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Biochemical differentiation in a mutant of *Dictyostelium discoideum* defective in cyclic AMP chemotaxis and in intercellular cohesion

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Summary

A temperature-sensitive mutant of *Dictyostelium discoideum* has been isolated based on its lack of chemotaxis toward cyclic AMP at the restrictive temperature, 27 °C. The mutant develops normally at the permissive temperature, 22 °C, but fails to aggregate or complete development at the restrictive temperature. The temperature-sensitive phenotype can be bypassed by allowing cultures to grow into late log phase or to starve for 60–90 min at 22 °C prior to a shift to 27 °C. At 27 °C, the mutant overproduces cell surface cyclic AMP receptors of both high and low affinity and is capable of spontaneous oscillations in light scattering in cell suspensions.

Despite its complete lack of morphological development, the mutant undergoes extensive biochemical differentiation. At the onset of starvation, it shows increased levels of *N*-acetylglucosaminidase, it expresses cyclic AMP receptors at the normal time and, although somewhat slowly, suppresses those receptors as if aggregation had been achieved. Metabolic pulse labellings with [^{35}S]methionine revealed that the mutant at 27 °C displays the same changes in the patterns of newly synthesized proteins observed during the vegetative-to-aggregation and the aggregation-to-slug stages of normal development. The only clear difference from wild type was the failure of the culmination-stage isozyme of β-glucosidase to appear.

The mutant is defective in establishment of intercellular cohesion mechanisms, correlated with poor agglutination by concanavalin A, at the restrictive temperature. The properties of the mutant place severe constraints on models regarding the role of chemoreception and intercellular cohesion in regulation of gene expression.

Key words: glycosylation, aggregation, slime mold, *Dictyostelium discoideum*, cyclic AMP, intercellular cohesion, chemotaxis.

Introduction

Upon starvation, amoebae of *Dictyostelium discoideum* enter a developmental program during which individual cells aggregate to form multicellular masses. These become organized into a pseudoplasmodium with an established spatial pattern of prestalk and prespore cells which are, in turn, the precursors of stalk and spore cells of the mature fruiting body (reviewed in Loomis, 1975, 1982). Cyclic AMP plays a central role in *D. discoideum* development (for reviews, see Gerisch, 1987; Janssens & van Haastert, 1987; Schaap, 1986; Williams, 1988). Aggregation is mediated by cAMP which is released in a pulsatile fashion by aggregation centers. Aggregation-competent amoebae have expressed surface receptors for cAMP (Malchow and Gerisch, 1974; Henderson, 1975; Green and Newell, 1975) and respond to it by positive chemotaxis, activation of adenylate cyclase and release of a pulse of cAMP which attracts more distant amoebae. This relay of the cAMP signal leads to an aggregation pattern in which cells form streams moving toward the aggregation centers. During postaggregation development, the number of cAMP receptors has decreased substantially, but cAMP signalling appears to continue. In addition to its role as a chemoattractant in *D. discoideum*, cyclic AMP has been shown to be a controlling agent for a number of developmentally regulated genes, contributing to repression of some, expression of others and modulation of expression of specific prestalk and prespore genes (Gerisch, 1987; Williams, 1988).

In signal transduction, extracellular cAMP has been shown to activate both adenylate and guanylate cyclases, to mobilize calcium stores, to alter phosphorylation of several proteins and to affect cytoskeletal polymerization. The events appear to be mediated by receptor control of GTP-binding proteins, inositol phosphates and cAMP-dependent protein kinases presumably regulated by intracellular cAMP synthesized in response to cAMP binding to surface receptors.

A major challenge is to relate these specific receptor-mediated processes to the specific biological events,

including spatial orientation and chemotaxis, signal relay and changes in gene expression. One approach is to isolate mutants with defects in one response to extracellular cAMP and examine the effects on other responses. We have taken this approach by selection of conditional mutants which fail to perform chemotaxis toward cAMP at a restrictive temperature (Barclay & Henderson, 1977, 1982, 1986). Here we describe the properties of a mutant which is thermosensitive for aggregation and has a novel phenotype when compared with the properties of previously reported cAMP chemosensory mutants.

Materials and methods

Culture conditions and mutant selection

DdC, a cycloheximide-resistant derivative of NC4, was the parent strain from which HT44 was isolated. The mutant was obtained by mutagenesis of DdC with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to 30–50% survival and selection among progeny capable of normal development at 22°C for those that were defective in chemotaxis toward cAMP at 27°C, as described by Barclay and Henderson (1977). Cultures were grown in shaken liquid suspensions in KPM (M/60-potassium phosphate, pH 6·1, 2 mM-$MgSO_4$) buffer containing 10^{10} *E. coli* B/r ml^{-1} or on SM agar plates in association with *K. aerogenes* (Sussman, 1966). Development was on 2% agar in KPM.

cAMP binding to whole cells

Equilibrium binding of cAMP to cells was measured at 4°C with [^{3}H]cAMP ± 50 μM unlabelled competing cAMP, using $2–5\times10^7$ cells as described by Henderson (1975). Scatchard plot analysis was as described in Barclay and Henderson (1986).

Chemotaxis assays

These were as described by Barclay and Henderson (1982). Briefly, a droplet of cells was placed on KPM agar between two filter paper strips, one soaked in 5×10^{-5} M-cAMP in KPM and the other in KPM alone. Escape of the cells from the original droplet toward the cAMP was scored as a positive result.

cAMP-induced light scattering oscillations

Exponentially growing cells were harvested and plated on KPM agar for 4–6 h, then harvested and resuspended in KPM at 2×10^7 ml^{-1}. The light-scattering assays were performed by Dr Michael Brenner according to Gerisch and Hess (1974) with 1 ml aliquots of the cells in a rapidly oxygenated cuvette. One addition of cyclic AMP was made, to a final concentration of 1×10^{-5} M, and optical density changes at 405 nm were recorded.

Temperature shift experiments

KPM agar plates were used for all developmental studies. When temperature shifts were planned, either the agar plate was preincubated at the appropriate temperature before plating of cells or the agar was poured in a very thin layer so that it would equilibrate fairly rapidly when plated cells were subsequently shifted.

Cell agglutinations

Slugs suspended in KPM (M/60-potassium phosphate, pH 6·1, 2 mM-$MgSO_4$) with 10 mM-EDTA were disaggregated mechanically by vigorous pipetting and passage through a 20-gauge syringe needle. For assay of mutual cohesion, the disaggregated amoebae were resuspended at $5–8\times10^6$ ml^{-1} in KPM buffer with or without EDTA and shaken at 22° or 27°C at 200 revs min^{-1}. The total number of single cells and cell doublets remaining was scored at 30 min intervals and the percentage agglutination calculated from the decrease in this value. To examine agglutination by lectins, similar conditions were used except that the cells were suspended at 2×10^6 ml^{-1}.

Enzyme and protein measurements

For enzyme assays, a total of 1×10^8 vegetative or differentiating cells were harvested, washed in cold water and frozen at −20°C until use. The samples were thawed at room temperature and sonified in an ice bath by two 20 sec pulses from a Branson sonifier. The sonicate was centrifuged for 5 min at 1000 *g* to remove turbidity and the supernatant assayed immediately for enzyme activity. *N*-acetylglucosaminidase and β-glucosidase assays were performed using the *p*-nitrophenyl pyranoside sugars at 35°C and measuring the amount of *p*-nitrophenol released as described by Loomis (1969) and Coston and Loomis (1968). Protein determination was by the dye binding method of Bradford (1976).

Incorporation of [^{35}S]methionine

Metabolic labelling, SDS–polyacrylamide gel electrophoresis and fluorography were performed as described by Prem Das and Henderson (1983).

Materials

Unless otherwise specified, biochemicals were obtained from Sigma Chemical Co. Tritiated cAMP and [^{35}S]methionine were from Amersham.

Results

Temperature-sensitive aggregation of HT44

Mutant HT44 was isolated by its failure to migrate through a filter in contact with a solution of the chemoattractant, cAMP, at the restictive temperature, 27°C. Mutant amoebae starved at 22°C stream (Fig. 1A) and aggregate into mounds that develop a tip and form fruiting bodies (Fig. 1C), all similar to the parent DdC except that development of fruiting bodies requires 30–32 h rather than 24 h as in DdC. In contrast, HT44 amoebae maintained at 27°C from the onset of starvation fail to aggregate and to form fruiting bodies even after 50–60 h (Fig. 1D). When amoebae are plated at high densities ($2–4\times10^8$ *vs* 5×10^7 cells per 47 mm diameter dish), they form irregular loose mounds (Fig. 1B) but do not develop further. The mutant grows normally at both 22° and 27°C on SM agar plates with *K. aerogenes* or in liquid suspension with *E. coli* B/r.

Thermosensitive development was, however, only observed when cells were harvested from early exponential growth phase ($2–4\times10^6$ ml^{-1}). Cells harvested from a 22°C culture in late exponential phase ($5–8\times10^6$ ml^{-1}) developed normally even at 27°C. This suggests that the thermolabile defect in HT44 is in an element normally expressed very early in development and which can be induced by the conditions in a late log phase culture. Two further experiments confirm the

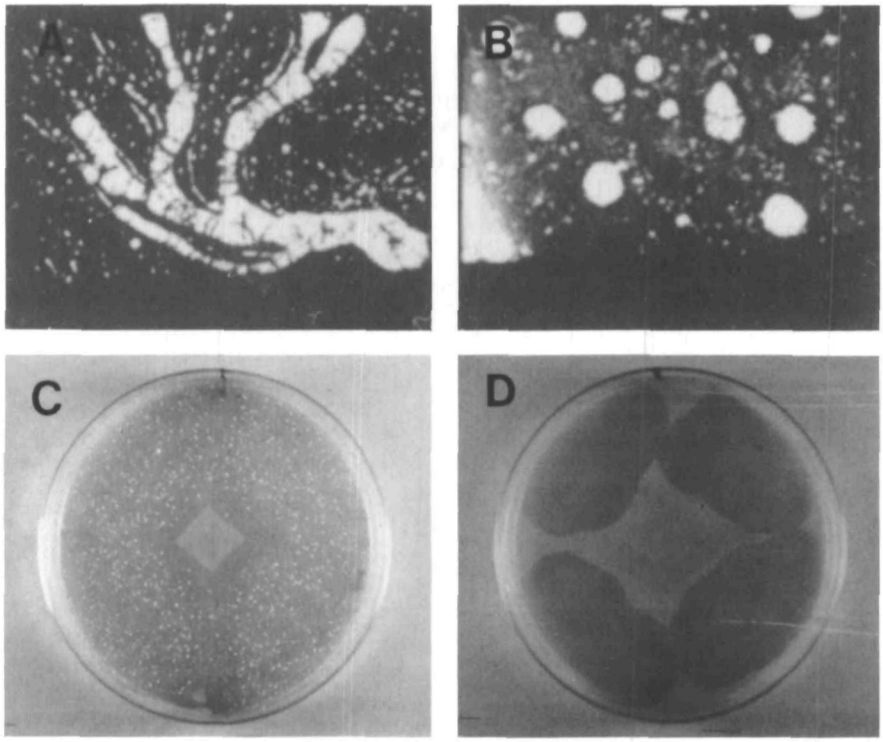

Fig. 1. Developmental phenotype of HT44 at 22°C and 27°C. Amoebae were harvested during early exponential growth in shaken suspension, washed free of bacteria and plated on KPM agar at a density of 5×10^7 (A,C,D) or 4×10^8 (B) per 47 mm area and incubated at either 22°C (A,C) or 27°C (B,D) and photographed after 8–10 h (A,B, 100× magnification) or after 32 h (C,D). C shows fruiting body spore heads from above. D shows that, while growth is only slightly retarded at 27°C, there is no development in the clear growth plaques.

very early defect. First, both DdC and HT44 develop aggregation competence when starved overnight at 7°C, and, when subsequently shifted to either 22° or 27°C, both begin to aggregate within minutes, forming large streams. Second, temperature-shift experiments were performed on cells harvested from early exponential cultures. Whereas HT44 development was completely blocked when it was incubated at 27°C from the onset of starvation, amoebae incubated for 60–90 min at 22°C and then shifted to the nonpermissive temperature aggregated and developed normally.

Chemotaxis toward cAMP

The method of isolation of HT44 predicted that the mutant would be temperature sensitive for chemotaxis. To test this, mutant and parent amoebae were collected after 9–10 h of starvation at the appropriate temperature and deposited in droplets on KPM agar between paper strips which had been soaked in buffer or 5×10^{-5} M-cAMP in buffer. The plates were incubated at either 22° or 27°C and monitored for escape of amoebae from the area of the droplet. DdC responded to cAMP independent of the temperature of starvation or assay. HT44 starved at 22° responded strongly (Fig. 2A), but those starved at 27°C showed only a very weak response, accumulation at the edge of the original droplet, even when tested at 22° (Fig. 2B).

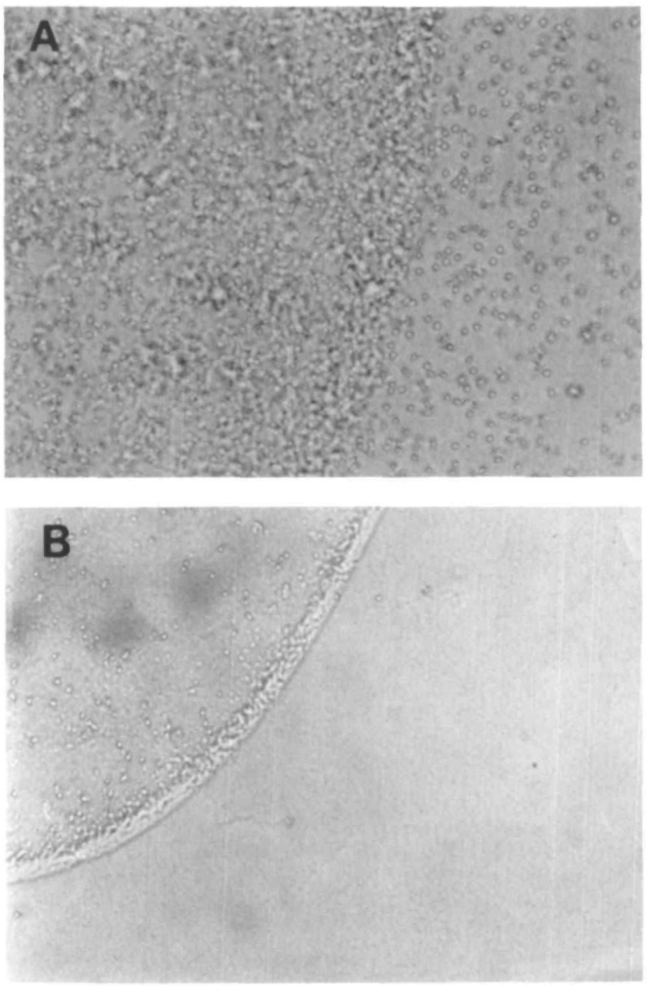

Fig. 2. Chemotaxis of HT44 after starvation at 22°C or 27°C. Amoebae starved on KPM agar for 9 h at 22°C (A) or 27°C (B) were washed and resuspended to 2×10^7 ml^{-1}. Droplets were deposited ca. 3 mm from filter papers (off the right side of panels) soaked with 5×10^{-5} M-cAMP and incubated 60 min at 22°C prior to photography.

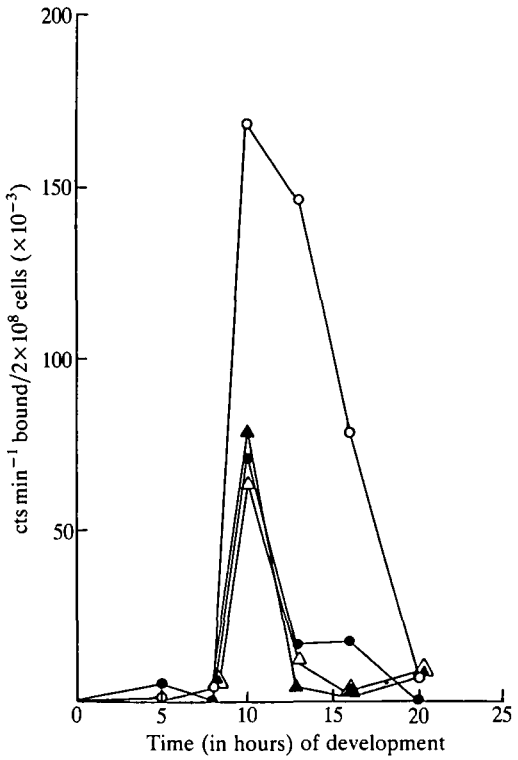

Fig. 3. Developmental regulation of cAMP receptors. Wild-type (triangles) and HT44 (circles) amoebae were harvested from growth at 2×10^6 ml^{-1}, washed, plated on KPM agar and incubated at 22 (closed symbols) or 27 (open symbols) °C. At the intervals shown, amoebae were harvested and assayed for binding at 10 nM-cAMP as described in Methods.

Cell surface cAMP receptors

To determine whether the defect in chemotaxis at 27°C was due to absence of cAMP receptors, DdC and HT44 amoebae were harvested at a series of times over a 20 h period of starvation at either 22° or 27°C and assayed for binding at 10 nM-cAMP. Fig. 3 shows that maximal binding of HT44 at 27°C is more than twice that at 22°C and that of DdC at either temperature. Further, at 27°C, mutant amoebae fail to regulate their receptors properly. In the wild type, and in HT44 at 22°C, the number of receptors per cell drops dramatically at the completion of aggregation. However, at 27°C, HT44 still exhibits as much binding at 18 h as did DdC at its maximum, though by 20 h the number of receptors has dropped to that of the positive control samples.

Green and Newell (1975) first showed that aggregation-competent amoebae have both high- and low-affinity surface receptors for cAMP. Since the results in Fig. 3 were obtained with a subsaturating concentration of cAMP, the enhanced cAMP binding by HT44 at 27°C could be due either to an increase in the affinity of the predominant low affinity sites or to an increase in total number of sites. Scatchard analyses were used to distinguish between these alternatives. Fig. 4 shows the Scatchard plots at 22° and 27°C for DdC (A), and for HT44 starved at 22°C (B) and 27°C (C). Both strains have both types of sites at both temperatures. Table 1 summarizes the Scatchard plot results from three such experiments. In HT44 at 27°C, the number of both high- and low-affinity sites has increased, and the K_d value for the low-affinity sites has increased at 27°C, a result observed in all such experiments.

Light scattering oscillations

In addition to chemotaxis, Gerisch and Hess (1974) first reported another assay for physiological function of receptors, oscillatory changes in light scattering in suspensions of starved cells, which reflect a positive cyclic AMP relay response. We were unable to obtain such a response from the parent DdC, as has been previously reported for this strain (Barclay and Henderson, 1986; Devreotes *et al.* 1987). However, HT44 did show such oscillations as also previously reported for a chemosensory mutant derived from DdC (Barclay and Henderson, 1986). Fig. 5 (A and B) shows that HT44 is able to respond to an initiating cAMP stimulus by producing light scattering oscillations. Both assays were performed at 22° after starvation at either 22° or 27°C. After similar starvation conditions, the 27°C amoebae were devoid of a chemotactic response (see above) but show oscillations of similar phase and amplitude at 27° as at 22°C.

Wild type cannot rescue HT44

Mixing of HT44 with the parent DdC in different proportions (100:1, 50:1, and 10:1 HT44 to DdC) indicated that the wild type cannot rescue the mutant at 27°C. Spores recovered from the fruiting bodies generated only wild-type plaques on bacterial lawns, i.e., they were not temperature sensitive for development. In

Table 1. *Scatchard analysis of cAMP-binding data*

	DdC		HT44	
	22°C	27°C	22°C	27°C
(a) K_d values (nM)				
High affinity sites	5·0	3·4	3·0	3·3
Low affinity sites	392	387	558	880
(b) Number of sites				
High affinity ($\times10^{-3}$)	1·6	3·3	2·0	7·1
Low affinity ($\times10^{-4}$)	3·7	2·8	4·1	6·5

Binding assays were performed as described in Methods. The results above represent the average of three independent experiments, and each gave essentially identical results.

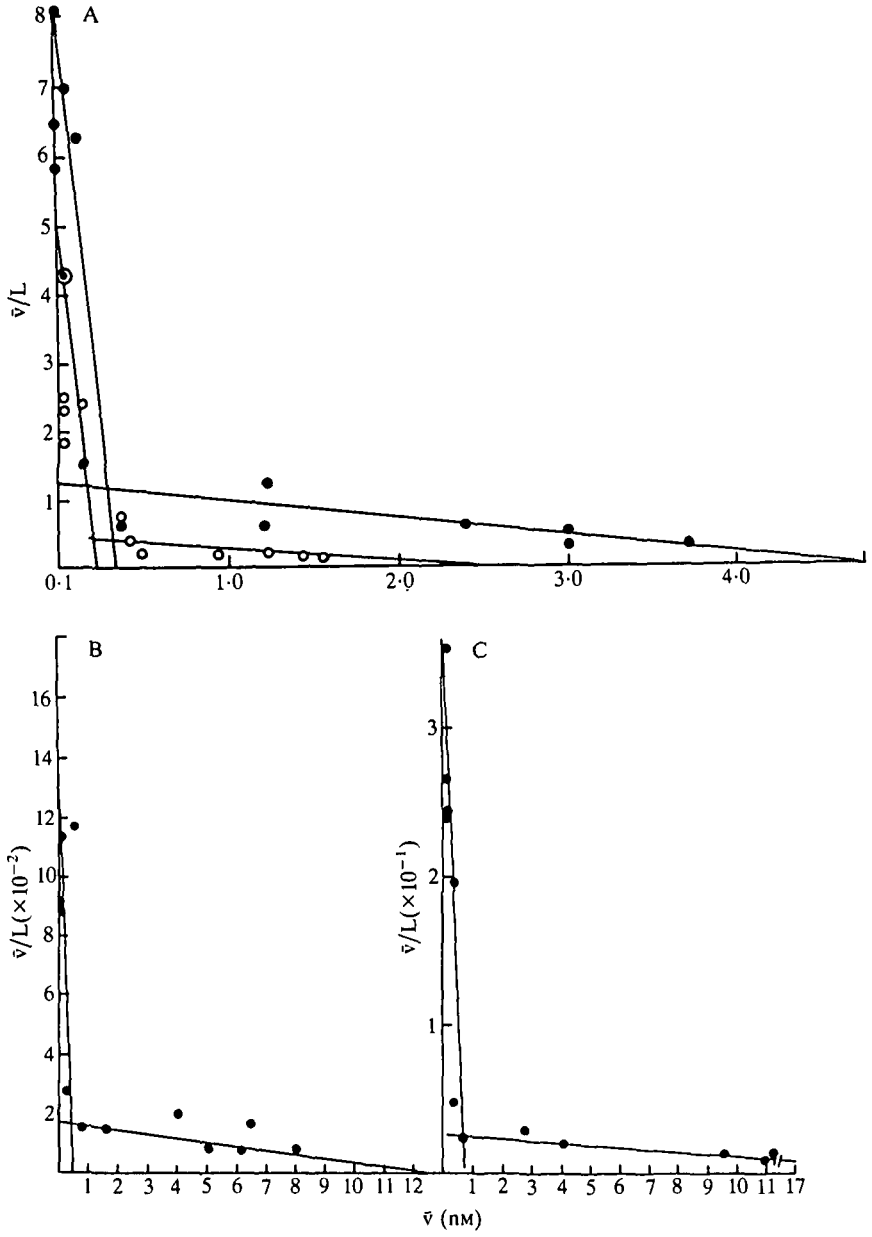

Fig. 4. Scatchard plots of equilibrium cAMP binding. Amoebae were starved for 9 h on KPM agar at 22° or 27°C, harvested, washed and assayed for binding at 4°C in 1·0 ml assay volumes containing 5×10^7 cells, 0·01 M-dithiothreitol, 0·2 mM-5′AMP and 0·4 to 420 pmoles of ^{3}H-cAMP. Nonspecific binding was determined at each cAMP concentration by inclusion of 100 μM-unlabelled cAMP. Panels are: (A) wild-type DdC starved at 22°C (solid symbols) or 27°C (open symbols); (B,C) HT44 starved at 22°C (B) or 27°C (C).

another type of experiment, parent and mutant amoebae were placed on opposite sides of a 0·1 or 0·2 μm pore size Amicon filter which was suspended over an agar support by a glass ring. The parent failed to rescue HT44 at 27°C, and HT44 did not inhibit the wild type at 27°C, suggesting the defect is not due to lack of a diffusible activator or production of a diffusible inhibitor.

Cell surface properties

The fact that the loose mounds formed by HT44, when plated at 27°C at high densities, do not develop further suggested the possibility that the cells had not expressed surface components required to establish the strong, EDTA-resistant cohesion characteristic of development. We tested the ability of cells to agglutinate in the presence and absence of 10 mM-EDTA at 22° and 27°C. Fig. 6 shows results with vegetative HT44 cells. At 22°C, the amoebae agglutinated to a similar extent independent of the presence of EDTA. At 27°C, there was less initial agglutination. In the absence of EDTA, the cells agglutinated fairly well at the earliest time point (60 min), but this declined over the next few hours (Fig. 6A). This early agglutination at 27°C was sensitive to EDTA (Fig. 6B).

Both DdC and HT44 were starved at 22° and 27°C for 16 h, by which time slugs form in wild type and in HT44 under permissive but not restrictive conditions. The cells were mechanically dispersed and tested for agglutination. In the absence of EDTA, DdC amoebae agglutinated equally well independent of temperature of starvation or assay (Fig. 7A). HT44 agglutinated well at 22° after starvation at 22°C but did not agglutinate well at 27° after 27°C starvation (Fig. 7B). The mutant expressed partial agglutination responses after starvation at 27° if then incubated for the assay at 22°C or

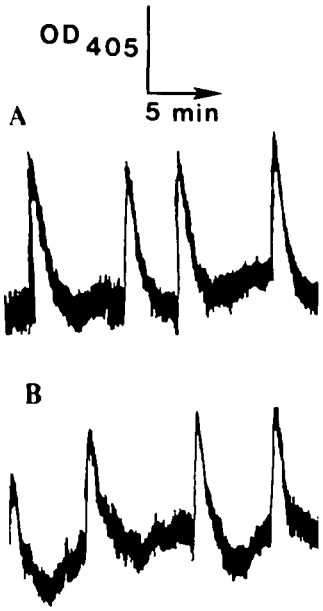

Fig. 5. Light-scattering oscillations. HT44 amoebae were starved on KPM agar for 5 h at 22 °C (A) or 27 °C (B), then harvested, washed, and monitored at 405 nm in rapidly oxygenated suspensions of 2×10^7 ml^{-1}. A single addition of cAMP (off scale left) initiated the oscillations.

vice versa. Results similar to those in Fig. 7 were obtained with cells starved 8–9 h and if the assay also contained 10 mM-EDTA (not shown).

To test whether the differences in endogenous agglutinability between DdC and HT44 were reflective of more general changes in the cell surface, agglutinations were performed in the presence of the lectins wheat germ agglutinin (WGA) and concanavalin A (con A). HT44 amoebae after 15 h of starvation at 22° or 27 °C were agglutinated well by WGA (Fig. 8A and B), as was the case with wild type. Incubation of dissociated amoebae with con A, however, revealed differences. DdC from 22° and 27 °C and HT44 from 22 °C agglutinated well (Fig. 8C shows the HT44 results). However, after starvation at 27 °C HT44 did not agglutinate in the presence of con A at either 22° or 27 °C (Fig. 8D), suggesting that the mutant has a defect in assembly or processing of asparagine-linked oligosaccharides.

Developmentally regulated enzymes

A number of enzymes show changes in specific activity at precise times during development (Loomis *et al.* 1976). In DdC at 22° and 27 °C and in HT44 at 22 °C, the specific activity of the very early enzyme *N*-acetylglucosaminidase (NAGase, Fig. 9) increased from the onset of starvation, reaching a maximum at 8–9 h and remaining constant thereafter. In contrast, in HT44 at 27 °C, the specific activity of NAGase increased fivefold but reached a maximum at a value two-thirds that of normally developing cells and decreased thereafter. A similar phenomenon has been observed with developmental mutants for expression of lysosomal α-mannosidase (Livi and Dimond, 1984). RNA and protein synthesis are needed for the increase in NAGase activity (Loomis, 1969).

One isozyme of β-glucosidase is expressed late in

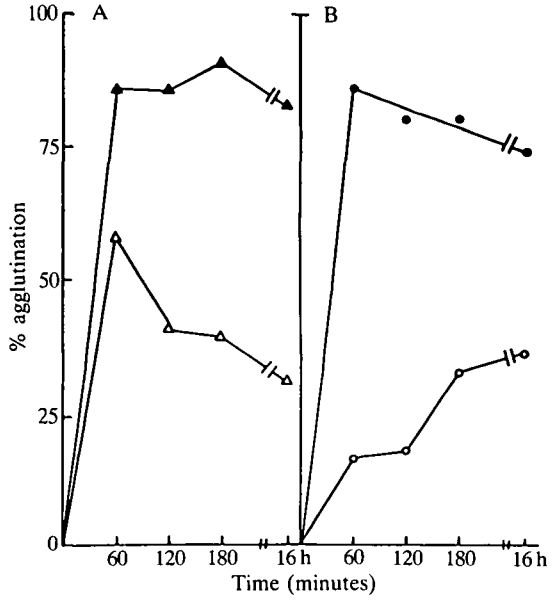

Fig. 6. Intercellular cohesion of HT44 amoebae in early development. Amoebae were harvested from growth in suspension at 22 °C, washed in KPM containing 10 mM-EDTA, and resuspended at 2×10^7 ml^{-1} in KPM without (A) or with (B) 10 mM-EDTA. The suspensions were shaken on a rotary platform at 200 revs min^{-1} at either 22 °C (solid symbols) or 27 °C (open symbols), and aliquots were removed at the indicated times for determining the number of single and doublet cells remaining. The decrease in this value is a measure of entry of the cells into large agglutinates and is shown as % agglutination.

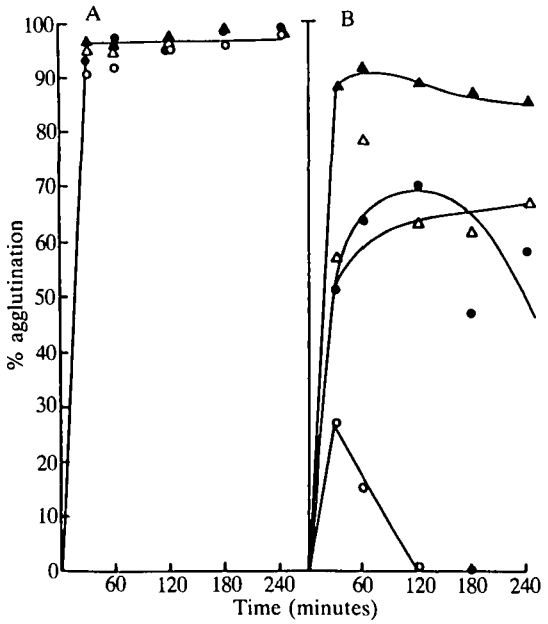

Fig. 7. Intercellular cohesion of HT44 amoebae after 16 h starvation. Amoebae plated and starved for 16 h were harvested, mechanically dispersed, washed in KPM buffer with 10 mM-EDTA and shaken at 200 revs min^{-1} in KPM without EDTA at 22° or 27 °C. (A) Wild-type DdC; (B) HT44. Amoebae starved at 22 °C were shaken at both 22 (solid triangles) and 27 (open triangles) °C. Amoebae starved at 27 °C were shaken at 22 (closed circles) and 27 (open circles) °C. Samples were taken at the indicated times and examined as described in Fig. 6.

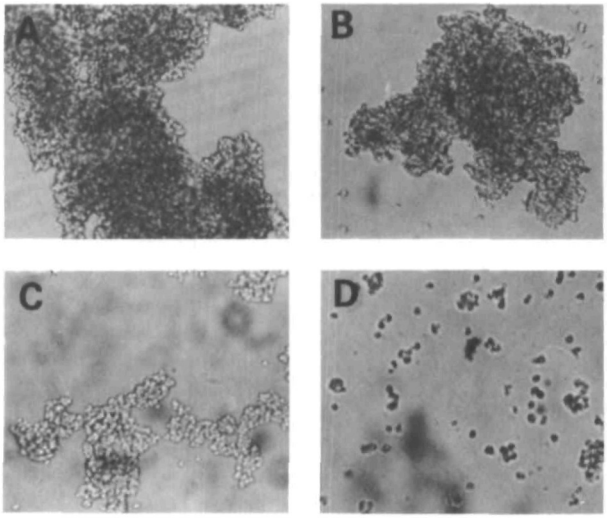

Fig. 8. Agglutination of HT44 amoebae by lectins. HT44 amoebae starved, as in Fig. 7, at 22°C (A,C) or 27°C (B,D) were washed and then resuspended at 2×10^6 ml^{-1} in the presence of 20 μg ml^{-1} of wheat germ agglutinin (A,B) or concanavalin A (C,D). The photographs show representative microscopic fields (400× magnification).

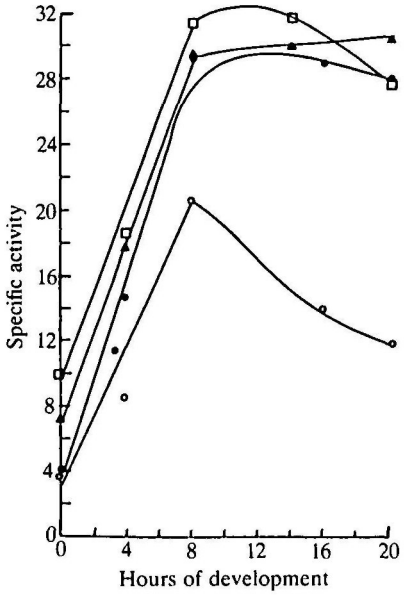

Fig. 9. Developmental regulation of *N*-acetylglucosaminidase activity. DdC (solid symbols) and HT44 (open symbols) amoebae were plated for development at 22°C (open boxes, closed triangles) or 27°C (circles). Samples were taken at the indicated intervals and assayed for *N*-acetylglucosaminidase as described in Methods.

development, at the beginning of culmination. Fig. 10 shows the expected decline in total β-glucosidase activity during early development and the appearance of the late developmental isozyme in DdC and in HT44 under permissive conditions. At the restrictive temperature, however, HT44 did not show this late increase.

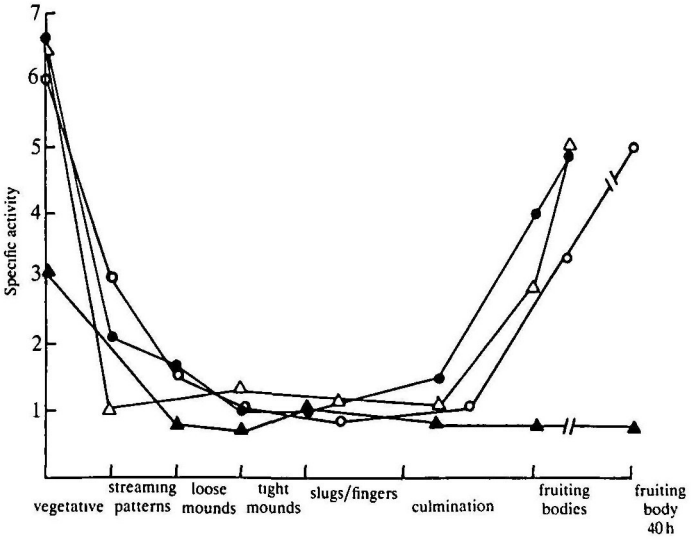

Fig. 10. Developmental regulation of β-glucosidase activity. DdC (circles) and HT44 (triangles) were starved as in the legend to Fig. 9 at 22°C (open symbols) or 27°C (closed symbols) and samples were assayed for β-glucosidase activity.

Protein synthesis in HT44

The NAGase and cAMP receptor data suggested that HT44 was not temperature sensitive in general protein synthesis and might undergo substantial biochemical differentiation at the restrictive temperature, despite the lack of morphogenesis. Rather than assay large numbers of additional specific biochemical events, we took advantage of the fact that, with wild type, there are orderly changes in the patterns of newly synthesized proteins which can be diagnostic of achievement of specific developmental stages (Alton and Lodish, 1977; Prem Das and Henderson, 1983). To examine the extent to which these changes occur in HT44, cells starving at 22° and 27°C were pulse labelled with [^{35}S]methionine for 30 min periods at different developmental time points, and the samples were analyzed by fluorography of SDS gels. HT44 incorporated [^{35}S]methionine into proteins at rates identical to DdC at the respective temperature (not shown). Figure 11 shows gel lanes from HT44 cells labeled at 27°C; the DdC profiles at 27°C for each stage were essentially identical (not shown).

Lanes 1 and 2 show the HT44 27°C band patterns for cells labeled after 1 and 8 h of starvation, respectively. At 8 h, DdC at both temperatures and HT44 at 22°C were undergoing streaming. While many minor bands appear to be the same, several changes in major bands are clearly seen and are the same as the wild-type vegetative-to-aggregation developmental transition. Lanes 3 and 4 directly compare band patterns for cells labelled at 8 and 16 h of starvation. At 16 h, the control populations had formed slugs. As before, while some bands are at the same relative molecular mass between the two lanes, several major bands establish the aggre-

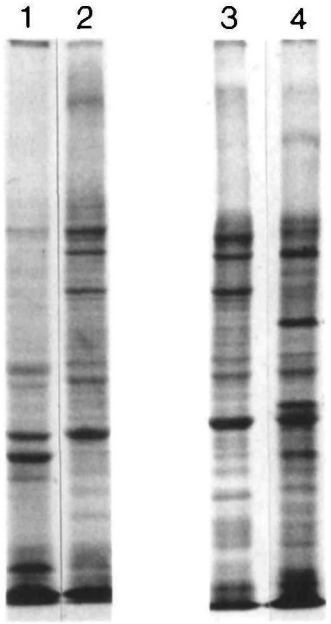

Fig. 11. Developmental regulation of protein synthesis. Exponentially growing HT44 cells were harvested, washed and plated on filters on KPM agar and incubated at 27°C. At 1 (lane 1), 8 (lanes 2 and 3) and 16 (lane 4) h, the filters were labelled with [^{35}S]methionine for 30 min at 27°C prior to SDS gel and fluorographic analysis of labelled proteins (ca. 100 000 cts min^{-1}) from an equal number of cells in each lane.

gation-to-slug transition in protein synthesis patterns. These results support the conclusion that the major biochemical switch to aggregation-stage and, later, to slug-stage protein synthesis occurs in the mutant at 27°C even though its morphological development is blocked prior to aggregation.

Discussion

We have exploited a selection method to obtain mutants that are conditionally unable to perform chemotaxis toward cAMP, based on their inability to move through a filter in contact with a solution of the chemoattractant (Barclay and Henderson, 1977). The mutant described in this report has a novel phenotype when compared with other mutants derived from the same selection scheme (Barclay and Henderson, 1982, 1986; Kesbeke and Van Haastert, 1988).

The mutant described here, HT44, grows normally, but mutant amoebae harvested from early log phase and plated at 27°C do not aggregate or make fruiting bodies. At the restrictive temperature, individual cells elongate and are motile, e.g., they collect at the edge of a droplet adjacent to a cAMP reservoir though their chemosensory response is too weak to allow escape from the area of the droplet (Fig. 2). When plated at high cell densities, the amoebae do assemble into loose mounds, but these do not form tips or show any visual signs of further development.

The phenotype of HT44 could be the result of a single mutation. The mutagenesis used to obtain HT44 allowed survival of 30–50% of the starting cells, to reduce the probability of multiple mutations, and temperature-sensitive mutants are usually a very low proportion of the total mutations. It is also highly unlikely that the developmental defects of cells starved for long periods are due to the presence of multiple mutations. The thermolabile period in HT44 is very short and is only during the initial stages of development. Mutant amoebae harvested from cultures which are past mid-log-phase growth, though still increasing in cell number, aggregate well when plated at 27°C and make normal fruiting bodies. There are two additional ways for HT44 to bypass the developmental block. First, amoebae starved for only 60–90 min at 22°C before a shift to 27°C develop normally. Second, if amoebae are starved overnight at 7°C (as described by Alcantara and Monk, 1974), when shifted to 27°C, they stream, aggregate and develop in a manner identical to the parent DdC.

At least two distinct control systems appear to be required for the transition of amoebae from growth to development. One is cessation of growth, most probably due to nutrient limitation. However, some early developmental events can be induced even when nutrients remain present. Ribosome run-off and synthesis of three early developmental proteins have been reported to occur even in nutrient broth if the cells were plated at a sufficiently high density (Margolskee *et al.* 1980). Also, Gerisch *et al.* (1985) have isolated a mutant that can enter development without removal of broth nutrients, though the developmental events did not occur until growth had ceased. In HT44, development does not occur at 27°C even when starvation is complete.

The second condition for development is usually observed as a requirement for a sufficiently high cell density. This can be replaced at least partially by cell-derived factors present in 'conditioned' medium (Clarke *et al.* 1987, 1988; Grabel and Loomis, 1978; Klein and Darmon, 1976; Mehdy *et al.* 1983; Mehdy and Firtel, 1985). The primary defect in HT44 cannot be the lack of such soluble factors since development of the mutant at 27°C cannot be rescued by presence of the wild type, and development of wild type in these mixtures rules out generation of an extracellular inhibitor by HT44.

The biological defect of HT44, observed several hours after the temperature-sensitive period, is a failure to aggregate, suggesting failure of the chemosensory mechanism. The cAMP cell surface receptor signal transduction systems in *D. discoideum* drive signal relay (synthesis and release of cAMP), chemotaxis, and several types of changes in gene expression. The current view is that all of these responses are mediated by GTP-binding G proteins. Equilibrium-binding studies of cAMP receptors yield nonlinear Scatchard plots indicating at least two affinity classes, and kinetic analyses indicate more than two species (Janssens and van Haastert, 1987; Gerisch, 1987). The sites of highest abundance and lowest overall affinity appear to regu-

late adenylate cyclase and the relay response, possibly via a G protein. The system that controls chemotaxis is believed to use G protein activation of phospholipase C which generates inositol triphosphate, thus releasing calcium which in turn activates guanylate cyclase (Newell *et al.* 1988).

The defect in HT44 is unlikely to be in the cAMP relay system since a single pulse of cAMP can entrain the population to begin spontaneous light scattering oscillations, and these reflect synthesis and release of cAMP to re-excite the population. The failure of HT44 to perform chemotaxis suggests a defect in the alternate response system, which also oscillates with optical density and includes alterations in cytoskeletal actin (McRobbie and Newell, 1983, 1984) and phosphorylation of myosin (Berlot *et al.* 1985; Rahmsdorf and Gerisch, 1978).

The phenotype of HT44 also indicates that it is not identical to any known mutants. Absence of cAMP receptors (Coukell *et al.* 1983; Klein *et al.* 1988) causes a complete failure to develop, but HT44 overproduces cAMP receptors of both classes. Some mutants that do not aggregate can be rescued by pulses of cAMP or by mixing with wild-type cells to supply the pulses (Darmon *et al.* 1977), but HT44 cannot be rescued by wild type. A strain containing a mutated *ras* gene shows normal regulation of adenylate cyclase but underproduces cGMP and has an increased basal level of inositol polyphosphates, correlated with a reduced chemotactic response to cAMP (Europe-Finner *et al.* 1988; van Haastert *et al.* 1987). The biological phenotype of the mutant *ras* transformant (Reymond *et al.* 1986) is like chemosensory mutant HB3 which generates multiple tips on cell mounds (Barclay and Henderson, 1986) and is distinct from that of HT44.

cAMP regulates expression of a number of developmental genes (reviewed in Gerisch, 1987). In early development, this includes a positive feedback loop for expression of cAMP receptors and the induction of gp80, a glycoprotein implicated in aggregation-stage cohesion. The properties of HT44 suggest that this component of the chemosensory system functions normally, as the cAMP receptors are expressed at the correct time after starvation. While the later decline in the numbers of these receptors is slower than under permissive conditions, they do decrease to wild-type levels. This decrease is not observed when wild-type cells are starved in shaken suspension to prevent late developmental events (Henderson, 1975). By this criterion, HT44 behaves as if aggregation had been completed.

The same conclusion is reached when newly synthesized proteins are examined. The wild type at both temperatures and the mutant at 22°C display stage-specific profiles of such proteins in which the pattern during aggregation is distinct from that of newly plated cells and the pattern at the slug stage differs from both of the earlier patterns. Despite the absence of any signs of aggregation, HT44 at 27°C showed these stage-specific changes.

A large number of *Dictyostelium* mutants have been shown to fit a dependent sequence model of development in which one biochemical stage cannot be achieved until the earlier ones are complete (Loomis *et al.* 1976). However, Livi and Dimond (1984) described aggregation-deficient mutants in which the accumulation of α-mannosidase terminated at intermediate levels. Their results also indicated that the early developmental increases in *N*-acetylglucosaminidase and α-mannosidase are controlled by different systems. Both of these results are incompatible with an all-or-none dependent sequence.

While the culmination-stage β-glucosidase does not appear in HT44 at 27°C, the appearance and loss of cAMP receptors, recently shown to reflect changes in the mRNA (Klein *et al.* 1988), and the stage-specific transitions in protein synthesis can only be accounted for by the existence of parallel but independent regulatory sequences. We are unaware of any other mutants with this phenotype.

The primary biochemical defect in HT44 is not yet known. The mutant fails to perform chemotaxis toward cAMP after starvation at 27°C. However, chemotaxis has been shown to be nonessential for aggregation since several chemotaxis-defective mutants are able to make small cell mounds and complete development to small fruiting bodies (Barclay and Henderson, 1986; Wallraff *et al.* 1984). It is likely, however, that generation of small mounds by these mutants is dependent upon the presence of a fully functional system for intercellular cohesion, in order to collect and retain cells which collide, and this may be the crucial defect in HT44.

At the restrictive temperature, HT44 is deficient in both EDTA-sensitive and EDTA-resistant cohesion. In accord with these defects, amoebae plated at very high densities produce, at best, loose mounds which do not further develop. This also correlates with poor agglutination of 27°C-starved amoebae by concanavalin A. This may reflect a defect in the early developmental changes which normally occur in the processing of asparagine-linked oligosaccharides (Ivatt *et al.* 1981, 1984). Two glycoproteins, gp80 (Hohmann *et al.* 1987*a*, 1987*b*) and antigen 117 (Sadeghi *et al.* 1987), implicated in intercellular cohesion during aggregation contain *N*-linked oligosaccharides. We have recently reported that endoglycosidase H-resistant, *N*-linked glycopeptides derived by pronase digestion of plasma membrane glycoproteins can inhibit intercellular cohesion of aggregation-stage amoebae (Ziska and Henderson, 1988), and glycosylation mutants are defective in aggregation stage cohesion (Boose *et al.* 1988). HT44 may provide insight into the relationship between protein glycosylation and cohesion.

Finally, the ability of HT44 to undergo a significant amount of temporal biochemical differentiation places severe restrictions on regulatory roles in this process which may be attributed to formation of stable intercellular contacts. Mutants like HT44 should allow more precise definition of mechanisms underlying contributions of cell–cell contacts as well as cAMP receptor-mediated signal transduction to the regulation of developmental gene expression.

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