, two monoclonal antibodies produced by independently isolated hybridomas were applied in addition to mAb 71. Evidence has been provided previously that all three antibodies are directed against the polypeptide moiety of the csA-glycoprotein (Bertholdt *et al.*, 1985). Results obtained with mAb 294 are shown in Figure 2. Neither in starved unstimulated cells nor after stimulation by cyclic-AMP pulses for 23 h was the glycoprotein detectable with this antibody, and also with mAb 448 no csA-glycoprotein was detected. In HG592, the parent strain of HG693, the csA-glycoprotein was recognized by both antibodies, as it was with mAb 71. The csA-glycoprotein was already weakly expressed in HG592 cells at the time of their harvest from nutrient medium, in accord with previous results (Gerisch *et al.*, 1985b), and the expression was strongly increased during development (Figure 2). To exclude the possibility that the glycoprotein was degraded during sample preparation by overproduction of a protease in HG693, and also to exclude the presence of any inhibitor of antibody binding in this strain, equal amounts of HG592 and HG693 were mixed and subjected together to SDS-polyacrylamide gel electrophoresis of the proteins. As shown in the last lane of Figure 2 no degradation of the glycoprotein from HG592 was observed in the mixture.

The finding that HG693 was not generally blocked in development prompted us to determine cell functions affected in coincidence with the defect in csA-glycoprotein expression. Figure 3 shows that aggregation-competent cells of HG592 formed EDTA-stable contacts, whereas the cell-to-cell adhesion of HG693 was strongly sensitive to 10 mM EDTA. Figure 3 further shows that HG693 cells acquired the elongated shape typical of the aggregation stage. Another developmentally regulated cell function involved in cell aggregation is the chemotactic reponse to cyclic AMP (Bonner *et al.*, 1969). Figure 4 shows that cells of HG693 developed chemotactic responsiveness to this attractant.

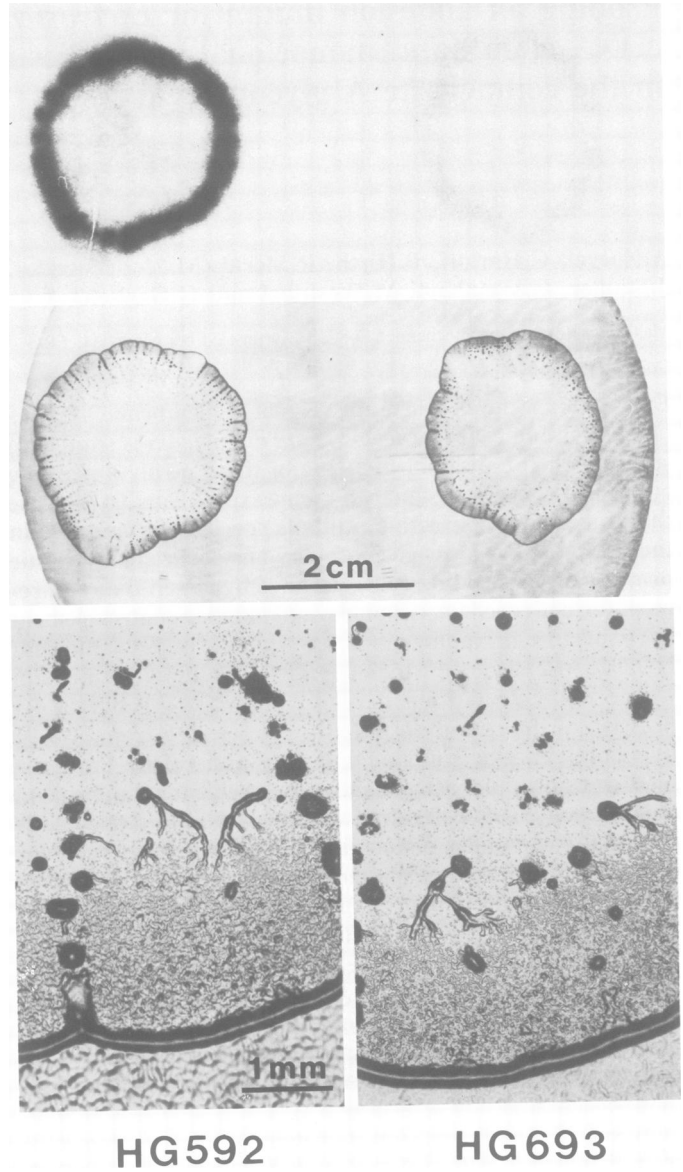

Fig. 1. Colony blots and aggregates on agar plates of the double bypass mutant HG592 **(left panel)** and the csA-defective mutant HG693 **(right panel)**. Top: autoradiogram of a colony immunoblot labeled with [^{125}I]mAb 71. Only the aggregates of HG592 are strongly labeled. Middle: proteins of the same blot stained with Ponceau S. Bottom: aggregates and fruiting bodies.

Normal expression in HG693 of seven out of eight developmentally regulated RNA species tested

Developmentally regulated membrane proteins play key roles in the cyclic-AMP signal system and in cell adhesion of aggregating cells. We have therefore isolated cDNA-probes for appropriate genes and their transcripts. One strategy used included the cloning

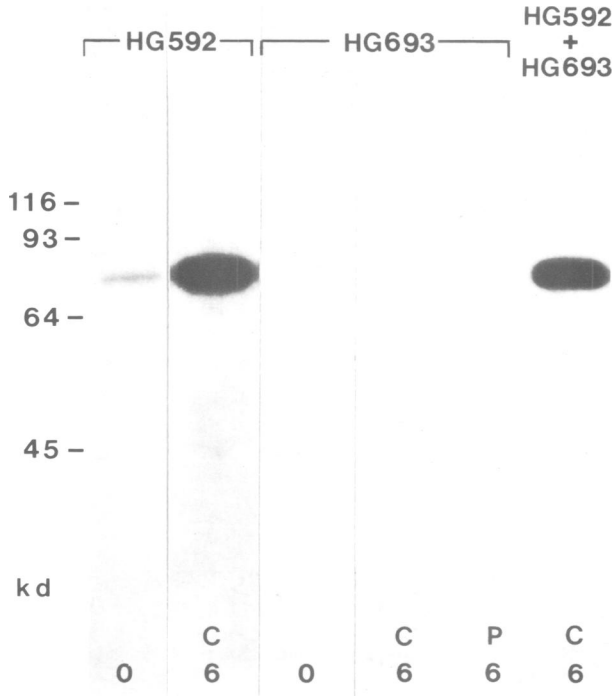

Fig. 2. Autoradiogram of immunoblotted proteins separated by SDS-polyacrylamide gel electrophoresis. Cells of mutants HG592 or HG693 were harvested either from nutrient medium (0) or after 6 h of starvation (6) with (P) or without (C) stimulation by pulses of cyclic AMP. Total cellular protein equivalent to 1×10^6 cells was applied per lane; in the last lane the equivalent of 5×10^5 cells of each mutant was applied.

of poly(A)$^+$ RNA from membrane-bound polysomes of cells harvested at the 6-h stage of development, the beginning of aggregation competence. The cDNA library was differentially screened with DNA complementary to poly(A)$^+$ RNA from membrane-bound polysomes of the 6-h stage of development and with DNA complementary to total cytoplasmic poly(A)$^+$ RNA from growth phase cells. Seven different cDNA clones which hybridized preferentially or exclusively with the probe from the 6-h stage were used in the study presented here. A second strategy was to screen a cDNA library in the λgt11 expression vector with antibodies specific for the polypeptide backbone of the csA-glycoprotein. Here we have used clone λc523 coding for a polypeptide recognized by mAb 294. Northern blots of minigels indicate that the stringency of regulation during early development of the AX2 strain varied between the RNA species (Figure 5). In the bypass mutant HG592 all RNA species were expressed at the 5-h stage, as they were in the AX2 wild-type strain, and five of them were already strongly expressed when the cells were harvested from nutrient medium. This observation reflects the bypassing of developmental controls in HG592.

In mutant HG693 seven of the RNA species accumulated to similar levels as in the AX2 and HG592 strains. The only RNA species that behaved differently was that recognized by μc523. Only insignificant labeling was obtained with RNA of the mutant under conditions which gave a strong signal with RNA from aggregation-competent cells of the AX2 and HG592 strains.

The recognition by mAb 294 of λgt11-hybrid phages carrying c523 DNA suggested that this insert contained part of the coding sequences for the csA-protein. This assumption was supported by the stringent developmental regulation of the transcripts. Since the csA-glycoprotein is known to be weakly expressed in suspension cultures of starved AX3 cells and to be drastically induced

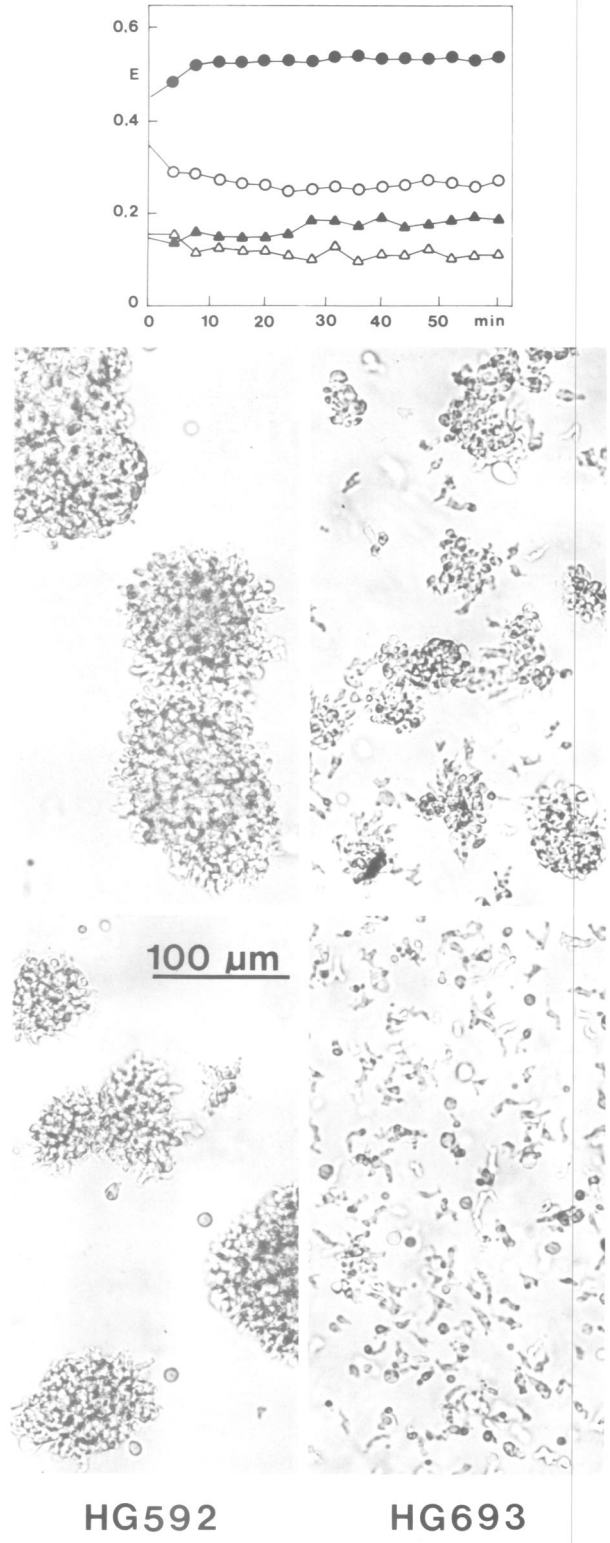

Fig. 3. Cell agglutination in mutants HG592 and HG693. Top: record of light scattering monitored in an agglutinometer. Ordinate: light scattering values E are arbitrary units; low values indicate that cells have formed agglutinates. Abscissa: time in min after transfer of the cells to the agglutinometer. Cells of mutant HG592 (△,▲) and HG693 (○,●) were harvested at 6 h of starvation in 17 mM phosphate buffer, pH 6.0. During starvation the cells had been stimulated by pulses of cyclic AMP. Subsequently the cells were washed and subjected to the agglutinometer either with 10 mM EDTA (▲,●) or without EDTA (△,○) added to the phosphate buffer. Middle: photographs of cells and agglutinates of HG592 (left) and HG693 (right) after 1 h without EDTA in the agglutinometer. Bottom: the same with 10 mM EDTA.

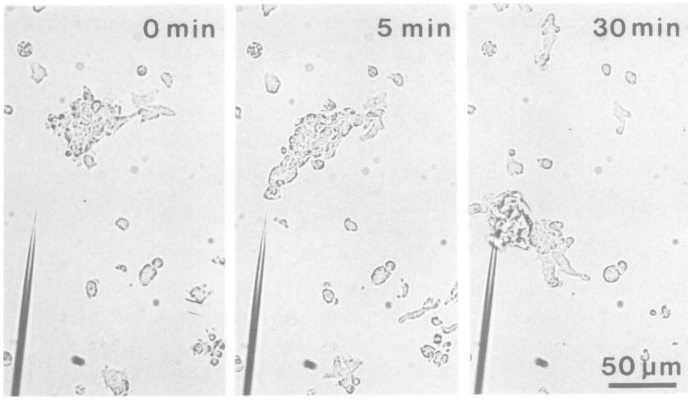

Fig. 4. Chemotactic response of HG693 cells to cyclic AMP. The cells were stimulated by a micropipette filled with 10^{-3} M cyclic AMP, and photographs were taken at the times indicated after positioning of the micropipette.

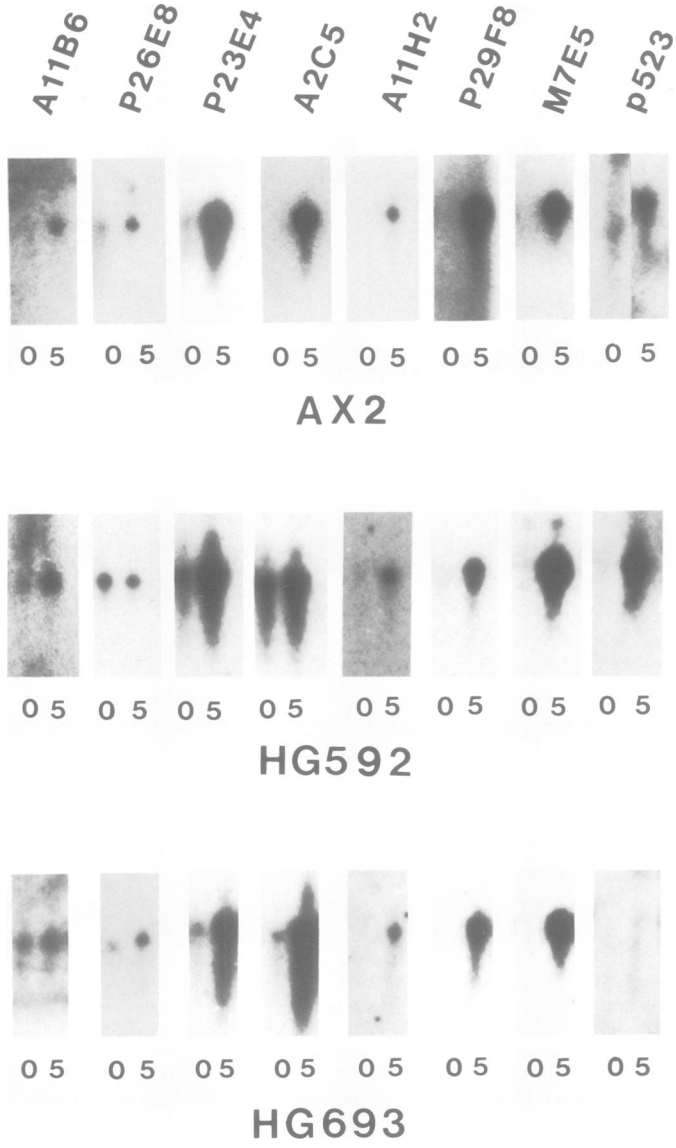

Fig. 5. Northern blots of total cytoplasmic RNA of *D. discoideum* strain AX2 (top), HG592 (middle) and HG693 (bottom). The cells were harvested from nutrient medium (0) or after 5 h of starvation (5), and the RNA was hybridized with eight different cDNA probes as indicated.

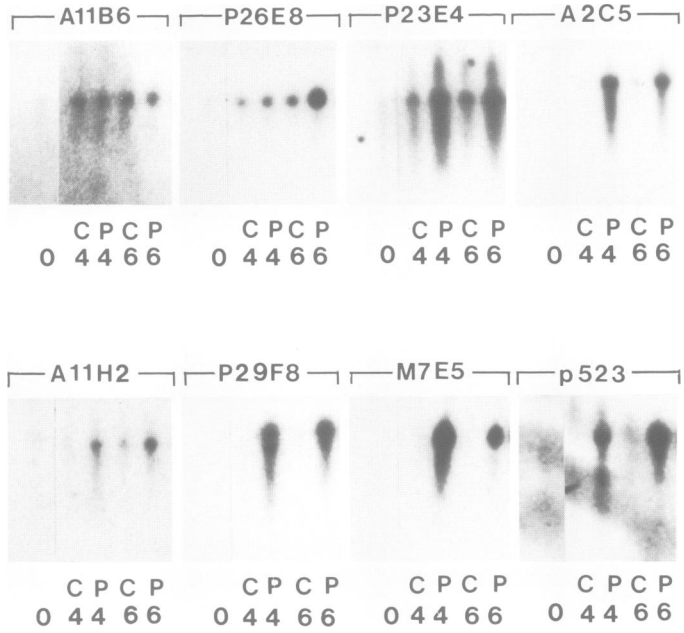

Fig. 6. Northern blots of total cytoplasmic RNA of *D. discoideum* strain AX3. The RNA was hybridized with the same cDNA probes as in Figure 5. The cells were harvested from nutrient medium (0) or after starvation for 4 h (4) or 6 h (6) with (P) or without (C) stimulation by pulses of cyclic AMP.

in these cells by pulses of cyclic AMP (Gerisch *et al.*, 1984, 1985b), we have examined regulation of the RNA species, including the one hybridizing with p523, in stimulated and unstimulated AX3 cells (Figure 6). As in the AX2 strain, in the AX3 strain differences were observed among the RNA species in the stringency of their developmental regulation (Figure 6). For example, RNA recognized by clone A11B6 was already substantially expressed in growth phase cells and steadily accumulated during development, while RNA species hybridizing to P29F8, M7E5 or p523 were not detectably expressed during growth and accumulated only slightly during the development of unstimulated cells. The stimulation of expression of these RNA species by cyclic-AMP pulses was correlated with the stringency of their regulation. Expression of the RNA hybridizing to clone A11B6 was only slightly enhanced by the cyclic-AMP pulses, whereas the RNA hybridizing to clone p523 was drastically induced by the stimulation.

Properties of cDNA clone λc523 isolated by labeling plaques with the csA-specific mAb 294

Phage λc523 carried a 1.3-kb insert, c523, that recognized in Southern blots of DNA from the AX2 strain one *Eco*RI fragment of 6.8 kb and two *Hind*III fragments of ~4.9 kb and >10 kb, respectively. Northern blots showed that the c523 DNA hybridized with a single RNA of 1.9 kb. Figure 7 shows more precisely than Figure 5 that the RNA recognized by c523 was not detected during growth and was strongly expressed in AX2 cells at 5 h of starvation (Figure 7). Only a faintly labeled band was seen with RNA of HG693 cells that were starved for 5 h with or without stimulation by cyclic-AMP pulses. The RNA recognized by c523 might be slightly larger in HG693 than in AX2.

Discussion

The goal of the work presented here was to relate a defect in the expression of the csA-glycoprotein to a failure of a specific

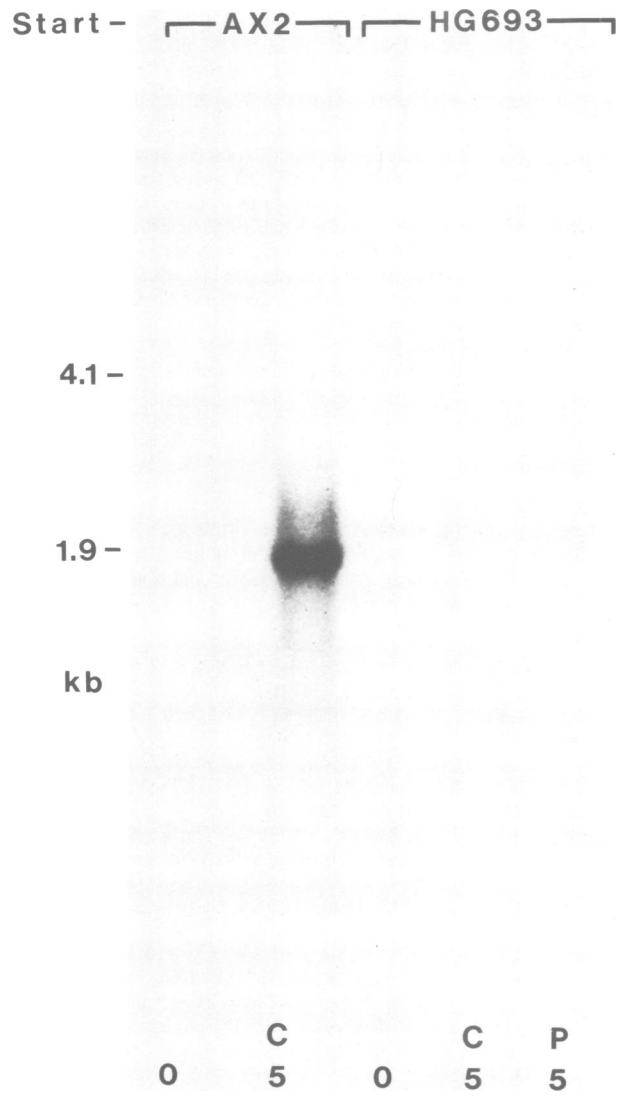

Fig. 7. Northern blot of total cytoplasmic RNA from AX2 and HG693 cells harvested either from nutrient medium (0) or after 5 h of starvation (5) with (P) or without (C) stimulation by pulses of cyclic AMP. The location of rRNAs (4.1 kb and 1.9 kb) is indicated.

cell function. The large number of regulatory genes involved in *D. discoideum* development makes it a tedious task to select structural gene mutants for proteins which are, like the csA-glycoprotein, under the control of these genes. Previous attempts to select csA-defective mutants from mutagenized wild-type cells yielded only pleiotropic mutants whose development was blocked prior to the expression of the csA-glycoprotein. Mutants selected by use of a carbohydrate-specific antibody (Murray *et al.*, 1984), were partially defective in glycosylation but expressed the csA-protein (Gerisch *et al.*, 1985a).

In the present study a double bypass mutant was used as a progenitor, and one mutant was obtained which appeared to lack specifically the csA-glycoprotein. Since this mutant, HG693, showed chemotaxis to cyclic AMP and underwent development beyond the aggregation stage it did not suffer from a defect in the overall control of development. The inability of aggregating cells of HG693 to form EDTA-stable cell contacts is in accord with previous findings indicating that EDTA-stable cell adhesion is blocked by Fab fragments from antibodies directed against the csA-glycoprotein (Müller and Gerisch, 1978). The ability of the mutant to form fruiting bodies agrees with other findings according to which the csA-glycoprotein is degraded during the multicellular stage following aggregation, suggesting that the function of this glycoprotein is limited to the aggregation stage. A question that remains to be answered is the relationship of EDTA-stable adhesiveness to the formation of streams by cells aggregating on a substratum. Previous studies have shown that the end-to-end adhesion of streaming cells is EDTA stable (Beug *et al.*, 1973). On the other hand, long streams are formed by a mutant exhibiting a defect in carbohydrate synthesis. This mutant produces greatly reduced amounts of a partially glycosylated csA-protein and accordingly exhibits weaker EDTA-stable adhesiveness (Murray *et al.*, 1984; Gerisch *et al.*, 1985b). HG693 also forms streams. Further work must clarify whether this stream formation is due to the EDTA-sensitive type of cell adhesion which is independent of the csA-glycoprotein, whether small amounts of the csA-glycoprotein not detected by the immunoblotting procedure are synthesized in the mutant, or whether weak EDTA-stable cell adhesion barely detected by the agglutinometer assay used can be mediated by cell surface components other than the csA-glycoprotein.

Examining the expression of eight different developmentally regulated poly(A)$^+$ RNA species showed that only one RNA was strongly suppressed in mutant HG693. The cDNA clone λc523 which recognized the suppressed RNA was selected from a λgt11 library by labeling plaques with mAb 294, an antibody highly specific for the polypeptide moiety of the csA-glycoprotein (Figure 2). Compared with the RNA species recognized by the other seven cDNA probes, which varied in the stringency of their developmental regulation, the RNA hybridizing to clone λc523 belonged to the most stringently regulated ones. Both this transcript and the csA-glycoprotein were undetectable in growth phase cells and were expressed in AX2 cells at 5 h of starvation. The RNA remained almost unexpressed in starved cells of strain AX3 cultivated in suspension and became strongly expressed in these cells after stimulation by pulses of cyclic AMP, in coincidence with expression of the csA-glycoprotein (Gerisch *et al.*, 1984, 1985b). Together, these results suggest that clone λc523 contains an insert homologous to the coding region of the csA-protein gene. The size of the RNA of 1.9 kb recognized by this insert is sufficient to code for a protein of mol. wt. 53 kd, the presumed size of the polypeptide moiety of the csA-glycoprotein (Hohmann *et al.*, 1985).

The selective and substantial suppression of RNA hybridizing with clone λc523 and the apparent presence of minute amounts of this RNA suggest that a regulatory region of the csA-protein gene is changed in HG693. Final proof in favour of or against this conjecture will be provided by sequencing the csA-protein genes from the wild-type and mutant.

Materials and methods

Culture of D. discoideum strains

Cells were cultivated at 23°C in nutrient medium with 1.8% maltose as described by Watts and Ashworth (1970). Cells of strains AX2 (clone 214) and AX3 were harvested at densities of not more than 5×10^6 cells/ml. Cells of mutants HG592 and HG693, which grew in the medium to maximal densities of $3-5 \times 10^6$/ml, were harvested at densities of not more than 2.2×10^6/ml. Development was initiated by washing cells in 17 mM Soerensen phosphate buffer, pH 6.0 ('non-nutrient buffer'). The cells were resuspended in the same buffer at a density of 1×10^7/ml, and agitated on a rotary shaker at 150 r.p.m. for AX2 and AX3 and at 220 r.p.m. for HG592 and HG693. Cells were allowed to develop either without stimulation, or with stimulation by cyclic-AMP pulses of 20 nM amplitude applied every 6 min.

For aggregation on agar and for colony blotting cells were cultivated with

Escherichia coli B/2 on nutrient agar containing 0.1% bacteriological peptone (Oxoid), 0.1% glucose and 2% Bacto-Agar (Difco) in non-nutrient buffer.

Preparation of antibodies

Monoclonal antibodies 33-294-17, 41-71-21 and 41-448-9 (Bertholdt *et al.*, 1985) are referred to as mAb 294, mAb 71 and mAb 448, respectively. Antibody IgG was purified from hybridoma culture supernatants on protein A-Sepharose columns. For iodination, 70 – 100 μg IgG were labeled in a total volume of 200 μl containing phosphate-buffered saline, pH 7.2, 0.5 mCi of [^{125}I]iodide (IMS 30, Amersham), and 15 μg chloramine T. After 45 s at room temperature, 100 μl of a saturated tyrosine solution was added and the [^{125}I]IgG separated with Dextran Blue and Phenolred on a 10 ml column of Sephadex G50 medium. For labeling of blots the IgG was diluted to $10^5 - 10^6$ c.p.m./ml.

Mutant selection and immunoblotting

Growth phase cells of HG592 were mutagenized by incubating 1×10^8 cells in 5 ml of 17 mM phosphate buffer, pH 7.0, with 8 mg of 1-methyl-3-nitro-1-nitrosoguanidine for 20 min at room temperature in the dark under gentle agitation. The survival rate was 8%. The cells were washed, dispensed into 10 flasks with 30 ml of nutrient medium, and shaken at 23°C for 5 days. At that time HG592 had expressed the csA-glycoprotein on the cell surfaces. 2×10^6 washed cells were incubated for 15 min at ~4°C under heavy shaking in 150 μl non-nutrient buffer containing 15 μg of mAb 71. The cells were washed and labeled under the same conditions with 50-fold diluted FITC-conjugated sheep anti-mouse IgG (Institute Pasteur Production). The labeled cells were washed in non-nutrient buffer, resuspended in phosphate-buffered NaCl (150 mM), pH 7.2, filtered through fine nylon gauze, and sorted in the cold using a FACS IV cell sorter. The 2% fraction of the cell population with the lowest fluorescence intensity was selected. Per flask of mutagenized cells 3×10^6 cells were sorted and the selected cells grown in a suspension of 5 ml of 1×10^{10} bacteria per ml of *Salmonella minnesota* R595 (Gerisch *et al.*, 1985a). After 2 days, 25 ml more of the bacteria suspension were added. After 5 days the cells were washed in non-nutrient buffer and starved for 6 h with stimulation by pulses of cyclic AMP. The cells were again labeled with mAb 71 and fluorescent antibody for sorting as described, and were directly cloned using a single-cell deposition system onto bacteria-coated nutrient agar plates (Francis *et al.*, 1985). Colonies were blotted onto BA 85 nitrocellulose filters (Schleicher and Schüll, 3354 Dassel, FRG), frozen on a metal plate cooled by dry ice, thawed and washed as described by Stadler *et al.* (1984) for immunoblotting. After labeling with [^{125}I]mAb 71 and autoradiography, blots were stained with 0.2% Ponceau S (Cat. No. 33429, Serva, Heidelberg) in 3% trichloroacetic acid (TCA) and washed in the TCA solution. The original plates were kept in the cold; enough cells remained on the agar surface after blotting to start cultures from mutants identified by immunoblotting as csA-defective. Cells of these mutants were recloned before use.

SDS-polyacrylamide gel electrophoresis was performed in 10% gels according to Laemmli (1970). Immunoblots were obtained according to Towbin *et al.* (1979) as described by Bertholdt *et al.* (1985).

Assays for cell adhesion and chemotaxis

Cell adhesion was quantitated by use of a microprocessor controlled version of the agglutinometer described by Beug and Gerisch (1972) in which light scattering could be recorded continuously. Cuvettes containing 1×10^7 cells/ml were rotated at 40 r.p.m. to expose the cells to constant shear forces. The decrease in light scattering due to cell aggregate formation under these conditions was used as a measure of cell-to-cell adhesion.

For the assay of chemotaxis, HG693 cells were starved for 5 h, washed with non-nutrient buffer and transferred to the Teflon surface of a Petriperm dish (Heraeus, 6450 Hanau, FRG). Micropipettes filled with cyclic AMP solution were used as described by Gerisch and Keller (1981).

cDNA cloning

Seven cDNA clones were obtained as described previously using poly(A)$^+$ RNA from polysomes bound to endoplasmic reticulum membranes of cells harvested at 6 h of starvation (Gerisch *et al.*, 1985b). The plasmid used was p2732B constructed by J.D.Monahan, Roche Institute, Nutley, NJ, and the *E. coli* host strain was BJ5183 (rec BC$^-$sbcB$^-$) (Hanahan, 1983). Plasmid DNA was isolated according to Birnboim and Doly (1979) and nick-translated inserts were used as probes in Northern blots. Clone λc523 was obtained by screening with mAb 294 a λgt11 library provided to us by Dr Richard Kessin, Columbia University, NY. The library made by Dr M.-L.Lacombe was a full length cDNA library from RNA that had been induced by cyclic AMP. For screening 10^4 phages per 12 × 12 cm plates were grown on *E. coli* RY1090 (Young and Davis, 1983). After 3 h at 43°C nitrocellulose filters previously soaked in 10 mM IPTG were laid onto the plates and incubation was continued at 37°C for 14 – 16 h. The filters were extensively washed with buffer containing 10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20 and 0.02% azide. The antibody was iodinated as described and the filters were incubated for 2 h with [^{125}I]mAb 294, $10^5 - 10^6$ c.p.m./ml, in the Tris-NaCl-Tween buffer. After washing several times with the buffer, the filters were exposed to Kodak X-Omat AR film. Phage DNA was isolated according to Maniatis *et al.* (1982) using RY1088 as host strain, and the insert DNA c523 of phage λc523 was recloned into the *Eco*RI site of the plasmid vector gemini 2 using DHI as host strain (Hanahan, 1983); the plasmid obtained was p523. DNA of the plasmid was isolated according to Holmes and Quigley (1981).

Isolation and hybridization of RNA and DNA from D. discoideum strains

RNA was extracted from cells at the indicated stages with phenol-chloroform. Northern blots were obtained either from minigels of 6 cm length or, for a detailed analysis, from gels of 20 cm length containing 1.2% agarose and 6% formaldehyde according to Maniatis *et al.* (1982). If not indicated otherwise, inserts of cDNA clones were used for hybridization. For Southern blotting DNA was extracted by lysis of purified nuclei at 65°C in a solution containing 0.2 M EDTA, pH 8.4, and 2% Sarcosyl and purified by centrifugation in a density gradient of CsCl with ethidium bromide (Noegel *et al.*, 1985). *Eco*RI and *Hind*III fragments were separated on 0.7% agarose gels in Tris-phosphate buffer, pH 7.8 (Maniatis *et al.*, 1982).

For hybridization, filters were incubated with nick-translated probes for 18 – 20 h at 37°C in 2 × SSC, 50% formaldehyde, 4 mM EDTA, 1% Sarcosyl, 0.1% SDS, 4 × Denhardt's and 0.12 M phosphate buffer, pH 6.8 (Mehdy *et al.*, 1983). The filters were washed in the same solution for 1 h at 37°C and autoradiographed on Kodak XAR film.

Acknowledgements

We are grateful to Drs Richard Kessin and Marie-Lise Lacombe for kindly providing us with the λgt11-cDNA library. We thank also Barbara Fichtner and Daniela Rieger for monoclonal antibody production and Klaus Weber for construction of the single-cell deposition system.

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Received on 21 October 1985