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*UDP-GALACTOSE POLYSACCHARIDE TRANSFERASE IN THE CELLULAR SLIME MOLD, DICTYOSTELIUM DISCOIDEUM: APPEARANCE AND DISAPPEARANCE OF ACTIVITY DURING CELL DIFFERENTIATION**

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Dictyostelium discoideum amoebae, upon entering the stationary growth phase, collect together into organized, multicellular aggregates which are ultimately converted into fruiting bodies that contain at least two differentiated components, spores and stalk cells. A schematic diagram of the morphogenetic sequence accompanies the data shown in Figure 4.

The syntheses of three polysaccharides are keyed to this sequence and their developmental kinetics are governed by the over-all rate of morphogenesis.[1, 2] None is synthesized by mutant strains unable to accomplish the later stages of fruiting body construction. One of the three is an acid mucopolysaccharide, composed of galactose, galactosamine, and galacturonic acid; together these sugars constitute at least 92 per cent of the dry weight of the purest preparations. This polysaccharide is serologically reactive against anti-*D. discoideum* spore sera and anti-pneumococcal (type VII) serum, and reaction with either antiserum is blocked by galactose and lactose (50% as active at equimolar concentrations), but by no other carbohydrate tested. This component, as measured by quantitative complement fixation or by bound nondialyzable galactose or galactosamine, first makes its appearance during the transformation of cell aggregates into migrating pseudoplasmodia. It is synthesized rapidly during actual fruiting body construction and ultimately reaches a peak of 1–2% of the dry weight.

These data led us to an examination of the enzymes that might be responsible for the synthesis of the mucopolysaccharide in order to determine if they, too, are controlled by the over-all morphogenetic program. Prior work[3–5] has indicated that galactose-containing polysaccharides are synthesized by the following transfer reaction:

$$\text{UDP-Galactose} + \text{Acceptor} \rightarrow \text{Galactosyl-Acceptor} + \text{UDP}.$$

It has been possible to demonstrate the presence of a UDP-galactose polysaccharide transferase, catalyzing the above reaction, in cell-free extracts of *D. discoideum*. The enzyme does indeed appear to be under morphogenetic control. Some of its properties and its developmental kinetics are described below.

Materials and Methods.—Organisms: *D. discoideum* strain NC-4 (haploid) wild type and mutants thereof were grown on SM agar in association with *Aerobacter aerogenes.*[6]

Substrate: UDP-galactose-C^{14} was prepared according to the method of Osborn *et al.*[5]

Analytical techniques: Total protein was determined by the Folin assay,[7] using a bovine serum albumin standard. Galactose was measured indirectly by the anthrone reaction,[8] corrected for glucose by glucostat assays,[9] and directly by the galactose oxidase assay;[10] galactosamine was determined by the Elson-Morgan procedure.[11]

Preparation of homogenates: Cells were harvested at appropriate developmental stages in 0.01 *M* Tris[12] buffer, pH 7.5, collected by centrifugation, and suspended at high concentration (20–50 mg per ml dry weight) in Tris buffer. The suspension was passed twice through a cold French pressure cell producing virtually complete cell breakage. The total extract or the 10,000 × *g* supernatant fluid obtained from it was employed for assay of galactose incorporation.

Assay conditions: The reaction mixture contained: 54 mM dimethylglutarate buffer, pH 7.5; 14 mM KCl; 1.8 mM $MgCl_2$; 50 mμM C^{14}-labeled UDP-galactose (4×10^5 cpm/μmole); acceptor (see below); and the enzyme. The final volume was 0.28 ml. After incubation for 1 hr at 30°C, 0.25 ml of 0.1 *N* HCl was added, and the mixture was heated 15 min in a boiling water-bath. Three ml of 95% ethanol were added and, after 10 min in the cold, the mixture was centrifuged and the supernatant fluid aspirated. After three washings with 80% ethanol, the pellet was taken up in alkaline 50% ethanol and dispersed on a planchet for counting. Treatment with dilute acid was necessary to minimize nonspecific adsorption of radioactivity to the ethanol precipitate.

Results.—Acceptor requirement: Incubation of UDP-galactose-C^{14} with extracts prepared from cells harvested during the period of active mucopolysaccharide synthesis (20–30 hr after the start of the morphogenetic sequence) resulted in a small but significant transfer of galactose into an ethanol-insoluble product (Table 1). This incorporation was markedly increased by addition of a suitable acceptor

TABLE 1

INCORPORATION OF C^{14}-GALACTOSE INTO THE ETHANOL-INSOLUBLE FRACTION

Reaction mixture	Galactose incorporation, mμmoles
Enzyme alone	0.13
Enzyme and acceptor	1.12
Enzyme and boiled acceptor	1.02
Enzyme and 10,000 × *g* supernatant fluid from boiled acceptor	0.99
Acceptor alone	0.01

Assays were carried out as described in *Materials and Methods.* The enzyme preparation was the 10,000 × *g* supernatant fraction obtained from cells actively synthesizing mucopolysaccharide. The acceptor was the total extract from mature fruiting bodies.

provided by the supernatant fraction of a boiled extract prepared from mature fruiting bodies (Table 1). It has previously been shown[2] that maximal levels of the galactose-containing mucopolysaccharide are present at this stage of development. The active acceptor was purified by a modification of a procedure used previously for the isolation of the mucopolysaccharide. The fruiting bodies were suspended in water, pressed, and boiled 15 min. After centrifugation, the pellet was re-extracted with boiling water and centrifuged. The two supernatant fractions were pooled, adjusted to pH 7.5, concentrated *in vacuo* at 50°C, and cleared by centrifugation. Ethanol was added to a concentration of 35 per cent. The precipitate that formed in the cold was removed, and the ethanol concentration of the supernatant was raised to 65 per cent. The resulting white-flocculent precipitate was washed with 95 per cent ethanol, dissolved in water, reprecipitated with 65 per cent ethanol, and again dissolved in water. Trichloracetic acid (TCA) was added at a final concentration of 10 per cent. After removal of the TCA-insoluble material, the supernatant was dialyzed against 0.9 per cent NaCl, alcohol

was added to a concentration of 65 per cent, and the final precipitate was dissolved in water. Such preparations were found to contain Folin positive material (10–20% of the dry weight as protein), negligible amounts of orcinol reactive material, and the remainder as the mucopolysaccharide by antigenic assay and by galactose and galactosamine determinations. Figure 1 shows that galactose incorporation was a linear function of the concentration of this acceptor fraction, here expressed in terms of its galactosamine content.

An acceptor fraction with two- to fourfold higher specific activity could be isolated by the same procedure from cells harvested at earlier stages of mucopolysaccharide synthesis, at a time when mucopolysaccharide content was approximately 50 per cent of the maximal level (cf. Fig. 4). This material was applied to a Sephadex G-200 (15 × 1.5 cm) column. The distribution of acceptor activity in the eluate is shown in Figure 2*a*. The first (excluded) peak which had the galactose and galactosamine content and the antigenic reactivity characteristic of the completed mucopolysaccharide displayed relatively low acceptor activity. The lower molecular weight component had no antigenic reactivity, but was a considerably better acceptor of galactose. Although the composition of this fraction was qualitatively similar to that of the immunologically active mucopolysaccharide, the ratio of galactosamine to galactose was considerably lower in the active acceptor fraction (13%) than in the less active mucopolysaccharide (40%) (Fig. 2*b*). The structural relationship between the two mucopolysaccharide fractions is not yet clear, but it is possible that, in the intact cell, the signal for cessation of galactose incorporation into the polysaccharide is provided by addition of N-acetyl galactosamine in terminal positions.

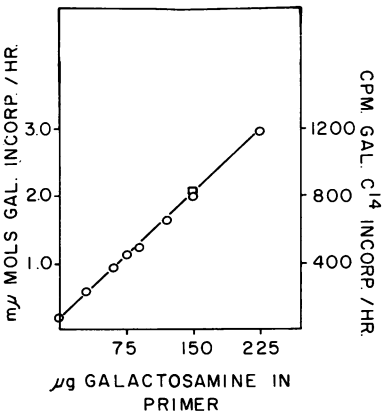

FIG. 1.—Relation between the rate of galactose-C^{14} incorporation and the concentration of acceptor. The latter was prepared according to the procedure given in the text. The enzyme preparation was a 10,000 × *g* supernatant containing 1.48 mg protein/assay tube. Incubation time: 1 hr.

Properties of the UDP-galactose transferase system: In the presence of added acceptor, incorporation of galactose into the alcohol-insoluble fraction was linear with time for 2 hr and was proportional to enzyme concentration up to 1.5 mg protein per assay tube. No clear requirement for Mg^{++} or Mn^{++} was observed, but the reaction was stimulated approximately 2.5-fold by K^+ at a concentration of $1.4 \times 10^{-2} M$.

Characterization of the reaction product: Examination of the isolated C^{14}-galactose-labeled reaction product provided additional evidence that acidic mucopolysaccharide acts as acceptor of the transferred galactose. After paper electrophoresis of the ethanol-insoluble product at pH 6.0 (Fig. 3), two peaks of radioactivity were observed. The bulk of the radioactivity of the product migrated toward the anode and was associated with a minor, somewhat heterogeneous component of the acceptor polysaccharide. This material was characterized by a low galactosamine: galactose ratio, was inactive in complement fixation, and corresponded to the low

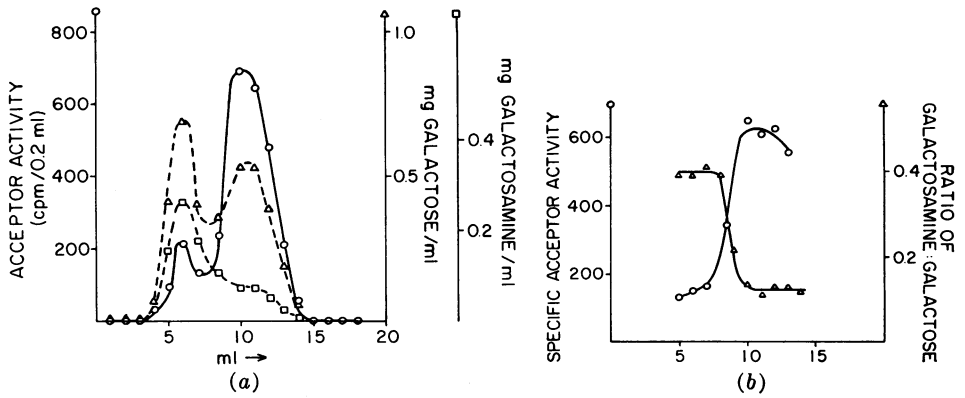

FIG. 2.—(*a*) Distribution of galactose and galactosamine content and acceptor activity in the eluate from a Sephadex G-200 column. One ml of acceptor preparation was applied to a 1.5 × 15-cm column and eluted with water. Initial drop rate: 55 sec. (*b*) Distribution of specific acceptor activity (cpm of galactose-C^{14} incorporated/hr/μg galactose in the acceptor) × 100, and the ratios of galactosamine : galactose in the eluate.

molecular weight, highly active acceptor fraction in chromatography on Sephadex G-200 (cf. Fig. 2). The smaller, electrophoretically nonmigrating peak of radioactivity showed a high galactosamine:galactose ratio and high complement-fixing activity, and corresponded to the high molecular weight acceptor fraction on Sephadex G-200. The failure of this fraction to migrate during paper electrophoresis is probably attributable to its high molecular weight, since it is known to con-

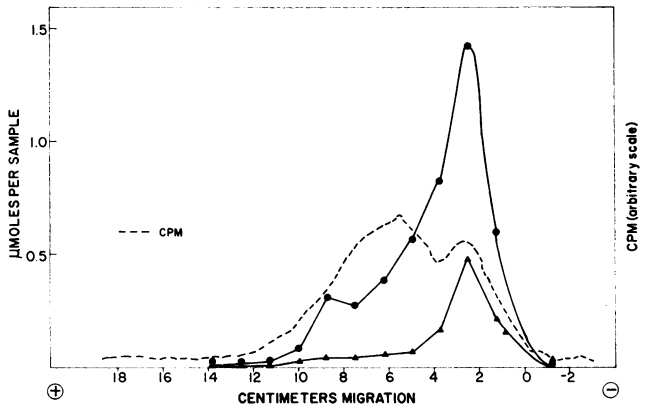

FIG. 3.—Paper electrophoresis of the reaction product. Electrophoresis was carried out on Whatman no. 1 paper in pyridine : acetic acid: H_2O (10 : 1 : 69) pH 6.0, at 50 volts/cm for 4 hr. The paper was scanned for radioactivity with a Baird-Atomic 4π Scanogram. The paper was cut into strips 1 cm in width, eluted, and assayed for galactose and galactosamine. The dotted line: radioactivity. The solid line with circled points: galactose; with triangles: galactosamine.

tain galacturonic acid. The product isolated from reactions using acceptor derived from mature fruiting bodies yielded electrophoretic patterns similar to those obtained with acceptor from earlier stages of mucopolysaccharide synthesis.

After complete acid hydrolysis of the labeled products, all of the radioactivity was recovered as free galactose, as judged by cochromatography with carrier, nonradioactive galactose.

Developmental kinetic of the enzyme activity: Mass cultures were harvested from the stationary growth phase, washed three times by centrifugation (950 × *g* for 5 min), suspended at a density of 2 × 10^7 amoebae/ml in 0.05 *M* phosphate buffer pH 6.0 with streptomycin (0.5 gm/liter), and shaken at 22°C for 3 hr. At the end of this time the viable bacterial count had decreased to about 2 × 10^3 ml, representing a contamination of 1 part in 10^6 by weight. (The dry mass of a bacterial cell is *ca.* 1% that of a *D. discoideum* myxamoeba.) Although extracts of *A. aerogenes* have some galactose-incorporating activity, it is undetectable at this level of contamination. The amoebae were then harvested and dispensed on 2 per cent noble agar containing 0.5 mg/ml streptomycin sulfate at a cell density of 2.5 × 10^8/plate (*ca.* 50% of their density on growth plates). Under these conditions the cells proceed through the morphogenetic sequence with a high degree of synchrony,[13] according to the time scale shown in Figure 4. Aliquots were harvested at intervals in 0.01 *M* Tris buffer pH 7.5, collected by centrifugation, and pressed. Both crude extracts and 10,000 × *g* supernatant fractions were assayed in the presence and absence of a standard acceptor preparation whose activity corresponds to the level shown in Figure 1 for a concentration of 75 μg galactosamine. The difference between endogenous and primed incorporations was taken to be a measure of the specific enzyme activity expressed as μμmoles galactose incorporated/hr/-mg protein. Figure 4 shows the developmental kinetics of this activity in *D. discoideum* wild type and represents the combined results of four separate experiments.

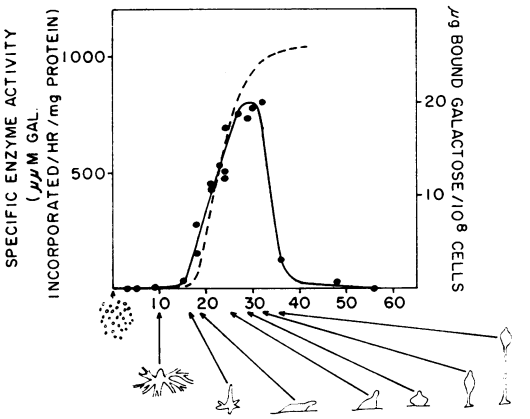

FIG. 4.—Developmental kinetics of the transferase activity in *D. discoideum* wild type. Abscissa: time in hours after deposition of cells on nonnutrient agar. Schematic diagrams: developmental stages attained. Dotted line: amount of mucopolysaccharide measured by bound, nondialyzable galactose content.[2]

UDP-galactose transferase activity was undetectable until after 15 hr of incubation, by which time the amoebae had completed aggregation and had been organized into migrating pseudoplasmodia (fourth structure in the schematic diagram). The activity subsequently increased over a 10–15 hr period and at its peak reached a level at least 400-fold greater than the limit of assay sensitivity. The peak preceded the appearance of mature fruiting bodies by a few hours. Subsequently the enzyme activity decreased sharply and fell to undetectable levels. Note that the initial rise occurred approximately an hour before the appearance of the mucopolysaccharide, here expressed in terms of its galactose content, and the subsequent fall in activity coincided with the cessation of polysaccharide synthesis.

The absence of activity in the periods prior to 15 hr and after 32 hr might conceivably be due to the presence of an enzyme inhibitor. Accordingly, mixtures of active and inactive extracts were assayed in order to determine if such an inhibi-

tion was operative. The data given in Table 2 lend no support to this possibility. The 3-hr extract, itself inactive, did not affect the activity of the 24-hr extract in the presence of a standard concentration of acceptor. The 36-hr extract whose specific enzyme activity was about 15 per cent of the peak value actually increased the rate of galactose incorporation by the mixture, presumably due to the presence of additional acceptor. (It should be noted that the concentration of acceptor added in these experiments was below a saturating level.) Similarly the 9-hr extract, though itself without enzyme activity, appeared to contain additional acceptor. This observation is surprising, since a nondialyzable mucopolysaccharide fraction is not detectable at this time. The nature of this early acceptor is under investigation.

Developmental kinetics of morphogenetically deficient mutants of D. discoideum: Three mutant strains were employed, with properties summarized below.

AGG-204: The myxamoebae grow normally, but fail to aggregate after cessation of growth. They do not synthesize the mucopolysaccharide nor do they form any of the differentiated cell types (spores and stalk cells) or other biochemical end products (pigment, cellulose, a number of antigens, etc.) characteristic of the wild-type fruiting body.[14]

Fr-2: The myxamoebae aggregate in typical fashion, but produce irregular mounds which fail to develop into pseudoplasmodia or fruiting bodies. The mucopolysaccharide is not synthesized nor are any of the other products listed above.[14]

Fr-17: The myxamoebae aggregate, producing flat amorphous mounds. Squashes and histological sections of the latter reveal the presence of large numbers of typical spores mixed in with short segments of stalk containing typical vacuolated cells. All the biochemical end products listed above appear in this mutant, including the mucopolysaccharide. In addition, Fr-17 reaches its ultimate developmental state in about half the time it takes the wild type. The individual biochemical and morphological end products appear in an accelerated succession. Thus, the mutation has affected not only the morphogenetic pattern, but its temporal control.[15]

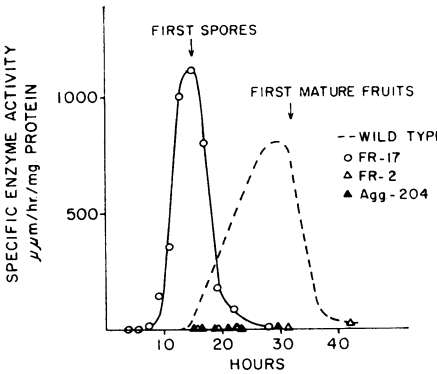

FIG. 5.—Developmental kinetics of transferase activity in three mutant strains of *D. discoideum.*

Figure 5 summarizes the developmental kinetics of the enzyme activities in the three strains. Neither AGG-204 nor Fr-2 displayed significant levels of activity during the period of incubation. In contrast, in Fr-17 the activity appeared much sooner, rose faster, and disappeared sooner than in the wild type. The acceleration was consistent with (*a*) the time of the appearance of the mucopolysaccharide in this mutant, and (*b*) the over-all acceleration of morphogenetic events in this strain.

It is not likely that the phenotypes of AGG-204 and Fr-2 result from mutations in the structural gene responsible for the synthesis of the transferase. Many other and distantly related metabolic activities are also blocked, and the morphogenetic

TABLE 2

C^{14}-GALACTOSE INCORPORATION BY MIXTURES OF ENZYME EXTRACTS

Extracted from Cells Incubated for (hr): 3	9	24	29	36	Mμmole Incorporated/hr	Sums of separate activities	Difference
Mg Protein Present: 2.7	2.0	1.48	1.2	0.95			
+	...	...	...	...	0.022	...	...
...	+	...	...	...	0.025	...	...
...	...	+	...	...	1.200	...	...
...	...	...	+	...	0.781	...	...
...	...	...	...	+	0.254	...	...
+	...	+	...	...	1.220	1.222	−0.002
...	+	+	...	...	1.503	1.225	+0.278
...	+*	+	...	...	1.610	...	+0.385
...	...	...	+	+	1.320	1.035	+0.285

* Heated at 100° for 3 min.

Assays were carried out as previously described. Enzyme preparations were 10,000 × *g* supernatants of pressed cells harvested at the indicated times. A standard amount of exogenous acceptor, containing 75 μg galactosamine, was added to each assay tube. As noted in Fig. 1, this is below saturating levels.

abnormalties begin long before the time of appearance of the transferase activity in the wild type. Thus, it seems more likely that the mutants in AGG-204 and Fr-2 have affected the control systems which trigger transferase synthesis and/or activity. Certainly this must be the case for the mutant Fr-17.

Summary.—UDP-galactose polysaccharide transferase activity has been demonstrated in the cellular slime mold *Dictyostelium discoideum*. It is absent in the vegetative cells and does not appear until relatively late in the developmental sequence, shortly before actual synthesis of a galactose-containing mucopolysaccharide. It reaches a peak of activity shortly before the end of fruiting body construction and then disappears. Two morphogenetically deficient mutants which fail to synthesize the mucopolysaccharide (as well as many other morphological and biochemical end products) fail to display significant levels of enzyme activity at any time. A third mutant, in which temporal control of morphogenesis has been altered, displays a consonant variation in the developmental kinetics of the transferase activity.

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