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Regulation of gene expression in *Dictyostelium discoideum* cells exposed to immobilized carbohydrates

Salvatore Bozzaro*, Carla Perlo[1], Adriano Ceccarelli[1] and Giorgio Mangiarotti[1]

Max-Planck-Institut für Biochemie, 8033 Martinsried bei München, FRG, and [1]Cattedra di Biologia Generale, Universitá di Torino, Via Santena 5 bis, 10126 Torino, Italy

*To whom reprint requests should be sent

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When amoebae of *Dictyostelium discoideum* develop on gels of polyacrylamide that are derivatized with glucosides, they become capable of aggregation at the same time as cells not exposed to glucosides. However, the aggregation centers and streams of adherent cells formed on immobilized glucosides suddenly disintegrate. The cells repeatedly re-aggregate, but never form tight aggregates as they do on other substrata. Tight aggregates formed in the absence of glucosides disperse after their transfer to glucoside gels, and the cells undergo aggregation-disaggregation cycles. The formation of tight aggregates is correlated with the expression of specific post-aggregative poly(A)$^+$ RNAs. These RNAs are not expressed in cells developing on glucoside gels, and the dispersal of tight aggregates on such gels is accompanied by the almost complete loss of these RNAs. A developmentally regulated membrane glycoprotein called contact site A, which is a marker of aggregation-competent cells, is normally expressed on glucoside gels. Cyclic AMP is also produced, indicating that the strong increase of adenylate cyclase activity during the pre-aggregation phase is not affected. In conclusion, cell contact with immobilized glucosides specifically inhibits post-aggregative gene expression and arrests development at the aggregation stage.

Key words: carbohydrate binding sites/cell adhesion/cyclic AMP/*Dictyostelium*

Introduction

Cell interactions following aggregation have been reported to regulate gene expression in *Dictyostelium discoideum* both at the transcriptional (Blumberg and Lodish, 1981; Landfear *et al.*, 1982; Mangiarotti *et al.*, 1983a; Mehdy *et al.*, 1983) and translational level (Newell *et al.*, 1971, 1972; Loomis, 1975; Alton and Lodish, 1977). During the formation of compact aggregates equipped with a tip, i.e., with the organizer region controlling subsequent development, genes are newly expressed as judged by the appearance of specific 'post-aggregative' mRNAs or proteins. Mechanical dissociation of tipped aggregates results in the loss of post-aggregative products. When the dissociated cells are allowed to reassociate into tight aggregates, post-aggregative transcription and translation begins again (Chung *et al.*, 1981; Mehdy *et al.*, 1983; Newell *et al.*, 1971, 1972; Loomis, 1975).

Evidence is accumulating that diffusible factors and cell surface components are involved in post-aggregative gene expression. cAMP (Town and Gross, 1978; Takemoto *et al.*, 1978; Landfear and Lodish, 1980; Chung *et al.*, 1981; Mangiarotti *et al.*, 1983b; Mehdy *et al.*, 1983) and a low mol. wt. substance called differentiation inducing factor (Kay and Trevan, 1981) have been proposed to control post-aggregative gene expression. Intercellular adhesion is also necessary (Wilcox and Sussman, 1978; Chung *et al.*, 1981; Mangiarotti *et al.*, 1983a; Mehdy *et al.*, 1983) but not sufficient (Kaleko and Rothman, 1982).

For analysis of the control mechanisms, conditions are required under which post-aggregative gene expression is amenable to experimental control and separable from pre-aggregative events. We have described an experimental system in which *D. discoideum* cells develop under a liquid layer on polyacrylamide gels derivatized with glucose, cellobiose or maltose (Bozzaro and Roseman, 1982, 1983a, 1983b; Fisher *et al.*, 1983). The liquid layer freezes development at the stage of tight aggregates (Gerisch, 1968). This means that cells not in contact with immobilized glucoside aggregate and

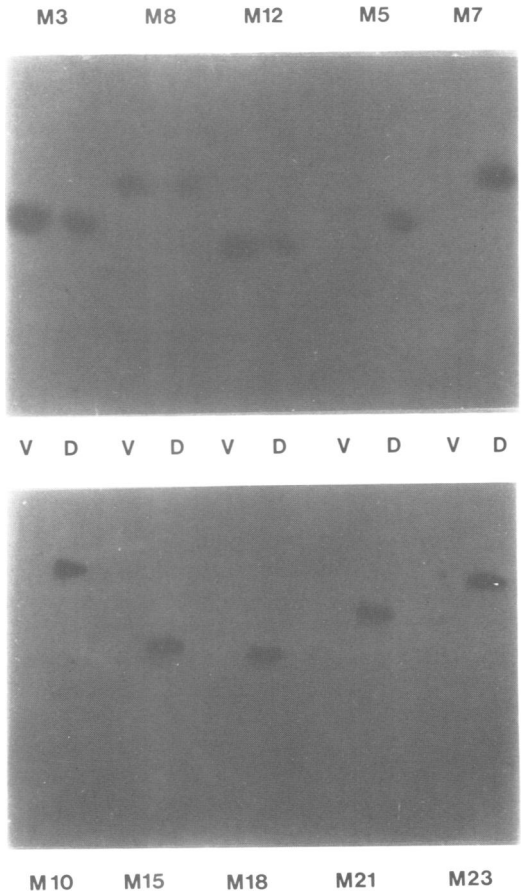

Fig. 1. Northern blot analysis of cloned DNAs. Poly(A)$^+$ RNA from either growth phase cells (V) or from cells harvested after 16 h of development (D) was hybridized, after electrophoresis and transfer to nitrocellulose, with ^{32}P-labeled DNA of 10 clones. Three of the DNA clones, M3, M8 and M12, reacted with single RNA species from both stages, the others only with RNA from the 16 h stage.

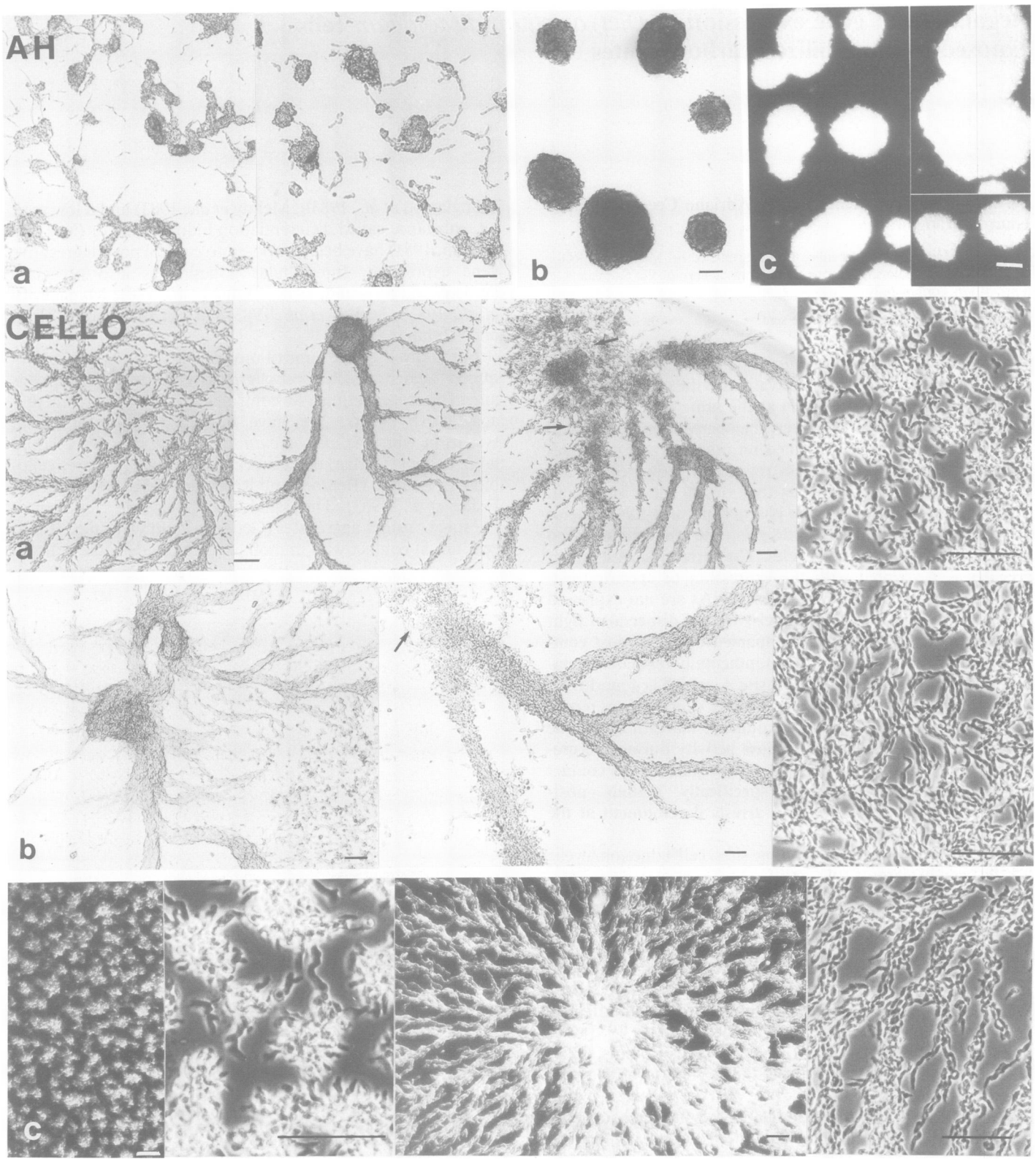

Fig. 2. Development on control polyacrylamide gels derivatized with aminohexanol (AH) or on glucoside gels derivatized with cellobiose (CELLO). Growth phase cells washed free of bacteria were transferred to the gels and photographed after 5.5 h (**a**), 8 h (**b**) or 16 h (**c**) of development. On the control gels, tight aggregates were formed within 8 h, whereas on the glucoside gels the cells aggregated under formation of end-to-end contacts (**b**, **c**, right panels), and disaggregated repeatedly. Zones of disintegration of an aggregation center (**a**, arrows) or of a stream (**b**, arrow) are indicated. Bars: 100 μm.

form compact, rounded clumps, but development does not proceed to the slug stage. The immobilized glucosides, which interact with carbohydrate binding sites on the cell surfaces (Bozzaro and Roseman, 1982, 1983a), allow the cells to form aggregation centers and streams, and to move chemotactically towards the centers, not however to form tight aggregates.

In this paper we report that exposure to immobilized gluco-

sides not only inhibits tight aggregate formation but also causes tight aggregates to disperse. We show that, concomitant with the formation of tight aggregates and their dispersal, specific post-aggregative poly(A)$^+$ RNAs are, respectively, synthesized and disappearing. In accord with the finding that cell aggregation into centers and streams is not inhibited, a marker of aggregating cells, the cell surface glycoprotein called contact site A (Müller and Gerisch, 1978), is normally expressed. cAMP, synthesized by aggregating cells and released as an intercellular signal into the medium (Devreotes, 1982), is produced in at least the amounts produced on non-glucoside derivatized gels. Thus, an important feature of immobilized glucosides is the specificity of their action on post-aggregative gene expression.

Results

Hybridization of cloned DNA with developmentally regulated and non-regulated RNA

DNA clones from a genomic library of *D. discoideum* strain AX3 have been screened by the hybridization-competition technique of Mangiarotti *et al.* (1981) with poly(A)$^+$ RNA from the growth phase stage and from the 16 h stage of development (Chung *et al.*, 1981; Mangiarotti *et al.*, 1981). The results obtained with strain AX3 were confirmed for V12M2, the strain best suited for the experiments reported in this paper. When poly(A)$^+$ RNA from the 16 h stage was used for hybridization total, unlabeled RNA from growth phase cells was added for competition. Thus RNAs present both during growth and development, or only during development, were distinguished. Northern blot analysis confirmed that each DNA clone hybridized to a single RNA species (Figure 1). Poly(A)$^+$ RNA hybridizing to clones M3, M8 or M12 was present in V12M2 both during the growth phase and after 16 h of development. RNA hybridizing to clones M5, M7, M10, M15, M18, M21 or M23 was present at the 16 h stage but absent from growth phase cells.

Inhibition of cell development on glucoside gels

Cells were transferred onto gels derivatized with cellobiose or aminohexanol, the spacer used for linking the sugar to polyacrylamide (Schnaar *et al.*, 1978; Bozzaro and Roseman, 1983a). On both gels aggregation began after 4 h, as it is typical of the V12M2 strain. The cells assumed an elongated shape, collected into streams and moved chemotactically towards aggregation centers (Figure 2). On the control gels derivatized with aminohexanol, aggregation was completed 2 h later, and tight, rounded aggregates were formed. The tight aggregates, which were only loosely attached to the substratum, did not disintegrate during the experiments, i.e., until 20–24 h after the beginning of starvation (Figure 2,AH,b–c).

On the glucoside gels, aggregation proceeded for 1.5 h (Figure 2, Cello, a). Thereafter, aggregation centers and streams suddenly disintegrated within a period of a few minutes. After the disintegration, cells showed no orientated movement until new centers were formed and aggregation resumed. The aggregation-disintegration cycle was repeated several times, but tight, rounded aggregates, as on control gels, were never formed (Figure 2, Cello, b–c).

To study the effect of immobilized glucosides on tight aggregates, cells were starved and gently shaken in suspension. Under these conditions they formed EDTA-resistant contacts, typical of aggregation-competent cells, within 4 h and tight aggregates within 6–7 h. The tight aggregates could not be dissociated into single cells by strong vortexing and repeated pipetting. However, they immediately started to disintegrate when deposited on cellobiose gels (Figure 3, Cello, a). At 30 min after plating, most of the cells were spread on the gel surface. Thereafter, new aggregation centers appeared and the cells re-aggregated, followed by cycles of disaggregation and re-aggregation (Figure 3, Cello, b–d).

On control gels the tight aggregates did not dissociate (Figure 3,AH), they rather fused into larger ones when brought into contact by moving the specimens for microscopical examination (Figure 3, AH,b–d).

Inhibition and reversal of post-aggregative gene expression on glucoside gels

Poly(A)$^+$ RNA was prepared from undeveloped cells, from starved cells that had developed into tight aggregates on control gels for 8 or 16 h, and from aggregating and disaggregating cells exposed to glucoside gels for 8 or 16 h (Figure 4, nos. 1–5). The RNAs were ^{32}P-labelled and hybridized with the same DNA clones as in Figure 1. Poly(A)$^+$ RNAs hybridizing with clones M5, M7, M10, M15, M18, M21 or M23 were detected neither in undeveloped cells (Figure 5, lane 1), nor in cells developing on glucoside gels (Figure 5, lanes 4 and 5). These RNAs were present in cells incubated on control gels for 8 h and, in equal amounts, in cells incubated on the same gels for 16 h (Figure 4, lanes 2 and 3). Poly(A)$^+$ RNAs hybridizing with DNA clones M3, M8 or M12 were found under all conditions in comparable amounts (Figure 5, lanes 1–5).

Reversal of post-aggregative gene expression was investigated by transferring tight aggregates for 8 h suspension cultures to control or glucoside-derivatized gels, as indicated by nos. 6–8 of Figure 4. The post-aggregative poly(A)$^+$ RNAs, expressed in the tight aggregates (Figure 5, lane 6), remained present for at least 8 h of incubation on control gels (Figure 5, lane 7). They declined, however, to trace amounts during the same time of exposure to glucoside gels (Figure 5, lane 8). No decline of non-developmentally regulated poly(A)$^+$ RNAs was found under these conditions (Figure 5, lanes 6–8).

cAMP production and expression of contact sites A

Strain V12M2 cells incubated on glucoside gels start to aggregate ~4–5 h after the end of growth. Time-lapse films show that chemotactic signals, known to be pulses of cAMP (Tomchik and Devreotes, 1981), are produced periodically by the centers, as they are produced in controls on aminohexanol gels (Bozzaro and Roseman, 1983b). The basal activity of adenylate cyclase increases by a factor of more than 10 between 2 and 4 h of development, followed by periodic increases of activity superimposed on the basal activity (Klein, 1976; Roos *et al.*, 1977). These changes in adenylate cyclase activity are reflected in an increase from the growth phase to aggregation of the average cellular cAMP concentrations (Pahlic and Rutherford, 1979).

Under our conditions the cAMP levels began to rise at ~3 h after the beginning of development (Figure 6). The rise of cAMP continued for two additional hours, followed by a fall to half of the peak value within the next 2 h. The cAMP production was higher on glucoside gels than it was on control gels (Figure 6). The decrease of cAMP occurred independently of whether tight aggregates were formed, as on control gels, or the cells continued to disaggregate and re-

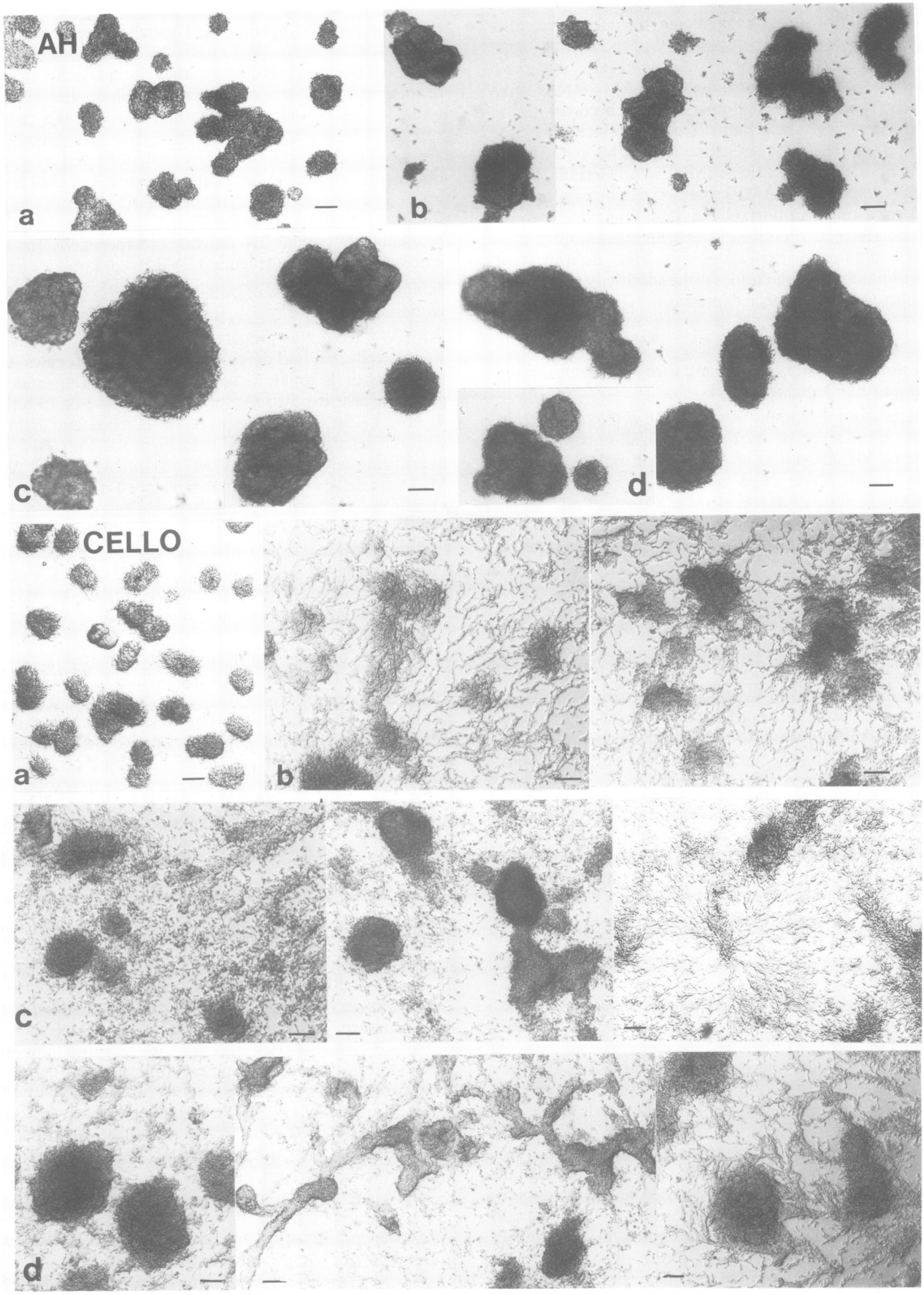

Fig. 3. Effects on tight aggregates of exposure to control gels (AH) or glucoside derivatized gels (CELLO). Tight aggregates from 8 h shaken cultures were transferred to the gels and photographed at 5 min **(a)**, 30 min **(b)**, 4 h **(c)** and 8 h **(d)** later. On the control gels, tight aggregates fused into larger clumps. On glucoside gels, the tight aggregates started to disintegrate within 5 min **(a)** and underwent several aggregation-disintegration cycles **(b – d)**. A few aggregates did not disperse totally **(c**, center panel; **d**, left panel) and their core often became an aggregation center. Bars: 100 μm.

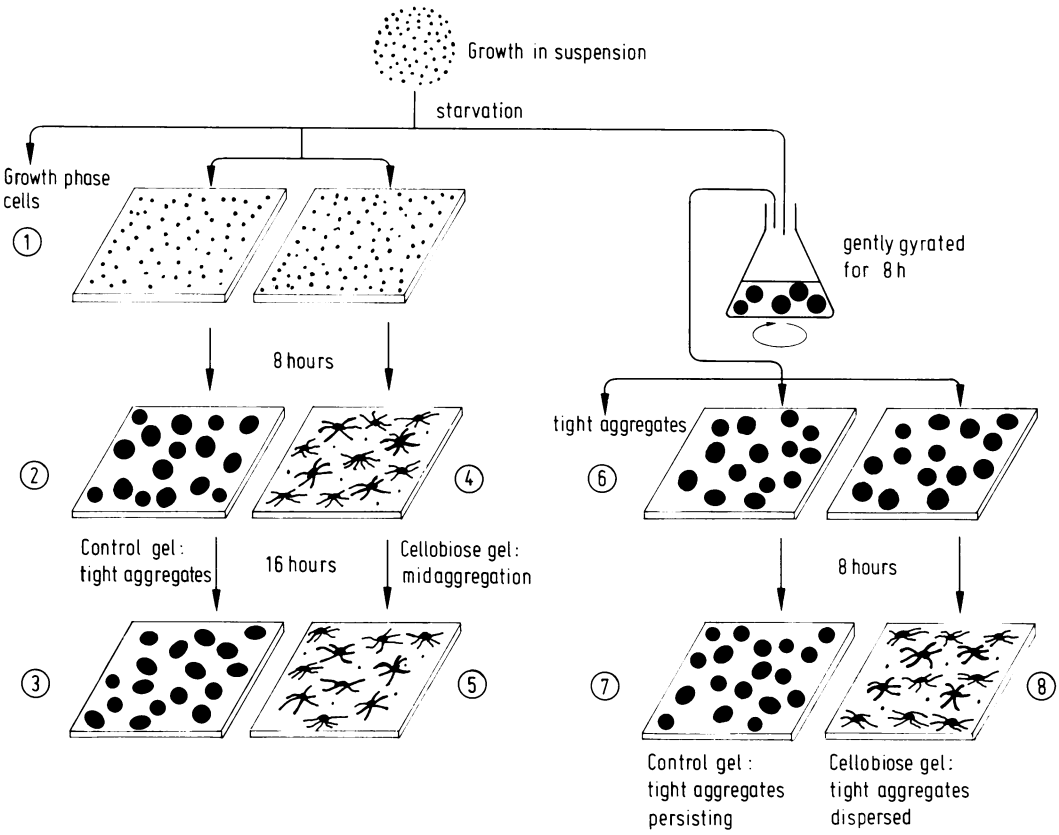

Fig. 4. Experimental protocol for the analysis of gene expression. Encircled numbers indicate the source of the poly(A)$^+$ RNA, which was hybridized with cloned DNA as shown in Figure 5, and correspond to the lane numbers in that Figure. **Left part (1–5)**, cells grown with bacteria in suspension were washed free of bacteria (**1**) and transferred onto aminohexanol gels for controls (**2,3**), or onto cellobiose derivatized gels (**4,5**). Tight aggregates formed on the control gels, or aggregating and disaggregating cells on the glucoside gels, were harvested after 8 h (**2,4**) or 16 h (**3,5**). **Right part (6–8)**, starved cells were allowed to form tight aggregates in a gently shaken suspension. At 8 h of starvation the tight aggregates (**6**) were transferred onto aminohexanol or cellobiose gels and incubated for another 8 h. On the control gels the tight aggregates persisted (**7**), whereas on the glucoside gels the cells reverted to the aggregation stage (**8**).

aggregate, as on glucoside gels.

EDTA-stable contact, as they are typical of the aggregation stage (Beug *et al.*, 1973), are formed at the same time by cells developing on glucoside gels and by control cells, indicating that not only the chemotactic system but also the adhesion system of aggregating cells becomes functional on glucoside gels (Bozzaro and Roseman, 1983b).

To provide direct evidence that genes activated in the pre-aggregation stage are expressed on glucoside gels, the regulation of the contact site A glycoprotein was investigated. The appearance of this glycoprotein normally coincides with the beginning of EDTA-stable contact formation. Under our conditions the glycoprotein, apparent mol. wt. 80 kd, became detectable after 4 h of development both on cellobiose and aminohexanol gels (Figure 7, top). Under both conditions the cells began to aggregate ~45 min later, and the glycoprotein persisted until 16 h or more after the beginning of development. When tight aggregates were dispersed following incubation on glucoside gels, the 80-kd glycoprotein remained present on the cells until 20 h of development or even longer (Figure 7, bottom).

Discussion

The finding that *D. discoideum* cells developing on glucoside-derivatized polyacrylamide gels become capable of aggregation but not of forming tight aggregates, has suggested that immobilized glucosides specifically interfere with post-aggregative events, thus arresting development at the aggregation stage (Bozzaro and Roseman, 1983b). This notion was supported by the dispersal of tight aggregates by immobilized glucosides which resulted in a reversal of development back to the aggregation stage. In accord with these effects none of the post-aggregative poly(A)$^+$ RNA species shown to be present in tight aggregates was expressed in cells developing on glucoside gels. Also, the post-aggregative poly(A)$^+$ RNA species were no longer detectable after dispersal of tight aggregates on glucoside gels, indicating turning-off of the respective genes and degradation of the RNA in the dispersed cells. In contrast to post-aggregative gene products, a typical aggregation marker, the contact site A glycoprotein, was synthesized on glucoside gels and was not lost during the dispersal of tight aggregates on such gels.

Taken together, these results indicate that exposing cells or tight aggregates to immobolized glucosides enables one to distinguish between development up to the aggregation stage and post-aggregative cell differentiation. There is, however, one exception. UDP-glucose pyrophosphorylase, an enzyme considered to be a typical post-aggregative gene product (Newell *et al.*, 1971, 1972; Town and Gross, 1978; Kaleko and Rothman, 1982), accumulates at its normal rate in cells plated on glucoside gels (Bozzaro and Roseman, 1983b).

Cells developing on glucoside gels form the EDTA-stable intercellular contacts typically located at the ends of the elongated aggregating cells. These cells also show normal chemotaxis to cAMP, and they synthesize cAMP up to levels

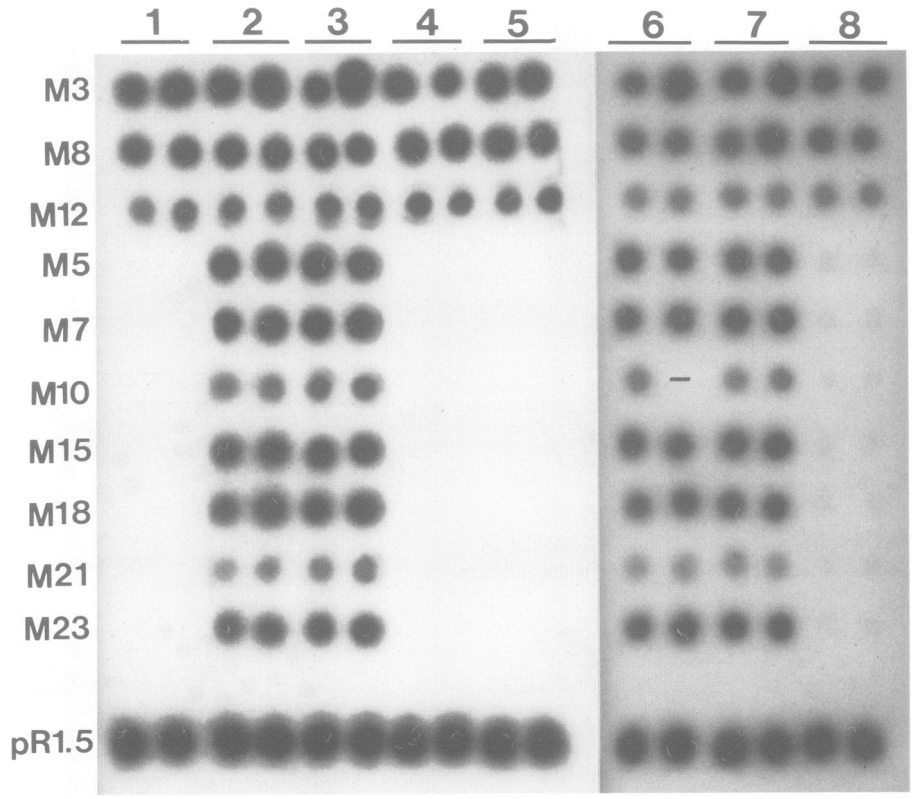

Fig. 5. Expression of poly(A)$^+$ RNA species on glucoside and control gels. DNA of the clones shown in Figure 1 was spotted onto nitrocellulose filters and hybridized with ^{32}P-labeled poly(A)$^+$ RNA from cells incubated according to the numbers in Figure 4. Clone pR 1.5 contained a fragment of 26S rRNA gene, and was used for calibration. One spot of M10(−) was left out because only little DNA was available.

even exceeding those in control cells. The cell contact of aggregating cells, cAMP signals, and/or some unknown effector (Kaleko and Rothman, 1982) acting during aggregation are sufficient to induce UDP-glucose pyrophosphorylase. These factors are not sufficient, however, for induction of the post-aggregative poly(A)$^+$ RNA species studied in this paper. Their expression is linked to an additional event closely associated with tight aggregate formation and blocked by immobilized glucosides. Apparently, gene expression considered to be 'post-aggregative' does not occur *en bloc*, but consists of processes that require different types of cell-to-cell contact or of diffusible factors.

The sheets of polyacrylamide gels to which the glucosides are bound are too large to be internalized. Thus the cells interact with the immobilized glucosides via binding sites on their surface, as they have been demonstrated by the attachment of *Escherichia coli* cells carrying lipopolysaccharides with glucose as the terminal sugar (Vogel *et al.*, 1980). Attachment to *D. discoideum* cells results in phagocytosis of the bacteria, suggesting that recruitment of food is the natural function of the glucose binding sites. Assuming that the sites of attachment to glucoside gels are the same as for binding bacteria, one can speculate that the immobilized glucosides mimic bacteria as a source of food.

Competition of cell-to-substratum adhesion with cell-to-cell adhesion appears to be one mechanism by which tight aggregates are destabilized on glucoside-derivatized gels, another mechanism is active cell movement away from the aggregates. This movement has been shown to occur during disaggregation on glucoside gels and is thought to be guided by a negative chemotactic response of one cell to another

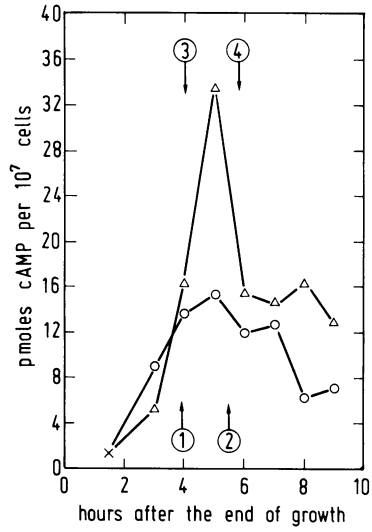

Fig. 6. Increase of cAMP during development on control (○) or glucoside (△) gels. Cells were transferred to the gels at 1.5 h of starvation (**x**). Arrows indicate the beginning of aggregation on the control (**1**) or on the glucoside (**3**) gels, the appearance of tight aggregates on the control gels (**2**), and the onset of the first round of dissociation on the glucoside gels (**4**). cAMP was determined as described in Materials and methods. Data represent the averages of two gels, each assayed in duplicate.

(Fisher *et al.*, 1983). Negative chemotaxis between *D. discoideum* cells has also been observed during growth on bacteria (Samuel, 1961; Keating and Bonner, 1977; Kakebeeke *et al.*, 1979), and has been considered to be a food-seeking device (Bonner, 1977). Thus, the behavior of cells in

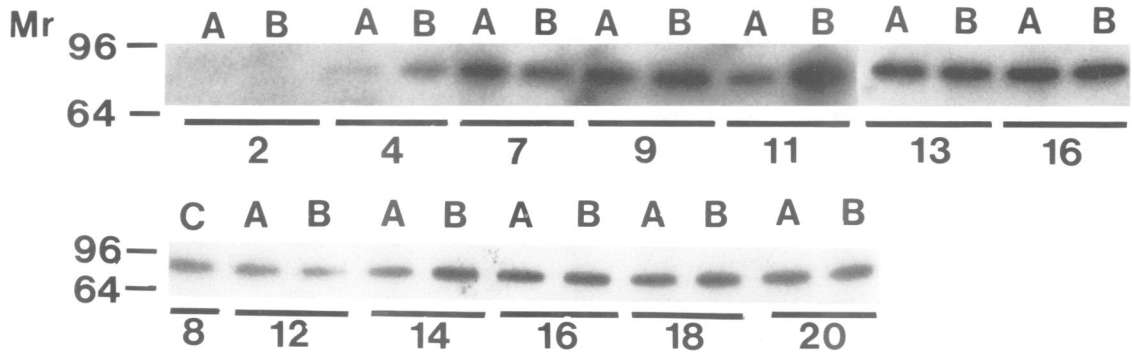

Fig. 7. Expression of the contact site A glycoprotein on control gels (**A**), glucoside gels (**B**), and in tight aggregates developed in suspension (**C**). **Top**: cells were transferred to the gels at the beginning of starvation. **Bottom**: tight aggregates developed for 8 h in suspension (**C**) were transferred to the gels. Numbers below the lanes indicate hours after the beginning of starvation. Gel pieces with cells or aggregates were heated in sample buffer according to Laemmli (1970), and the extracts from individual pieces were loaded onto SDS-polyacrylamide gels. The glycoprotein was visualized by immunoblotting with iodinated monoclonal antibody. Mol. wt. markers are indicated in kilodaltons.

contact with bacteria or with glucoside-derivatized gels suggests that the glucose binding sites not only mediate cell attachment but also act as receptors whose activation leads to mutual repulsion of the responding cells.

Most likely the primary action of immobilized glucosides is their interference with the intimate association of cells in tight aggregates, and the disappearance of post-aggregative poly(A)$^+$ RNAs is a consequence of cell dispersal. This view is based on the finding that mechanical dissociation of tight aggregates also inhibits post-aggregative gene expression (Chung *et al.*, 1981; Mehdy *et al.*, 1983). The blockade of developmentally regulated gene expression, as it is induced by immobilized glucosides, resembles a phenomenon called 'erasure': markers and functions of differentiated *D. discoideum* cells are lost when such cells are exposed to nutrients (Soll and Waddel, 1975; Finney *et al.*, 1983). However, erasure, as it is induced by particulate or soluble nutrients taken up by the cells, results in complete reversal of development up to the growth phase stage. Reversal to an intermediate stage, i.e., under maintenance of markers of aggregating cells, is thus a peculiar reaction to glucosides whose internalization is prevented by covalent linkage to sheets of polyacrylamide.

In summary, exposure of cells to immobilized glucosides provides an experimental system by which to manipulate post-aggregative gene expression without affecting pre-aggregative developmental control. The system might be useful in the characterization of factors responsible for the activation of genes at the switch point from aggregation to post-aggregative development.

Materials and methods

Preparation of derivatized polyacrylamide gels

Polyacrylamide gels derivatized with aminohexanol, cellobiose or glucose were prepared as described (Schnaar *et al.*, 1978; Bozzaro and Roseman, 1983a). The amount of bound cellobiose or glucose was, respectively, 1 and 0.7 μmol/cm^2 gel of 0.25 mm thickness, and the area of each gel piece was 0.6 cm^2. In all experiments, aminohexanol dervatized gels were used as control gels and cellobiose derivatized gels as glucoside gels, except in the experiment shown in Figure 6, where glucose derivatized gels were used. Before use the gels were equilibrated in 17 mM Soerensen phosphate buffer, pH 6.0, and, after absorption of the excess fluid by filter paper, placed in plastic dishes of 50 mm diameter (Falcon No. 3001).

Cell culture and development on derivatized gels

Cells of *D. discoideum* strain V12M2 were grown in shaken suspensions of *E. coli* B/r and starved in 17 mM Sorensen phosphate buffer, pH 6.0, at a cell concentration of 1 x 10^7/ml (Gerisch, 1961; Bozzaro and Roseman, 1983a). Development to tight aggregates was achieved by gently agitating the starved cells for 8 h in 30 ml suspensions in 100-ml Erlenmeyer flasks at 150 r.p.m. on a gyratory shaker. 50 μl of cell suspensions at the beginning of starvation or of tight aggregates were transferred on top of derivatized gels. Since the cells developed under a liquid layer, tight aggregates did not form tips and thus did not develop into slugs (Gerisch, 1968; Bozzaro and Roseman, 1983b). For the experiment shown in Figure 1, cells were washed free of bacteria, and 1 x 10^8 cells were plated on a Millipore filter (type AA, 4.5 cm diameter), resting on two filter pads saturated with the phosphate buffer. All experiments were done at 22 ± 2°C.

DNA-RNA hybridization

Poly(A)$^+$ RNA was prepared as described by Mangiarotti *et al.* (1981), labeled with [^{32}P]ATP and polynucleotide kinase according to Williams and Lloyd (1979), and used for screening of DNA clones as described (Mangiarotti *et al.*, 1981). When labeled poly(A)$^+$ RNA from cells at the 16 h stage was used, total unlabeled RNA from growth phase cells was added for competition.

Northern blot analysis was performed after electrophoretic separation of RNA species in a formaldehyde gel according to Rave *et al.* (1979) as modified by Mangiarotti *et al.* (1981). Nick-translated DNA probes were used for hybridization as described by Alwine *et al.* (1977).

For dot hybridization, solutions of 250–500 μg cloned DNA per ml were spotted in 3 μl aliquots on nitrocellulose pretreated for DNA fixation, and hybridization with 0.1 μg of ^{32}P-labeled poly(A)$^+$ RNA, specific activity 5–8 x 10^7 c.p.m./μg, was performed as described (Mangiarotti *et al.*, 1981).

Determination of cAMP

Developing cells were incubated on derivatized gels for various times. Thereafter, whole gels were transferred with the cells under mixing into 1.5 ml Eppendorf microtubes containing 50 μl of 2 N perchloric acid. After centrifugation, neutralization of the supernatant with potassium carbonate, and acetylation, cAMP was determined in the extract according to Harper and Brooker (1975), using the NEN cAMP RIA kit.

Detection of the contact site A glycoprotein

Cells were placed on derivatized gels at the beginning of starvation, tight aggregates after 8 h of development. At various times the gels were frozen together with the cells, thawed and heated in sample buffer for subsequent SDS-polyacrylamide electrophoresis in 12% gels according to Laemmli (1970). Proteins were blotted and the contact sites A labeled with [^{125}I]monoclonal antibody 12-120-94 (Ochiai *et al.*, 1982).

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