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Characterization and Regulation of Galactose Transport in *Neurospora crassa*

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Two galactose uptake systems were found in the mycelia of *Neurospora crassa*. In glucose-grown mycelia, galactose was transported by a low-affinity (K_m = 400 mM) constitutive system which was distinct from the previously described glucose transport system I (R. P. Schneider and W. R. Wiley, J. Bacteriol. **106**:479–486, 1971). In carbon-starved mycelia or mycelia incubated with galactose, a second galactose transport activity appeared which required energy, had a high affinity for galactose (K_m = 0.7 mM), and was shown to be the same as glucose transport system II. System II also transported mannose, 2-deoxyglucose, xylose, and talose and is therefore a general monosaccharide transport system. System II was derepressed by carbon starvation, completely repressed by glucose, mannose, and 2-deoxyglucose, and partially repressed by fructose and xylose. Incubation with galactose yielded twice as much activity as starvation. This extra induction by galactose required protein synthesis, and represented an increase in activity of system II rather than the induction of another transport system. Glucose, mannose, and 2-deoxyglucose caused rapid degradation of preexisting system II; fructose and xylose caused a slower degradation of activity.

In *Neurospora crassa*, several sugar transport systems have been described in both conidia and mycelia. A constitutive system which transports glucose and 3-*O*-methyl glucose has been found in mycelia by Scarborough (13) and by Schneider and Wiley (15), who have designated it system I. The transport is carrier mediated, has a rather low substrate affinity (K_m for glucose is 8 to 25 mM), and probably does not operate against a concentration gradient. This may be the same as the constitutive mycelial system for L-sorbose transport described by Crocken and Tatum (1). A similar glucose transport system has been reported in conidia by Neville et al. (12).

A second mycelial transport system, which was found to transport glucose and 3-*O*-methyl glucose, was also reported by Scarborough (14) and by Schneider and Wiley (15, 16) and was designated system II. This system is repressed by high concentrations of glucose and derepressed by starvation, has a high affinity for glucose (K_m of ca. 40 μM), and can concentrate sugars against a considerable gradient. Scarborough (14) showed that L-sorbose is also transported by this system, and Klingmüller and Huh (6) confirmed these findings. Neville et al. (12) found a high-affinity glucose transport system in conidia which seems comparable to system II.

Studies by Slayman and Slayman (18) indicate that transport of sugars by system II is coupled to transport of H^+ ions across the membrane. Technically, this would mean that system II is not an active transport system, but rather a facilitated diffusion system which cotransports sugars and protons. The site of energy input is a proton extrusion pump which maintains an H^+ ion gradient which in turn supplies the electrochemical potential necessary for accumulation of sugars against a sugar gradient.

L-Sorbose transport has been studied in conidia (3–6), and three systems seem to be operational under different conditions: a system in germinated conidia which have been starved (probably the same as system II), a constitutive system in ungerminated conidia, and a system in ungerminated conidia pretreated with L-sorbose. A lactose transport system in mycelia has been described by Lester et al. (7), and the existence of a derepressible fructose transport system in mycelia has been inferred by Schneider and Wiley (15, 16).

As part of a general study of galactose metabolism and its regulation in *N. crassa*, we have investigated galactose transport in mycelia of this organism. This paper reports on the characterization of a derepressible, high-affinity galactose transport system, shows that the system transports several other sugars as well, and presents evidence that this system is identical to the previously reported system II. We also show that system II is not only derepressible, but may

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be induced specifically by galactose. In addition, both repression and degradation of the system may be affected by a number of glucose analogs, not all of them substrates for the transport system.

MATERIALS AND METHODS

Reagents and chemicals. D-[1-^{14}C]galactose was obtained from Schwartz/Mann. D-[^{14}C]xylose and L-[^{14}C]sorbose were purchased from Amersham Corp. 3-*O*-[^{14}C]methyl-D-glucose was obtained from New England Nuclear Corp. All unlabeled sugars and all other reagents were purchased from Sigma Chemical Co. All sugars used in this study are of the D configuration unless otherwise indicated.

Cultures and growth conditions. Wild-type *N. crassa*, RL3-8A, from the Rockefeller collection, was used throughout. Conidial suspensions were inoculated into 600 ml of Vogel minimal medium N (20) plus 2% glucose in a 2-liter flask. Cultures were grown for 18 to 22 h on a rotary shaker at 30°C. Transfer to other media (for starvation or induction) was accomplished by rapid vacuum filtration, washing with minimal salts solution, and suspending in the appropriate incubation medium. This was performed in less than 1 min, and the mycelia were never permitted to dry out. Studies on the low-affinity system used mycelia directly from the growth medium. For most kinetic experiments involving the high-affinity system, mycelia were incubated either at 30°C for 2.5 h in minimal salts solution (derepressed mycelia) or for 3.5 h in minimal salts plus 2% galactose (galactose-induced mycelia).

Sugar uptake assay. The appropriate cultures were harvested rapidly by vacuum filtration, and washed with distilled water. For assay of the high-affinity system, ca. 1 g (wet weight) of mycelia was suspended in 49 ml of 10 mM citrate buffer (pH 5.0) in a 125-ml flask. All assays were conducted at 30°C in a reciprocating shaker water bath. After about 1 min, for the mycelia to equilibrate, the assay was initiated by the addition of 1 ml of a 50-fold-concentrated solution of the radioactive sugar at a specific activity of 1 to 10 μCi/mmol. The derepression and induction time courses were assayed at the following final sugar concentrations: 5 mM galactose; 5 mM xylose; 25 mM L-sorbose. For competition studies the mycelia were suspended in 40 ml of buffer, and the radioactive sugar plus the competing sugar were added in 10 ml of buffer prewarmed to 30°C. The low-affinity system was assayed in a similar manner, except that the mycelia were suspended in 40 ml of 50 mM phosphate buffer (pH 6.9) and the labeled sugar was added in 10 ml of prewarmed buffer. In all cases, 5-ml portions were removed at four 30-s points, rapidly filtered, and washed with distilled water. The mycelial pads were transferred to scintillation vials; 2 ml of water was added to disperse the samples, which were then counted in 15 ml of scintillation fluid (Aquasol) on a Nuclear Chicago Unilux counter. A 10-ml portion was also taken from the assay mixture, filtered, and washed. It was then dried overnight at 90°C and weighed. The results from each assay were plotted, and a straight line was fitted by inspection. Sugar uptake results are presented as nanomoles per minute per milligram (dry weight) of mycelium. Intramycelial sugar concentrations were calculated by assuming 2.5 μl of water per mg of mycelium (dry weight) (8). Data from kinetic experiments were plotted by the method of Lineweaver and Burke (10).

To determine whether the galactose inside the mycelia had been metabolized, we incubated the mycelia under standard assay conditions for 8 min with 5 mM labeled galactose, washed them rapidly with distilled water, and immersed them into 50 ml of boiling 70% ethanol. After 5 min of boiling, the ethanol extract was filtered, and the volume was reduced by flushing with nitrogen. The extract was then chromatographed on Whatman no. 1 paper using *n*-propanol-ammonia-water (6:3:1), a system which resolves free galactose from galactose phosphates.

RESULTS

Number of systems for galactose uptake. Two systems for galactose uptake were observed, depending upon growth conditions of the mycelia, particularly on the nature of the carbon source provided. Mycelia grown on glucose or mannose possessed a low-affinity, presumably constitutive, system for galactose uptake. This uptake activity had a broad pH optimum, from 6.5 to 8.0, which was not affected by the nature of the buffer used (citrate, phosphate, Tris). Accordingly, 50 mM phosphate (pH 6.9) was used to assay this activity. The uptake appeared to be carrier mediated, for it demonstrated saturation kinetics, with an apparent K_m of 400 mM and a V_{max} of 90 nmol/min per mg (dry weight) of mycelium at 30°C and pH 6.9. The kinetic parameters of this system seemed to be affected by the assay conditions, for in 10 mM citrate buffer (pH 5.0), the apparent K_m for galactose was 140 mM and the V_{max} was 30 nmol/min per mg (dry weight).

Galactose uptake by this low-affinity system was inhibited by glucose. The inhibition was variable and not competitive, however, and never greater than 50%, even with a 15-fold excess of glucose (0.3 M, which is 10 to 30 times the glucose K_m for system I). Similarly, 3-*O*-methyl glucose was a weak inhibitor of the low-affinity galactose uptake system. In the reverse experiment, an 18-fold excess of galactose (0.36 M) was only able to inhibit 3-*O*-methyl glucose uptake by 35% in glucose-grown mycelia. Thus, the low-affinity system for galactose uptake is distinct from the low-affinity system for glucose and 3-*O*-methyl glucose (system I) which has previously been characterized (13, 15). Mannose, fucose (6-deoxygalactose), and tagatose (the ketose analog of galactose) were weak inhibitors of galactose uptake by the low-affinity system, although the nature of the competition and the kinetics of competition were not determined.

Fructose, xylose, L-arabinose, and L-sorbose in 15-fold excess and 2-deoxygalactose in 10-fold excess did not inhibit galactose uptake in glucose-grown mycelia.

If glucose-grown mycelia were incubated in the absence of a carbon source, a second galactose uptake activity appeared. This activity also appeared when the mycelia were incubated with galactose and, in fact, twice as much galactose uptake activity appeared after incubation with galactose as with starvation alone (see below). Accordingly, for most of the following experiments, we used galactose-induced mycelia. The following results provide a characterization of this second uptake system and its regulation.

Linearity of uptake. Figure 1 shows the time course of galactose uptake by galactose-induced mycelia. Uptake was linear, with a rate of 18 nmol/min per mg (dry weight), for at least 7 min, at the end of which the intramycelial concentration of labeled sugar was calculated to be more than 10 times the extramycelial concentration. After 8 min of incubation, extraction of the mycelium and separation of the labeled compounds of paper chromatography showed that 93% of the radioactivity in the mycelium was present as free galactose, and 7% was present as phosphorylated intermediates. Thus, *Neurospora* is able to transport galactose quite rapidly and against a considerable concentration gradient, which argues strongly for an energy-requiring step in or coupled to the transport process.

Effects of pH, ionic strength, and buffers. Galactose uptake by derepressed or galactose-induced mycelia showed a fairly narrow pH optimum around 5.0; a shift of 1 pH unit in either direction reduced the uptake rate by about 40%. Increase in ionic strength was inhibitory: 50 mM KCl inhibited uptake about 40%, whereas 50 mM KH_2PO_4 inhibited almost 70%. Low concentrations of citrate and tartrate inhibited galactose uptake only slightly. Acetate buffer, on the other hand, was almost completely inhibitory (see below). It was therefore concluded that 10 mM citrate buffer (pH 5.0) was optimal for the assay of galactose uptake.

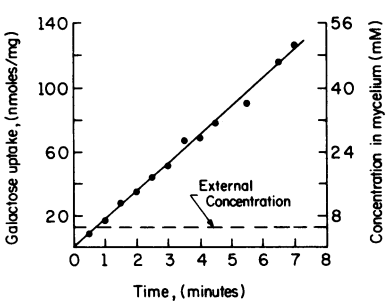

FIG. 1. *Linearity of uptake of galactose by galactose-induced mycelia as a function of time. Conditions as given in the text, except that the total assay volume was 100 ml. Galactose concentration was 5 mM.*

Effects of inhibitors. Respiratory inhibitors impaired galactose uptake significantly in derepressed mycelia. 2,4-Dinitrophenol (0.5 mM) and NaN_3 (1 mM) each inhibited uptake by more than 70%, and KCN (1 mM) inhibited uptake by almost 60%; thus, an energy requirement existed for galactose transport. In contrast, neither 1 mM iodoacetate nor 1 mM parachloromercuribenzoate affected galactose uptake. This argues against sulfhydryl groups being essential to the transport process. Deoxycorticosterone (1 mM), which is known to inhibit other transport systems in *Neurospora* (9), also inhibited galactose transport by 75%. Acetate buffer, in concentrations greater than 10 mM, inhibited galactose uptake by more than 90%. This was in good agreement with the results of Marzluf and Metzenberg (11), who showed that acetate probably affects several transport systems.

Kinetics and competition studies. In galactose-induced mycelia, galactose uptake was concentration dependent and obeyed saturation kinetics, thus implying a carrier-mediated uptake. The apparent K_m was 0.7 mM, and the V_{max} was 21 nmol/min per mg (dry weight) of mycelium. At the range of galactose concentrations employed to study this system, the contribution from the constitutive, low-affinity uptake system (K_m = 140 mM under these conditions) was negligible.

Galactose uptake in galactose-induced mycelia was competitively inhibited by xylose, glucose, talose, 3-*O*-methyl glucose, 2-deoxyglucose, and L-sorbose. It was also shown that galactose is a competitive inhibitor of both xylose and L-sorbose uptake. Results of these and other experiments are summarized in Table 1. The following sugars and sugar derivatives did not inhibit galactose uptake, even in 20-fold excess: glycerol, dihydroxyacetone, D-erythrose, D-ribose, D-arabinose, L-arabinose, D-fructose, D-tagatose, D-glucosamine, inositol, galactitol, α-methyl galactoside, D-sedoheptulose, lactose, and melibiose. D-Fucose (6-deoxygalactose) and 2-deoxygalactose inhibited galactose uptake very weakly, with K_i's $\geq$ 50 mM.

Counterflow. If two different molecular species are transported into a cell across a membrane by a common carrier, then a high external concentration of one of these species should stimulate the efflux of previously accumulated molecules of the other species (19). This phenomenon, known as counterflow, is frequently

employed to demonstrate that two molecular species share the same transport system. Figure 2 summarizes several counterflow experiments. Since the preloading with radioactive labeled sugar takes a considerable time under these conditions, L-sorbose, which is not metabolized (2, 13), was used. Derepressed mycelia were incubated for 31 min with 0.625 mM labeled L-sorbose, and then a large excess of the sugar to be tested was added. The label remaining in the mycelia was then monitored for another 30 min. The efflux of L-sorbose from preloaded mycelia was stimulated by galactose, xylose, mannose, and by 25 mM unlabeled L-sorbose. Similar results were obtained with talose and 2-deoxyglucose. In contrast, neither fructose nor L-arabinose was able to stimulate L-sorbose counterflow.

TABLE 1. *Sugars transported by system II*[a]

Substrate	K_m[b]	K_i for Gal	K_i for Sorb	K_i for Xyl	V_{max}[c]
Glucose	—[d]	0.054	—	0.071	—
3-*O*-methyl-glucose	—	0.14	—	0.13	—
2-Deoxyglucose	—	0.17	—	0.26	—
Mannose	—	—	0.20	0.37	—
Xyl	1.05	1.03	0.67	—	50
Gal	0.7	—	1.14	1.16	21
Talose	—	1.13	1.33	—	—
L-Sorb	4.5	6.0	—	5.8	55

[a] Abbreviations: Gal, Galactose; Sor, sorbose; Xyl, xylose.

[b] K_m's and K_i's are all given as millimolar concentrations.

[c] V_{max}'s are given as nanomoles per minute per milligram (dry weight) and are for Gal-induced mycelia. V_{max}'s for derepressed (starved) mycelia are 50 to 60% of these values.

[d] —, Not determined.

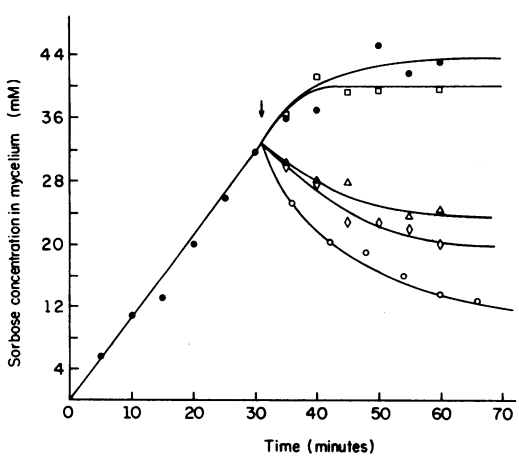

FIG. 2. *Counterflow of L-sorbose from derepressed mycelia. Conditions as described in text. Final concentrations of competitors:* (□) *fructose, 10 mM;* (△) *galactose, 5 mM;* (◇) *unlabeled L-sorbose, 25 mM;* (○) *mannose, 2 mM.* (●) *Control. All additions were made at 31 min, except that nothing was added to the control.*

Derepression and induction of galactose transport. Figure 3 gives a time course of galactose transport activity in glucose-grown mycelia transferred to either minimal medium or to minimal containing 2% galactose. Starvation for a carbon source caused a large, relatively rapid increase in galactose transport activity, and this activity was increased twofold when galactose was included in the incubation medium. When already derepressed mycelia (after 2 to 4 h of carbon starvation) were transferred to galactose-containing medium, galactose transport activity increased twofold (data not shown). In the course of many experiments, the measured rate of galactose uptake ranged from 8 to 14 nmol/min per mg (dry weight) of mycelia in starved (derepressed) mycelia and from 18 to 27 nmol/min per mg in galactose-induced mycelia. The initial value of 0.5 to 1 nmol/min per mg (Fig. 3) corresponded to the activity of the constitutive, high K_m (140 mM) galactose uptake system previously described.

Cycloheximide (4 μg/ml) prevented the increase of galactose transport activity when glucose-grown mycelia were transferred to starvation conditions, which suggested that protein synthesis was necessary for the expression of the transport system. Cycloheximide also prevented the twofold increase of transport activity after transfer of derepressed (starved) mycelia to media containing 2% galactose.

The question arose as to whether we were looking at two different transport systems, one

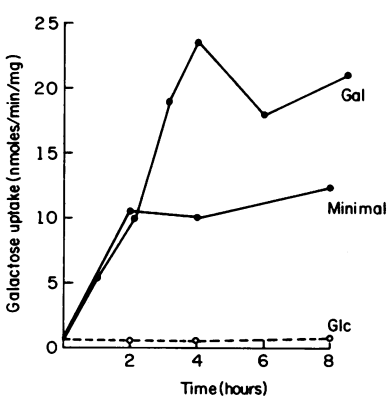

FIG. 3. *Appearance of galactose transport activity. At time zero, glucose-grown mycelia were transferred to either Vogel minimal medium (20), minimal medium plus 2% galactose (Gal), or minimal medium plus 2% glucose (Glc).*

derepressed by starvation and one induced by galactose, or whether this was one system which may be variably derepressed or induced. Kinetic studies showed that the apparent K_m for galactose was the same in starved mycelia as in galactose-induced mycelia, which argued for one transport system. Further evidence for this theory came from the study of the transport of xylose and L-sorbose, both of which we have shown to be competitors for the galactose transport system. Each of these transport activities followed a similar induction pattern to that of galactose: they were both induced twofold higher in the presence of galactose than by starvation alone.

The kinetics for xylose transport and L-sorbose transport were also examined. For each of these sugars, starved mycelia and galactose-induced mycelia showed the same apparent K_m's, but the V_{max}'s were twofold higher in the galactose-induced mycelia. It would thus seem that a specific galactose transport activity is not induced by galactose, but rather the same general system II transport activity found in starved mycelia is somehow doubled.

In an effort to determine the nature of this galactose-induced synthesis of the transport system, we tested the following sugars and sugar derivatives for their ability to stimulate transport system synthesis in derepressed cells (all were at 1% unless otherwise indicated): L-arabinose, D-xylose, galactitol, sorbitol (2%), galacturonic acid, D-tagatose, L-sorbose (2%), 3-*O*-methyl glucose, 2-deoxygalactose, and lactose (2%). None of these was any more effective than starvation in stimulating the synthesis of the galactose transport system.

Galactose concentrations below 1 mM were not able to induce galactose transport above starvation levels. Increasing external galactose concentrations in threefold steps from 1 to 110 mM (2%) increased the rate at which galactose transport activity appeared (data not shown), although the final plateau level of galactose transport was not affected.

Repression of the transport system. Besides glucose (Fig. 3), only 2-deoxyglucose and mannose were able to keep the mycelia fully repressed (Fig. 4). Fructose permitted partial derepression, whereas xylose, while permitting full derepression during the period the mycelia were adapting to growth on the carbon source, produced partial repression in mycelia actively growing on the sugar. In a separate series of experiments, we determined that when *Neurospora* mycelia were grown on glucose and transferred to xylose, no growth (as measured by the dry weight of the cultures) occurred for about 2 to 3 h, which correlated well with the onset of repression of system II activity. Mycelia grown on 2% xylose had a galactose uptake rate of 3 to 4 nmol/min per mg (dry weight), which was about one-third that of derepressed mycelium. L-Sorbose, sorbitol, and 3-*O*-methyl glucose (all at 1%) were unable to repress synthesis of system II.

Repression was concentration dependent; 1 mM 2-deoxyglucose did not repress synthesis of the transport system, but concentrations of 10 mM and higher caused complete repression.

Degradation of the transport system. When either derepressed (starved) or galactose-induced mycelia were transferred to glucose-containing medium, the high-affinity monosaccharide transport system was rapidly and irreversibly degraded. Cycloheximide did not prevent this degradation, although it prevented the diluting out of the specific activity caused by growth, and so the degradation seemed slightly slower. If the mycelia were washed and suspended in galactose, however, the transport activity reappeared. Because this reappearance was prevented by cycloheximide, and therefore presumably required de novo protein synthesis, it would appear that glucose caused irreversible loss of transport activity, and not merely temporary inactivation. These results for galactose transport agree closely with those of Schneider and Wiley (15) for 3-*O*-methyl glucose transport, and provide further confirmation for the identity of these two systems.

The kinetics of this degradation are examined

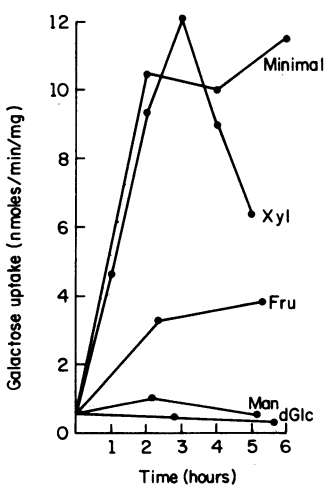

FIG. 4. *Repression of galactose transport activity. At time zero, glucose-grown mycelia were suspended in minimal medium or in minimal medium plus 2% xylose (Xyl), 2% fructose (Fru), 2% mannose (Man), or 1% 2-deoxyglucose (dGlc).*

in Fig. 5. Glucose, mannose, and 2-deoxyglucose all caused a rapid degradation of galactose transport activity, with first-order kinetics and a half-time of about 50 min. Fructose and xylose also caused degradation of the transport system, fructose with a half-time of 90 min, and xylose with a half-time of 104 min. L-Sorbose and 3-*O*-methyl glucose (1%), as well as transfer to medium containing galactose or medium lacking any carbon source, caused no decrease in transport activity during the course of a 3-h incubation.

DISCUSSION

The data for the constitutive, low-affinity galactose uptake system are still not complete, but it seems likely that in glucose-grown mycelia, galactose is not accumulated via the previously reported system I. This system is known to transport glucose and 3-*O*-methyl glucose (13, 15) and may also transport L-sorbose (1). However, because glucose and 3-*O*-methyl glucose are not competitive inhibitors of galactose uptake, and because galactose does not inhibit 3-*O*-methyl glucose uptake significantly, we concluded that the low-affinity galactose transport activity which we have described is a new, previously unreported constitutive sugar transport system in *Neurospora*. This transport system is quite specific for galactose: of the sugars tested, most were not competitors for the system, and only a few galactose analogs showed even weak inhibition of galactose uptake.

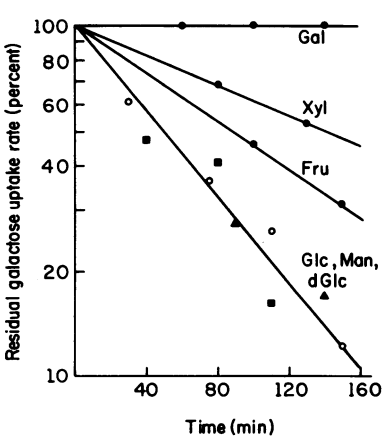

FIG. 5. *Degradation of galactose transport activity. Galactose-induced mycelia were suspended in minimal medium containing galactose (Gal), xylose (Xyl), fructose (Fru), glucose (Glc, ○), mannose (Man, ▲), or 2-deoxyglucose (dGlc, ■). All sugars were at a concentration of 2% except 2-deoxyglucose, which was at 1%. Cycloheximide (4 μg/ml) was added to all cultures to prevent growth.*

The second galactose uptake system, which is found in starved or galactose-induced mycelia, has a higher affinity for galactose, is energy dependent, and can concentrate the sugar against a substantial gradient. Other workers have previously demonstrated the existence, in mycelia of *N. crassa*, of a derepressible, energy-requiring, high-affinity transport system for glucose and 3-*O*-methyl glucose (13, 15), and L-sorbose (6, 14). We have now shown that galactose, xylose, mannose, 2-deoxyglucose, and talose are also transported by this system. This transport system, designated system II by Schneider and Wiley (15), is thus a rather general monosaccharide transport system.

Two lines of evidence have been presented for assigning these new sugars to system II; each of these sugars inhibits competitively the uptake of two others with comparable K_i's, and they are all able to induce counterflow of previously accumulated L-sorbose. In addition, we have shown that galactose, xylose, and L-sorbose transport all follow the same patterns of derepression and induction, and also that the degradation of galactose transport activity agrees well with published accounts for system II.

Schneider and Wiley (15) were unable to demonstrate competition by galactose of glucose uptake, and therefore concluded that galactose was transported by a separate, derepressible system from system II. However, they were looking for competitors with K_i's comparable to the K_m for glucose, and therefore saturated the system with glucose and looked for inhibition by a 10-fold excess of galactose. Because the K_m for galactose is 10 to 50 times higher than the glucose K_m (depending on whose data are used), such an experiment would show only slight inhibition by galactose.

The regulation of system II is proving to be complicated. The observation that galactose, and only galactose, could induce system II twice as much as carbon starvation was quite unexpected. It is reasonable for a transport system of broad specificity to be expressed under conditions of carbon starvation. It is not obvious, however, why a transport system with broad specificity should be induced by only one of the transported sugars, and one for which the transport system has a rather low affinity. Yet there is persuasive evidence that this indeed is the case, and the data argue against other possible interpretations.

Because cycloheximide prevents galactose induction of the transport system, we feel that the increased activity of the system is not due to transstimulation (19), i.e., a more rapid turnover of transport sites as a result of intramycelial

galactose loading. This phenomenon, although frequently a property of transport systems, should not require protein synthesis. In addition, if the increased transport activity produced by galactose were due to transstimulation, other nonmetabolizable substrates of the system should also stimulate galactose uptake, and this is not the case, for neither 3-*O*-methyl glucose nor L-sorbose stimulated galactose uptake, even after extensive loading. Nor did galactose induce a galactokinase which metabolizes the intramycelial galactose very rapidly because the intramycelial measurements of galactose-induced mycelia showed almost all the label (after 8 min) was present as free galactose.

The increased transport activity observed after incubation is probably not due to the induction of a second, galactose-specific transport activity. Because galactose, xylose, and L-sorbose are all transported with the same apparent K_m's in galactose-induced as in derepressed mycelia, and because the transport rate was approximately twice as high for all three sugars in galactose-induced mycelia, there was probably twice as much of the same system under conditions of galactose induction.

It is possible, of course, that we are looking at the same net amount of transport system in galactose-induced mycelium, but that it transported sugars at twice the rate of derepressed mycelia. Because this increased transport rate is dependent on protein synthesis for its expression, we must conclude that some protein or group of proteins is capable of increasing the rate of sugar transport.

The effects of glucose on the regulation of system II are complex. Previous reports have provided evidence that glucose prevents transcription of messenger for the transport system, hastens degradation of preexisting messenger, and causes degradation of the transport system itself (16, 17). In addition, because glucose is the sugar for which the transport system has the highest affinity, its presence prevents the uptake of any other sugar.

We originally thought that the glucose-stimulated degradation of system II might be caused by a membrane-bound protease and that this phenomenon and its control were distinct from glucose repression of transcription. Now, however, there seems to be evidence that these phenomena may be related. Repression of transcription and degradation of the transport system seem to be effected by the same spectrum of sugars; furthermore, there is a correlation between the degree of repression and the rate of degradation. Those sugars causing full repression (glucose, mannose, 2-deoxyglucose) also cause the fastest degradation of the system, whereas those sugars which only repress partially (fructose, xylose) degrade the system at lower rates.

This might all be understandable if system II were turning over rapidly, as does the tryptophan transport system in *Neurospora* (21). However, if galactose-induced mycelia are incubated with cycloheximide in the presence of galactose, or even in the absence of any carbon source, there is no degradation of system II (Fig. 5). Thus, glucose-caused degradation is not due to a rapid turnover of the transport system. In addition, because glucose causes rapid degradation even in the presence of cycloheximide, this degradation of the transport system must be due to some preexisting inactive molecule which is somehow activated by glucose or some metabolite of glucose.

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