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The Control of Saccharide Synthesis during Development of Myxamoebae of *Dictyostelium discoideum* Containing Differing Amounts of Glycogen

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1. Myxamoebae initially containing 5.59mg of glycogen/10^8 cells accumulate approx. 25% more cell-wall polysaccharide, 100% more mucopolysaccharide, 200% more glucose and 300% more trehalose during their development than do myxamoebae initially containing less than 0.3mg of glycogen/10^8 cells. 2. These observations restrict the number of possible control mechanisms operating to regulate carbohydrate metabolism during development. 3. Cells accumulating a large amount of trehalose (approx. 400μg/10^8 cells) have the same amount and pattern of changes in specific activity of trehalase and trehalose 6-phosphate synthase as do cells accumulating a smaller amount of trehalose (approx. 100μg/10^8 cells). 4. These two populations of cells do, however, differ markedly in the amount of UDP-glucose and glucose 6-phosphate that they contain. 5. It is concluded that this change in the intracellular pools of the metabolic precursors of trehalose accounts for the increased amount of trehalose synthesized by cells derived from myxamoebae containing an increased glycogen content.

Hames & Ashworth (1974) have reported that myxamoebae of *Dictyostelium discoideum* strain Ax-2, on entrance into the developmental phase of their life cycle, rapidly degrade most of the glycogen they may have accumulated during the growth phase and oxidize the products to CO_2. However, the data further suggested that not all of the myxamoebal glycogen need be metabolized in this way and that some can be used to synthesize non-glycogen carbohydrates. It is known that during differentiation a number of novel carbohydrates (cell-wall polysaccharide, trehalose, and a mucopolysaccharide; collectively referred to as end-product saccharides) are synthesized (White & Sussman, 1961, 1963*a*,*b*; Ceccarini & Filosa, 1965; Wright *et al.*, 1968; Cleland & Coe, 1969). These studies were restricted to populations of cells which had been grown on bacteria and all the authors quoted above agree that under such conditions the pattern of carbohydrate synthesis and accumulation is both qualitatively and quantitatively reproducible. However, myxamoebae grown on bacteria contain a characteristic and largely invariant amount of reserve material to use for end-product saccharide synthesis. The observed constant pattern of carbohydrate synthesis might therefore have had more to do with the constancy of the amount of precursor available than the precision of the control mechanisms regulating carbohydrate synthesis and development.

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We report, in the present paper, that marked differences in carbohydrate synthetic patterns can be observed during the development of cells initially containing different amounts of glycogen, and that there are marked differences in the way in which different metabolic pathways respond to this stress. Since cells develop according to a very similar morphogenetic pattern despite variations in glycogen content (Garrod & Ashworth, 1972) and end-product saccharide synthesis there is therefore no necessary connection between the controls regulating carbohydrate metabolism and development as suggested by Wright (1973).

Experimental

Materials

ATP, NAD^+, $NADP^+$, UDP-glucose, glucose 6-phosphate, anthrone, *o*-dianisidine, hexokinase, peroxidase, galactose oxidase, and DEAE cellulose were obtained from Sigma (London) Chemical Co. Ltd., London W.5, U.K.; phosphoglucomutase, glucose 6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were obtained from the Boehringer Corp. (London) Ltd., London W.5, U.K.; trehalose was obtained from BDH Chemicals Ltd., Poole, Dorset, U.K.

Dowex 1 (X8; AG; Cl^- form) was obtained from Bio-Rad Laboratories, St. Albans, Herts., U.K.;

Dowex 1 (formate form) was prepared from Dowex 1 (Cl^-) as described by Weeks & Ashworth (1972).

All other chemicals were of the highest purity commercially available and obtained either from BDH Chemicals Ltd. or Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K.

Methods

Growth and differentiation of myxamoebae. Myxamoebae of *D. discoideum* Ax-2 (ATCC 24397) were grown, harvested, allowed to differentiate and cell extracts prepared as described previously (Hames & Ashworth, 1974).

Determination of cellular glycogen content. Cellular glycogen content was determined as described previously by Hames *et al.* (1972).

Determination of mucopolysaccharide. Cell extracts were prepared as described previously (Hames & Ashworth, 1974), 9ml of extract was required for each mucopolysaccharide determination. Then 5.2ml of 0.3M-$Ba(OH)_2$ and 4.0ml of 5% (w/v) $ZnSO_4$ were added to 4.5ml of cell extract mixing well after each addition. Insoluble material was removed by centrifugation at 1000*g* for 15min. The supernatant was evaporated to dryness at 50°C under vacuum and the residue dissolved in 2ml of water. After neutralization with KOH a sample of this solution was assayed for free galactose and galactosamine (pre-hydrolysis sample) by using the galactose oxidase assay (Roth *et al.*, 1965), except that 0.3ml of 1% *o*-dianisidine in 95% ethanol was used in the assay mixture instead of benzidine, and absorbance measurements were made at 400nm instead of 295nm 20min after the acidification which is carried out to stop the reaction. This procedure was found to give more reproducible results than that described by Roth *et al.* (1965) and gave no cross reaction with up to 30mM-glucose.

Then 0.5ml of 1.0M-HCl was added to 4.5ml of the same cell extract and the mucopolysaccharide was hydrolysed at 95°C for 4h. After cooling the sample in ice for 10min, insoluble material was removed by centrifugation at 1000*g* for 15min, the supernatant was evaporated at 50°C under vacuum and the residue was dissolved in 3ml of water. After neutralization, 2.6ml of 0.3M-$Ba(OH)_2$ and then 2.0ml of 5% (w/v) $ZnSO_4$ were added to the solution, mixing well after each addition. The solution was left in ice for 30min and then centrifuged and evaporated to dryness as before. The residue was then dissolved in 2ml of water and a sample assayed for free galactose and galactosamine (post-hydrolysis sample) by using the galactose oxidase method described above.

The difference in free galactose and galactosamine content of the pre- and post-hydrolysis samples represents the amount of mucopolysaccharide present in the sample.

Determination of cell wall polysaccharide. For this procedure 1ml of 90% (w/v) KOH was added to 2ml of cell extract and the suspension was heated at 100°C for 20min. Insoluble material was removed by centrifugation at 17000*g* for 10min and extracted with 1ml of 30% (w/v) KOH at 100°C for a further 20min. After re-centrifugation, the pellet was washed twice with 2ml of water each time, and finally dissolved in 67% (w/v) H_2SO_4 (2h at room temperature). A sample of this solution was then assayed for hexose content by the anthrone method of Hassid & Abraham (1957).

Chromatography of trehalose. Trehalose was isolated from cell extracts by chromatography, eluted and assayed as described by Hames & Ashworth (1974).

Assay of trehalase. Trehalase was assayed by the method of Ceccarini (1966) with cell samples harvested in 0.016M-sodium potassium phosphate buffer, pH6.0, and frozen at −70°C.

Assay of trehalose 6-phosphate synthetase. The cells from four Millipore filters were harvested in 3ml of ice-cold 10mM-Tris–HCl, pH7.5, containing 1mM-dithiothreitol, sonicated, and assayed immediately for trehalose 6-phosphate synthetase activity by measuring the formation of UDP as described by Roth & Sussman (1968).

Determination of glucose 6-phosphate and UDP-glucose. Samples were prepared by harvesting the cells from 12 Millipore filters in 4.5ml of ice-cold water, adding 0.25ml of ice-cold 60% (w/v) perchloric acid, and then sonicating with a 100W MSE ultrasonic disintegrator (peak-to-peak amplitude of 7 μm) for six 15s periods with continuous cooling in an ice–salt bath to prevent the temperature rising above 4°C. The entire procedure took approx. 10min/sample.

After sonication, the mixture was incubated for 30min at 0°C and the resulting precipitate was removed by centrifugation. The supernatant was neutralized with ice-cold 5M-KOH, re-centrifuged to remove insoluble potassium perchlorate, and then freeze-dried. The residue was redissolved in 2ml of water and 0.2ml samples were used to assay glucose 6-phosphate by using the fluorimetric procedure described by Weeks & Ashworth (1972). The remainder of the sample was used for UDP-glucose isolation and measurement as described by Weeks & Ashworth (1972) except that 10ml of 1.5M-ammonium formate were used to elute UDP-glucose from the Dowex 1 (formate) columns, and that each fluorimetric assay for UDP-glucose contained 1nmol of Tris–HCl buffer, pH8.5.

Determination of sorocarp glucose and trehalose distribution. Mature fruiting bodies (40h) were harvested from Millipore filters rapidly in water and the suspension passed through four layers of wet muslin, thus trapping the stalks but not the spores. The entrapped stalks were washed well and then resuspended in water. Stalk and spore suspensions were then

analysed for glucose and trehalose as described previously (Hames & Ashworth, 1974). Comparison of the glucose and trehalose contents of stalk cells and spores on the basis of cell number is not feasible since the stalk cells are encased in the stalk matrix rendering them impossible to count. Likewise, protein content cannot be used as a basis because the stalk cells are known to metabolize more of their protein during development than spore cells (Gregg *et al.*, 1954). Thus comparison has been made on the basis of myxamoebal number; for example a stalk-cell glucose content of 1.42μg/10^8 myxamoebae should be interpreted as the glucose content of all the stalk cells produced by the development of 10^8 myxamoebae.

Results

End-product saccharide concentrations of myxamoebae initially containing different glycogen contents

(1) *Mucopolysaccharide.* The mucopolysaccharide content of mature fruiting bodies is markedly influenced by the initial glycogen content of the myxamoebae; myxamoebae initially containing 5.590± 0.048mg of glycogen/10^8 cells synthesize twice the amount of mucopolysaccharide as myxamoebae initially containing 0.070±0.008mg of glycogen/10^8 cells (Fig. 1, Table 1). No mucopolysaccharide is detectable until culmination (17h). Synthesis of this polymer then occurs at similar rates irrespective of the initial myxamoebal glycogen content.

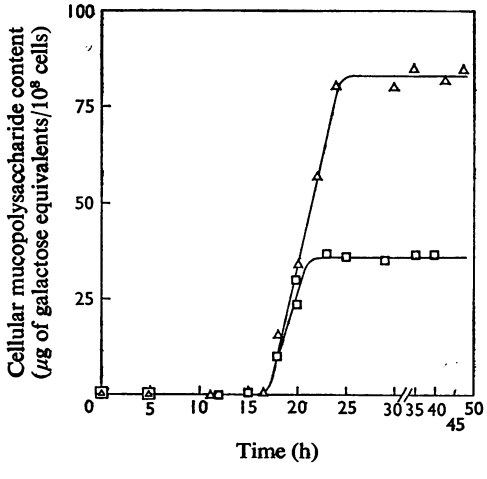

Fig. 1. *Accumulation of mucopolysaccharide during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.06mg of glycogen (□) or 5.65mg of glycogen (△).

(2) *Cell wall polysaccharide.* Axenically grown myxamoebae of strain Ax-2 contain a significant concentration of alkali-insoluble carbohydrate which is maintained until fruiting-body construction when cell-wall polysaccharide synthesis occurs (Fig. 2). It is not known whether this alkali-insoluble carbohydrate present in the cells before culmination is chemically identical with the cell-wall polysaccharide.

During fruiting-body construction, axenically grown myxamoebae synthesize approximately the same amount of cell-wall polysaccharide irrespective of their initial glycogen contents (Fig. 2, Table 1); thus 70-fold changes in myxamoebal glycogen content result in less than a 25% difference in the amount of cell-wall polysaccharide accumulated.

(3) *Trehalose.* During the development of bacterially grown cells, trehalose remains at a low concentration until fruiting-body construction (Ceccarini & Filosa, 1965), but axenically grown myxamoebae synthesize trehalose both during late aggregation and culmination (Fig. 3).

The trehalase of *Neurospora crassa* is highly specific for trehalose as substrate, but to confirm that the material assayed as trehalose in Fig. 3 is indeed this disaccharide, samples were chromatographed with authentic trehalose, eluted, and reassayed with trehalase. This procedure gave complete recovery of the presumed trehalose, thus confirming its chemical nature.

Fig. 3 and Table 1 show that the amount of trehalose synthesized by developing myxamoebae is dependent on the initial glycogen content of the cells; fourfold variations in trehalose content of the mature sorocarp can be obtained. The accumulation of trehalose follows a biphasic pattern irrespective of the amount of trehalose accumulated. Although the final amount of trehalose accumulated was constant for a given myxamoebal glycogen content (Table 1), the 15–20h plateau concentration of trehalose is subject to some variation. Assay of the amount of trehalose in separated stalk and spore cells from sorocarps containing different amounts of glycogen has shown that under all conditions at least 98% of the sorocarp trehalose is present in the spore cells.

(4) *Glucose.* Most of the myxamoebal glycogen that is degraded during the development of axenically grown myxamoebae has been broken down by 20h, but at this time a sudden increase is observed in the intracellular concentration of glucose (Fig. 4). Marked differences are observed in the amount of glucose accumulated by cells derived from myxamoebae containing high or low concentrations of glycogen initially; Fig. 4 shows the two extreme cases. The glucose pool diminishes slowly after fruiting-body formation. Assay of the amount of glucose in separated stalk and spore cells from sorocarps containing different amounts of glycogen has shown that

Table 1. *Relation between myxamoebal glycogen content and the sorocarp content of end-product saccharides*
Results are given as means±S.E.M.

Myxamoebal glycogen content (mg/10^8 cells)*	Sorocarp mucopolysaccharide content (μg/10^8 cells)†	Sorocarp cell-wall polysaccharide content (μg/10^8 cells)†	Sorocarp trehalose content (μg/10^8 cells)†	Sorocarp glucose content (μg/10^8 cells)†
0.070±0.008 (4)	42.19± 1.22 (6)	237.37± 3.38 (5)	—	—
	37.53± 1.11 (4)	234.00±12.49 (3)		
0.308±0.002 (2)	—	—	100.40± 3.72 (3)	21.10±0.84 (3)
			108.94± 1.48 (3)	24.32±0.27 (3)
1.947±0.044 (3)	54.83± 6.42 (5)	191.34± 8.45 (5)	—	—
		201.25±12.37 (4)		
2.670 (1)	—	204.39± 6.71 (5)	—	—
3.490±0.130 (3)	56.76± 2.24 (5)	235.67±12.33 (3)	—	—
5.060±0.042 (3)	70.01± 2.86 (5)	—	378.95± 6.62 (3)	66.48±0.57 (2)
			421.59±22.14 (3)	66.16±3.72 (3)
5.590±0.048 (5)	79.03± 2.77 (5)	281 ± 8.98 (5)	390.33± 6.37 (3)	60.26±0.87 (3)
	85.24±12.13 (6)	290.95± 8.38 (4)		

* Each set of values represents the mean glycogen content for several experiments with the number of experiments given in brackets.

† Each set of values represents a separate experiment with the number of determinations/experiment given in brackets.

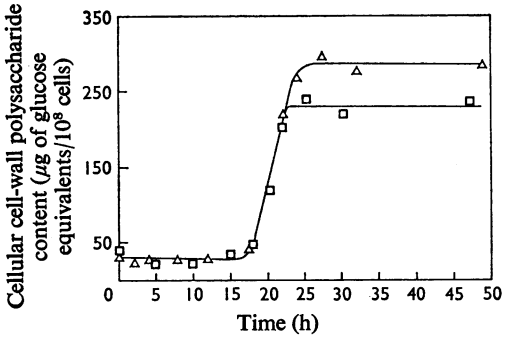

Fig. 2. *Accumulation of cell-wall polysaccharide during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.07mg of glycogen (□) or 5.70mg of glycogen (△).

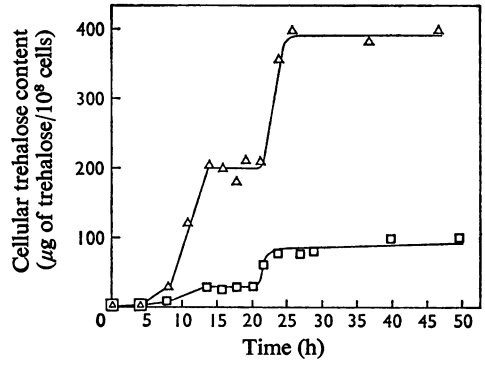

Fig. 3. *Accumulation of trehalose during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.31mg of glycogen (□) or 5.65mg of glycogen (△).

under all conditions at least 97% of the sorocarp glucose is present in the spore cells.

Control of trehalose accumulation during development

Trehalose accumulation was examined in preference to the accumulation of the other end-product saccharides since it showed a large variation in response to changes in the concentration of vegetative myxamoebal glycogen, the synthetic pathway leading to trehalose is short and well known, and the rate-limiting enzymic step is well characterized (Roth & Sussman, 1966; 1968).

To elucidate the control mechanisms operating on trehalose accumulation during development, we have assayed the activities of the enzymes relevant to the process, and determined the pool sizes of important metabolic intermediates in myxamoebae accumulating markedly different amounts of trehalose.

(1) *Trehalase and trehalose 6-phosphate synthase activities during development.* Vegetative myxamoebae grown axenically definitely possess both trehalose synthetic and trehalose degradative (trehalase) enzymes unlike cells grown bacterially which are reported by some workers (Roth & Sussman, 1968)

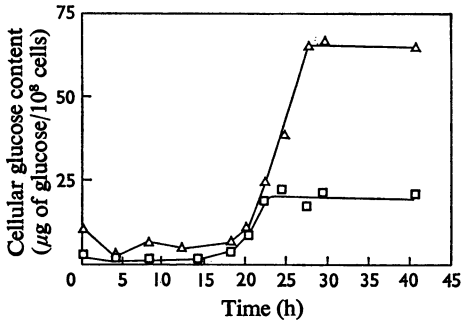

Fig. 4. *Accumulation of glucose during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.13mg of glycogen (□) or 4.98mg of glycogen (△).

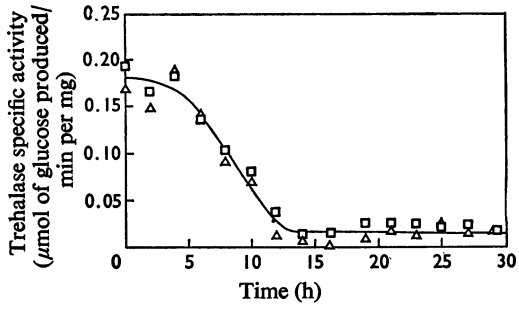

Fig. 5. *Trehalase activity during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.29mg of glycogen (□) or 5.95mg of glycogen (△).

but not others (Killick & Wright, 1972) to lack trehalose synthase activity. The reason for this discrepancy may lie in the stability of trehalose 6-phosphate synthase; Killick & Wright (1972) have shown that this enzyme is cold labile, activity decaying much more rapidly at 2°C than at 25°C. Roth & Sussman (1968) assayed trehalose 6-phosphate synthase in cell samples which had been frozen for several hours, whereas in the present paper we have followed Killick & Wright (1972) and used fresh, unfrozen cell extracts.

Although vegetative myxamoebae grown axenically possess high activities of trehalase, the enzyme disappears rapidly during aggregation, and soon reaches a low activity which is then maintained throughout the rest of development (Fig. 5). This pattern of activity is qualitatively similar to that reported to occur during the development of cells grown bacterially (Ceccarini, 1967) although cells grown axenically possess approximately tenfold higher trehalase activity than cells grown bacterially at all stages of development. Garrett *et al.* (1972) reported that the trehalase of *N. crassa* probably exists in an active and an inactive form, activation occurring after freezing and thawing in 0.05M-phosphate buffer, pH5.6. We were unable to show any difference in trehalase activity between fresh and frozen extracts with our buffer system and thus the high activity of trehalase in myxamoebal extracts is probably not an artifact.

The pattern of trehalase activity during the development of axenically grown cells is unaffected either qualitatively or quantitatively by variations in the vegetative myxamoebal glycogen content (Fig. 5).

The changes in trehalose 6-phosphate synthase activity during development are completely different from those of trehalase; axenically grown myxamoebae possess only low specific activities of the enzyme and activity increases during development,

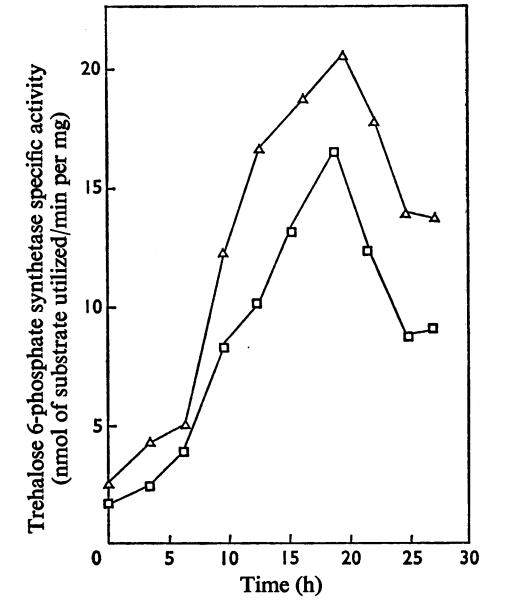

Fig. 6. *Trehalose 6-phosphate synthase activity during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.29mg of glycogen (□) or 5.95mg of glycogen (△).

reaching a peak just before fruiting-body construction (Fig. 6). A similar pattern of activity is observed during the development of bacterially grown cells (Roth & Sussman, 1968), and although the peak activity observed during the development of axenically grown myxamoebae is often broader for cells initially containing high glycogen contents than for

Table 2. *Concentration of UDP-glucose and glucose 6-phosphate, and trehalose 6-phosphate synthase activity during the development of myxamoebae containing different amounts of glycogen initially*

Results are expressed as means ± S.E.M. with the number of determinations in parentheses for cells at the 11 h developmental stage.

Myxamoebal glycogen content (mg of glucose equivalents/10^8 cells)	Cellular glucose 6-phosphate content		Cellular UDP-glucose content		Peak trehalose 6-phosphate synthase activity (nmol of UDP-glucose used/min per mg of protein)
	nmol/10^8 cells	mM*	nmol/10^8 cells	mM*	
0.24 ± 0.03 (4)	3.37 ± 0.32 (3)	0.068 ± 0.006 (3)	14.72 ± 2.44 (3)	0.294 ± 0.049 (3)	16.21 ± 0.61 (2)
6.58 ± 0.46 (4)	11.93 ± 1.41 (3)	0.239 ± 0.028 (3)	42.33 ± 5.69 (3)	0.846 ± 0.114 (3)	20.21 ± 0.71 (2)

* Assuming that the intracellular volume of 10^8 cells is 0.05 ml.

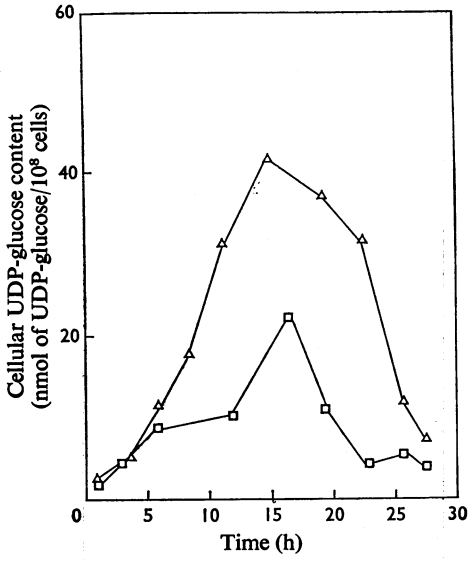

Fig. 7. *Cellular UDP-glucose content during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.26 mg of glycogen (□) or 7.80 mg of glycogen (△).

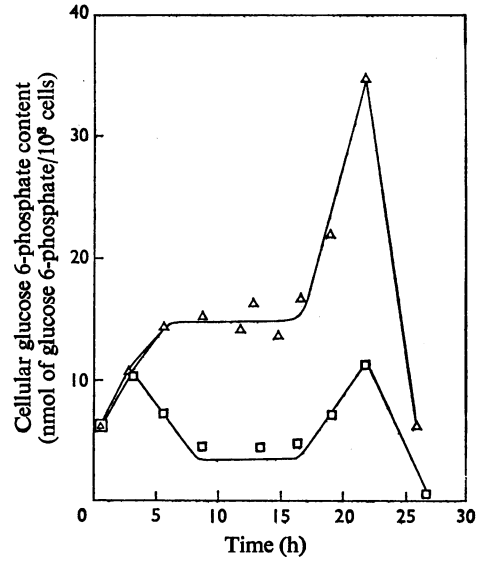

Fig. 8. *Cellular glucose 6-phosphate content during the development of axenically grown myxamoebae*

Myxamoebae were grown in axenic medium and contained initially, per 10^8 cells: 0.26 mg of glycogen (□) or 7.80 mg of glycogen (△).

cells initially containing low glycogen contents, the peak specific activities observed have not differed by more than 25% (Fig. 6, Table 2).

(2) *UDP-glucose and glucose 6-phosphate pools during development.* UDP-glucose and glucose 6-phosphate, the substrates for trehalose 6-phosphate synthase, have been reported to increase in cellular concentration during the development of bacterially grown myxamoebae, only decreasing during fruiting-body construction when end-product saccharide synthesis is occurring (Wright *et al.*, 1964; Pannbacker, 1967*a*).

During the development of axenically grown myxamoebae, the UDP-glucose pool varies in a qualitatively similar manner to that described for bacterially grown myxamoebae (Fig. 7). The glucose 6-phosphate pool, however, reaches a steady-state concentration after the first few hours of development, transiently increases at culmination, and finally decreases to a basal concentration (Fig. 8).

Both UDP-glucose and glucose 6-phosphate pools are influenced markedly by the vegetative myxamoebal glycogen contents. Thus midway during development, cells initially containing 6.58 ± 0.46 mg

of glycogen/10^8 cells possess UDP-glucose and glucose 6-phosphate pools three times the size of those in myxamoebae initially containing 0.24 $\pm$0.03 mg of glycogen/10^8 cells (Figs. 7 and 8, Table 2).

(3) *Kinetic parameters of trehalose 6-phosphate synthase.* To determine whether the changes in the intracellular concentration of UDP-glucose and glucose 6-phosphate would significantly affect the activity of trehalose 6-phosphate synthase *in vivo* the K_m values of this enzyme for these substrates was determined. In both cases Lineweaver–Burk plots of $1/v$ against $1/[S]$ gave straight lines and the K_m values thus deduced were found to be 1.0×10^{-3}M (for UDP-glucose) and 5.6×10^{-3}M (for glucose 6-phosphate). These values are in good agreement with those reported by Roth & Sussman (1968) with extracts of cells derived from bacterially grown myxamoebae.

Discussion

More is known of carbohydrate metabolism than any other biochemical aspect of the developmental phase of *D. discoideum*. Characteristically, development in this organism results in the synthesis of three main carbohydrates not present to any large extent during vegetative growth and these (trehalose, cell wall polysaccharides, and an acid mucopolysaccharide) have been termed end-product saccharides, since once synthesized they are not degraded during the developmental phase. In addition, glucose accumulates during fruiting-body construction and the glucose-pool size remains constant or decays only slowly in the mature sorocarp (Ceccarini & Filosa, 1965; White & Sussman, 1963*a*; Hames & Ashworth, 1974) possibly serving as an energy source to maintain the low metabolic rate of dormant spores, as well as being concerned with spore germination (Cotter & Raper, 1970). Since the glucose is localized almost entirely in the spores of the sorocarps, the slow decrease in sorocarp glucose content that is sometimes observed must be due to its metabolism by the spores. Glucose accumulation at culmination may therefore be just as important as the synthesis of end-product saccharides and so the term end-product saccharides as used here will include glucose. If saccharides other than those described above are made and retained in the mature sorocarp, it is unlikely that they occur in large amounts since the total hexose content of mature sorocarps is approximately equal ($\pm$15%) to the sum of the glycogen, glucose, trehalose, cell-wall polysaccharide and mucopolysaccharide hexose contents in bacterial grown cells (Sussman & Sussman, 1969), and in axenically grown cells containing different amounts of glycogen initially.

Sussman & Sussman (1969) have reviewed the evidence for the existence of transcriptional and translational controls of the synthesis *de novo* of some enzymes involved in the synthesis of these end-product saccharides. The increased rates of synthesis of these enzymes during development is presumably a means whereby the metabolic flux through the synthetic pathway may be increased, leading to the differentiated state. Wright (1966) has shown, however, that an alteration in the cellular concentration of an enzyme will only cause an alteration in overall metabolic flux if the enzyme concerned is catalysing the rate-limiting step of the overall metabolic sequence and is working at its maximal velocity. If the second of these two conditions is not satisfied it would be possible to increase the overall metabolic flux by alterations in the concentration of the substrate and/or effectors of that enzyme.

Determination of the controlling factors of any one pathway *in vivo* is complicated by the fact that it is experimentally difficult to alter the flux through that pathway. In the present paper we have described procedures whereby such alterations are possible, thus providing us with the opportunity to examine not only the factors controlling the flux through one particular pathway, but also the control relationships between pathways.

Control of end-product saccharide synthesis

In myxamoebae grown axenically in media lacking added glucose there is net gluconeogenesis during development (Hames & Ashworth, 1974). In myxamoebae grown in the presence of glucose and thus containing large amounts of glycogen there is evidence that a significant fraction of the radioactivity of myxamoebal U-^{14}C-labelled glycogen can be recovered in the end-product saccharides synthesized during development. Thus, although the immediate precursor of all end-product saccharides so far studied is glucose 6-phosphate or UDP-glucose (Roth & Sussman, 1966; Ward & Wright, 1965; Newell & Sussman, 1969) the ultimate origin of the glucose differs according to the glycogen content of the cells. The amounts of trehalose, cell-wall polysaccharide, mucopolysaccharide and glucose which accumulate during the development of axenically grown myxamoebae of *D. discoideum* strain Ax-2 are a function of the myxamoebal glycogen content; myxamoebae initially containing 5.59$\pm$0.048 mg of glycogen/10^8 cells accumulate approx. 25% more cell-wall polysaccharide, 100% more mucopolysaccharide, 200% more glucose and 300% more trehalose than myxamoebae initially containing less than approx. 0.3 mg of glycogen/10^8 cells (Table 1).

The observation that the percentage increase is specific for each saccharide, rather than the accumulation of each saccharide being increased proportionally indicates that (1) the controls which determine the quantity of each end-product saccharide accumulated are not coordinate and (2) that large variations

in the amounts and proportions of important end-product saccharides finally accumulated do not impair the ability of the myxamoebae to develop normally and produce fruiting bodies. This suggests that the pathway leading to each end-product saccharide is specifically controlled and that simple control of the UDP-glucose pool, as suggested by Wright & Gustafson (1972), is insufficient to account for the amount of end-product saccharides synthesized.

Newell & Sussman (1969) reported that UDP-glucose pyrophosphorylase is polymorphic and proposed that each end-product saccharide may be synthesized in a separate location with its own UDP-glucose pyrophosphorylase and, presumably, its own pool of UDP-glucose. According to this scheme, not only could the rate of synthesis of any one end-product saccharide be governed by the activity of the attendant UDP-glucose pyrophosphorylase (provided it is rate limiting), but termination of synthesis after a certain amount of end-product had been synthesized could be achieved by inhibition of the corresponding UDP-glucose pyrophosphorylase, without affecting the synthesis of the other end-product saccharides. Although a scheme of this kind could account for the results presented in the present paper, Pannbacker (1967*b*) found no evidence for the compartmentalization of UDP-glucose pyrophosphorylase; whether extracted by freezing or gentle homogenization, the activity was completely soluble when spun at 30000*g* for 30min. We found no evidence for compartmentalization of UDP-glucose pyrophosphorylase, and conclude that the control of end-product saccharide synthesis is both specific for each saccharide and that the control operates after UDP-glucose formation. The pathways leading to the synthesis of the mucopolysaccharide and cell-wall polysaccharides are insufficiently understood for us to discuss the molecular basis of the changes seen in the concentrations of these end-product saccharides. Trehalose biosynthesis is, however, known to occur (Roth & Sussman, 1966, 1968) via the reactions:

$$\text{UDP-glucose} + \text{glucose 6-phosphate} \rightarrow \text{trehalose 6-phosphate} \quad (1)$$

$$\text{Trehalose 6-phosphate} \rightarrow \text{trehalose} + P_i \quad (2)$$

No complex acceptors are involved, and since trehalose 6-phosphate has never been observed to accumulate during development (Roth & Sussman, 1966; Sargent & Wright, 1971), trehalose 6-phosphate synthetase (see eqn. 1) is considered to be the rate-limiting enzyme. Initiation of trehalose synthesis always occurs at approximately the same time during the development of axenically grown myxamoebae irrespective of the quantity of trehalose made. Trehalose 6-phosphate synthetase is not inhibited by as much as 40mM-trehalose (Roth & Sussman, 1966); approximately the same concentration as observed in spores produced from myxamoebae initially containing 5.590 ± 0.048mg of glycogen/10^8 cells (Table 1). Glucose 6-phosphate has been found by some workers (Roth & Sussman, 1968), but not us, to inhibit trehalose 6-phosphate synthetase at very high ($>3 \times 10^{-2}$M) and unphysiological concentrations, but this cannot limit the amount of trehalose synthesized since the cellular glucose 6-phosphate concentration is decreasing at the time of fruiting-body construction. Also the cells still contain high trehalose 6-phosphate synthetase activity at the time when trehalose synthesis ceases. Thus there appears to be no specific mechanism to terminate trehalose synthesis other than depletion of the substrates, UDP-glucose and glucose 6-phosphate, which always occurs during sorocarp formation, irrespective of their previous concentrations. Since initiation and termination of trehalose synthesis always occur at specific times in the developmental phase, the amount of trehalose synthesized depends on the rate of trehalose synthesis which in turn depends on the activity of the synthetase enzyme.

Trehalose 6-phosphate synthetase has been shown to exist in two forms; active enzyme and masked enzyme, the latter apparently being a complex between enzyme and an unidentified protein and found in extracts of developing cells only before the preculmination period of development (Killick & Wright, 1972). It is not clear whether this masked form of the enzyme exists *in vivo* or whether it is an artifact of experimental procedure, but the implication is that the increase in synthetase specific activity, as described by us and others (Roth & Sussman, 1968), is due mainly, if not wholly, to the unmasking of the masked form of the enzyme such that when assayed at preculmination (18h) all the enzyme present in the cell extract is in the active form (Killick & Wright, 1972). Whatever the reason for enzyme masking, it is clear that determination of the peak specific activity of trehalose 6-phosphate synthetase at preculmination is a valid measure of the cellular content of active enzyme during development.

However, in addition to trehalose 6-phosphate synthetase, myxamoebae also contain the enzyme trehalase which will degrade trehalose to glucose (Ceccarini, 1966). Thus increased trehalose accumulation could be the result of decreased cellular trehalase activity. Since no effectors of trehalase are known and trehalose is itself the substrate, increased trehalose accumulation could only be the result of decreased cellular content of trehalase. We have shown that this is not the reason for increased accumulation of trehalose by axenically grown myxamoebae initially containing high concentrations of glycogen since both the qualitative and quantitative pattern of trehalase specific activity during development is invariant irrespective of myxamoebal glycogen content.

We conclude that the amount of trehalose normally accumulated by developing cells of *D. discoideum* is limited not by the cellular content of either trehalose 6-phosphate synthetase or of trehalase, but rather by the availability of the substrates UDP-glucose and glucose 6-phosphate. The reason for the biphasic nature of trehalose accumulation (Fig. 3) during the development of strain Ax-2 remains unclear.

Wright & Gustafson (1972) have come to similar conclusions on the basis of computer simulations of carbohydrate metabolism of *D. discoideum* strain NC-4 grown on bacteria. However, since we regard many of the assumptions underlying this model as inapplicable to our experiments (Hames & Ashworth, 1974) and find different patterns of variation in cellular concentration of UDP-glucose and glucose 6-phosphate from those predicted by Wright & Gustafson (1972), this agreement would seem largely fortuitous.

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