Regulation of Sugar Transport in
Neurospora crassa

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Sugar uptake systems in *Neurospora crassa* are catabolically repressed by glucose. Synthesis of a low K_m glucose uptake system (system II) in *Neurospora* is derepressed during starvation for an externally supplied source of carbon and energy. Fasting also results in the derepression of uptake systems for fructose, galactose, and lactose. In contrast to the repression observed when cells were grown on glucose, sucrose, or fructose, system II was not repressed by growth on tryptone and casein hydrolysate. System II was inactivated in the presence of 0.1 M glucose and glucose plus cycloheximide but not by cycloheximide alone. Inactivation followed first-order kinetics with a half-time of 40 min. The addition of glycerol to the uptake medium had no significant effect on the kinetics of 3-0-methyl glucose uptake, suggesting that the system was not feedback inhibitable by catabolites of glycerol metabolism.

In bacteria (4) and yeast (1) most sugar uptake systems are inducible or feedback inhibitable, or both. Glucose transport systems on the other hand are generally synthesized constitutively.

Scarborough (10, 11) has shown that glucose transport in *Neurospora crassa* is mediated by two kinetically distinct systems: an active transport system with a K_m of 10 μM and a facilitated diffusion system with a K_m of 8 mM. The low K_m system was not apparent in cells grown on 50 mM glucose.

Work conducted simultaneously in our laboratory essentially confirmed the work of Scarborough (10). We designated the high K_m system, system I, and the low K_m system, system II (Bacteriol. Proc., p. 145, 1970). Some of the kinetic characteristics of systems I and II are described in the preceding paper (12). The high K_m system was inhibited by NaN$_3$ and 3-0-methyl glucose (3-0-MG) was shown to be accumulated against a concentration gradient. These studies and studies on transmembrane fluxes in the presence of NaN$_3$ suggested that both system I and system II were active.

The increased activity of system II in cells grown in fructose, 1 mM glucose (10, 11), or starved of a carbon source (12) could have resulted from either activation or derepression (synthesis) of system II. The results described in this report suggest that the increased activity of

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system II resulted from de novo synthesis. Derepression of distinct uptake systems for fructose, galactose, and lactose also occurred during starvation for a carbon and energy source. No evidence for feedback inhibition of system II was obtained.

MATERIALS AND METHODS

The strain of *N. crassa* used in these studies was wild-type strain Em a. The methods and materials used for culture of the mold and for assay of sugar uptake were described in the accompanying paper (12). Actinomycin D was purchased from Calbiochem (Los Angeles). All other chemicals were reagent grade.

RESULTS

Time course of synthesis of system II. The increase in the rate of uptake of several sugars during carbon starvation is shown in Fig. 1. These results were obtained from cells that were grown for 16 hr on 0.1 M glucose, washed and transferred to sugar-free Vogel's medium. After incubation at 30 C for various periods of time, portions of the fasting cells were assayed for the capacity to take up [^{14}C]glucose from a 10^{-4} M solution. Similar measurements were made for fructose, galactose, and lactose uptake. Previous data showed that the uptake of these sugars was mediated by four distinct systems and that the uptake from medium containing 10^{-4} M glucose was a measure of the activity of system II (12). The rates of uptake of the four sugars were quite different; in each case, however, they increased
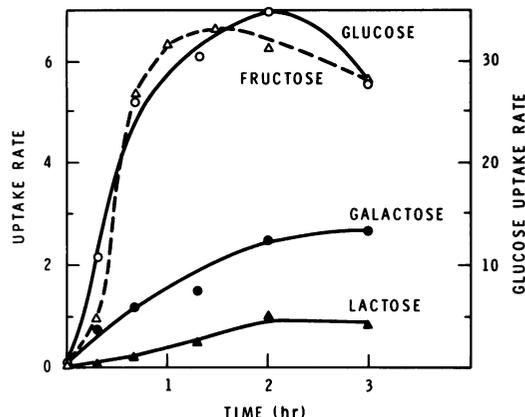
FIG. 1. *Rate of uptake of 10^{-4} M glucose (O), fructose (△), galactose (●), and lactose (▲) as a function of time after transfer of cells from glucose medium to sugar-free medium. Portions were removed from the starving culture and assayed for the uptake of the ^{14}C-labeled sugars. Uptake rates were calculated by least squares analysis of six 2-ml samples assayed at 30-sec intervals. Uptake rate was measured as nanomoles of sugar accumulated per milligram (dry weight) of cells per minute.*

FIG. 2. *Effect of cycloheximide on synthesis of system II during carbon starvation. A glucose-grown culture was transferred to sugar-free media, with (●) and without (O) 4 µg of cycloheximide per ml. Portions of the cells were removed at 20 and 40 min, washed, and assayed for uptake of 10^{-4} M 3-0-MG (specific activity, 0.05 µCi/µmole). Uptake rate was measured as counts per minute accumulated per milligram (dry weight) of cells per minute.*

during starvation. The increase in the activity of all the sugar uptake systems was inhibited by glucose but unaffected by the presence of galactose or lactose in the medium. The data suggested that the uptake systems were derepressed during carbon starvation. Since this strain grows very slowly on galactose and lactose (doubling time of more than 10 hr), it is possible that the lack of repression by galactose and lactose is related to the inability of the organism to rapidly metabolize these sugars.

Requirement of protein and RNA synthesis for derepression of system II. If the increased ability to transport sugars observed during fasting represents derepression of synthesis of the uptake systems, the addition of an inhibitor of protein synthesis should prevent this increase. Cycloheximide is an antibiotic which inhibits protein synthesis in *Neurospora* by 98% (14). The effect of cycloheximide on the derepression of system II activity is shown in Fig. 2. The antibiotic inhibited the increase in system II activity, as well as systems for fructose, galactose, and lactose uptake. The implication of these data are that the increased sugar transport during carbon starvation requires de novo synthesis of the uptake systems.

The requirement of ribonucleic acid (RNA) synthesis for derepression was studied by using a modification of the technique of Turner, Terry, and Matchett (14). Cells were suspended in 20 µg of actinomycin D per ml in 10 mM tris(hydrox-
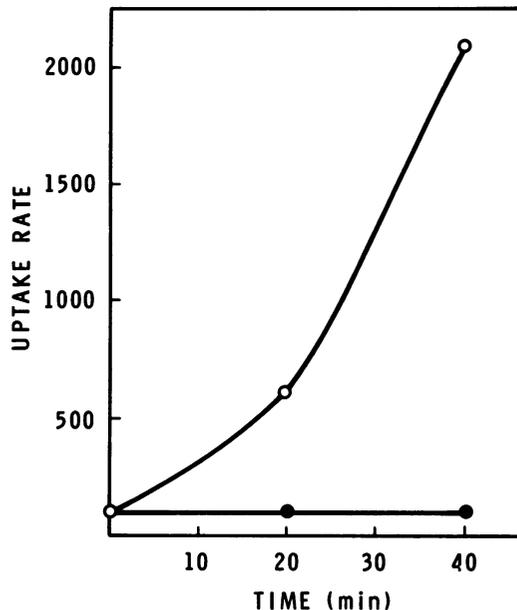
ymethyl)aminomethane ethylenediaminetetraacetic acid (Tris-EDTA), pH 8, at 40 C for 4 min before washing and suspending in sugar-free Vogel's medium containing 4 µg of actinomycin D per ml. Control cells were treated in the same manner except for the omission of the antibiotic. Portions were removed at appropriate intervals and assayed for uptake of 10^{-4} M 3-0-MG. The data from five such experiments are shown in Table 1. Actinomycin D consistently inhibited derepression; the degree of inhibition, however, was variable. Turner, Terry, and Matchett showed that actinomycin D inhibited RNA synthesis in *Neurospora* about 50% (14). The lack of complete inhibition apparently resulted from the fact that the cells were impermeable to the antibiotic. Although the data are equivocal, it seems clear that RNA synthesis is required for the derepression of system II.

Effect of various carbon sources on derepression of system II. The effect of the carbon and energy source on derepression of system II was studied in an experiment summarized in Table 2. Vogel's medium containing the carbon source to be tested was inoculated with conidia; the germinated cultures were harvested after 17 to 22.5 hr

TABLE 1. *Effect of actinomycin D on derepression of system II*

Expt	Time after actinomycin D treatment (min)[a]	Uptake rate as per cent of control
1	15	8
	30	15
2	15	55
	30	38
3	40	43
4	40	29
5	20	70
	40	71
Mean		41

[a] Cells were treated with ethylenediaminetetraacetic acid.

TABLE 2. *Effect of various carbon sources on the synthesis of system II[a]*

Carbon source	Cell dry weight (mg/ml)	Duration of growth (hr)	3-0-MG uptake rate (nmoles per mg per min)
Glucose	3.2	17	.011
Fructose	3.1	17	.047
Sucrose	3.1	17	.012
Xylose	1.0	17	1.1
Glycerol	0.75	22.5	0.88
Casein hydrolysate[b] . .	2.0	19	2.1
Tryptone	2.3	17	2.1
None[c]			1.7

[a] Concentrations of all carbon sources were 2%; cultures were inoculated with conidia and grown at 30 C for the period indicated; uptake of 10^{-5} M 3-0-MG was measured at 30 C over a period of 3 min.

[b] Acid-hydrolyzed casein.

[c] Cells were grown for 17 hr on glucose, suspended in glucose-free medium, and starved for 90 min before assay.

of growth at 30 C. The cells were washed and assayed for rate of uptake of 10^{-5} M 3-0-MG. Derepression was completely inhibited by growth on sucrose and glucose, and almost completely by growth on fructose. The cells grew at a maximal rate on these three sugars (doubling time of 2.5 hr). The rate of growth on xylose or glycerol was about half that on glucose, and the cells were about one-half derepressed relative to starved cells. As mentioned previously, galactose and lactose are metabolized very slowly by this strain of *Neurospora* and consequently have no effect on derepression. Tryptone and casein hydrolysate supported a growth rate comparable to that observed on glucose but did not repress synthesis of system II. Since derepression occurs in the absence of an exogenous energy source, it seems reasonable to assume that the energy derived from endogenous reserves are preferentially utilized to synthesize the uptake system.

An experiment designed to examine the effect of glucose concentration on synthesis of system II is illustrated in Fig. 3. The cells were grown for 16 hr in 0.1 M glucose, transferred to culture flasks containing the various concentrations of glucose and allowed to continue growth. After 90 min of growth, the cells were washed and assayed for the rate of uptake of 10^{-4} M 3-0-MG. Figure 3 shows a plot of the activity of system II as a function of glucose concentration during the 90-min growth period. These data show that glucose repression occurs at concentrations less than 1 mM and is maximal at concentrations greater than 2 mM.

Degradation of system II. Recent evidence demonstrated that inactivation of enzymes (13) and uptake systems (15) is an important aspect of regulation in eucaryotes. An experiment designed to investigate the role of specific protein degradation in the regulation of system II is shown in Fig. 4. Glucose-grown cells were washed and starved by incubation in sugar-free medium for 1 hr. Portions were removed and assayed for synthesis of system II, as indicated by the uptake of 3-0-MG. At the end of 1 hr, the culture was divided, cycloheximide was added to one half, and 0.1 M glucose was added to the other half. Incubation of the two cultures at 30 C
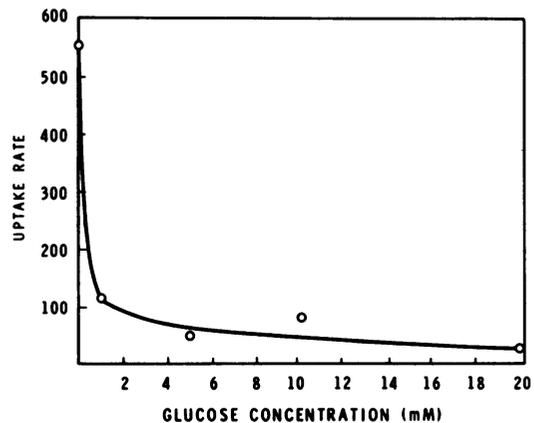

FIG. 3. *Initial rates of 10^{-4} M [^{14}C]3-0-MG uptake as a function of glucose concentration. The specific activity of 3-0-MG was 0.05 $\mu Ci/\mu mole$. Glucose grown cells were washed and suspended in media containing the appropriate concentration of glucose. The cells were grown at 30 C for 90 min, harvested, and washed; the uptake rate of 3-0-MG was measured as described in Fig. 1. Uptake rate was measured as counts per minute accumulated per milligram (dry weight) of cells per minute.*
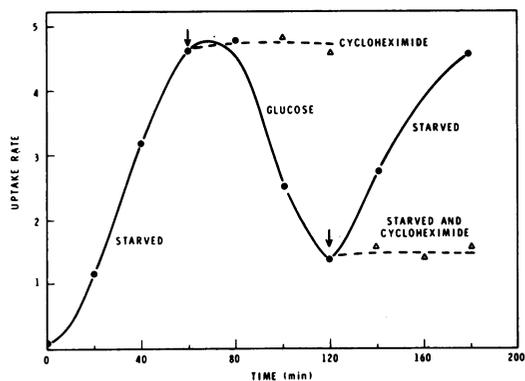
FIG. 4. *Effect of cycloheximide and glucose on system II activity. Glucose-grown cells were starved for 1 hr; during this time, they were assayed for the initial rate of 3-0-MG uptake. At 1 hr (first arrow), the culture was divided into two subcultures; cycloheximide (4 µg/ml) was added to one culture and glucose (0.1 M) was added to the other. Samples were removed at the appropriate intervals, washed, and assayed for the rate of 3-0-MG uptake ([^{14}C]3-0-MG, 10^{-4} M; specific activity, 0.05 µCi/µmole). At 2 hr (second arrow), the culture growing in glucose was subdivided into two cultures. Both subcultures were incubated in sugar-free media: one with 4 µg of cycloheximide per ml and the other without cycloheximide. Uptake rate was measured as nanomoles of 3-0-MG accumulated per milliliter of cells per minute.*

FIG. 5. *Semilogarithmic plot of 3-0-MG (10^{-4} M; specific activity, 0.5 µCi/µmole) uptake as a function of time after addition of 0.1 M glucose to a derepressed culture. The cells were starved for 2 hr, after which 0.1 M glucose was added to the culture. Portions of the cells were removed from the culture, washed, and assayed as described in Fig. 1. Data are shown for two experiments. Uptake rate was measured as counts per minute per milliliter of cells per minute.*

was continued, and portions were removed for assay of system II activity at various intervals. During the first hour, the cells synthesized system II, and the transport rate of 3-0-MG increased (Fig. 4). Derepression was interrupted by the addition of cycloheximide or glucose to the medium. System II persisted after the addition of the antibiotic but was rapidly degraded in the presence of glucose. These results suggest that system II, unlike the tryptophan transport system of *Neurospora* (15), does not turnover continuously but is specifically inactivated in response to the presence of glucose.

After 1 hr of incubation in glucose-containing medium, the cells were harvested, washed, and suspended in sugar-free medium with and without cycloheximide. Resynthesis of system II was prevented by cycloheximide, suggesting that breakdown had occurred and that protein synthesis was necessary to restore uptake activity (Fig. 4). Metabolism of glucose seems to be required for the degradation since inactivation was not observed in derepressed cultures incubated in 3-0-MG. Further degradation of system II does not appear to require protein synthesis since glucose mediated degradation was unaffected by the presence of cycloheximide in the medium.

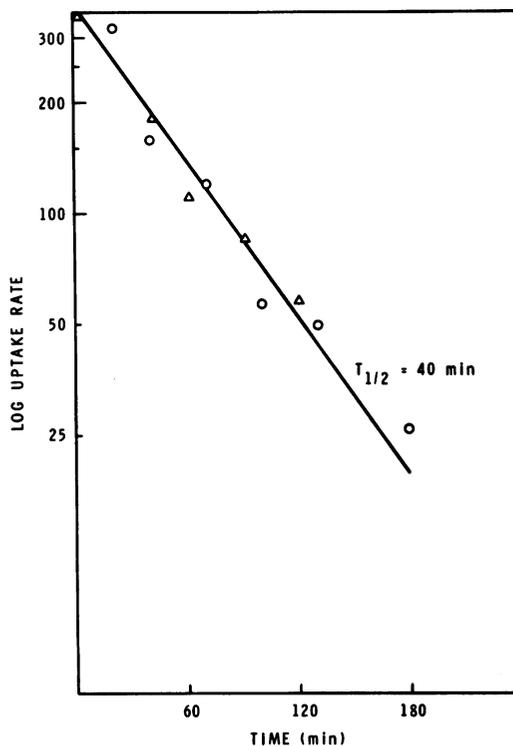Figure 5 is a semilogarithmic plot of 3-0-MG uptake rate as a function of time after addition

of 0.1 M glucose. The data shown in Fig. 5 are from two experiments. The inactivation of system II follows first-order rate kinetics, with a half-time of 40 min. This is significantly longer than the half-time of 15 min for turnover of the tryptophan transport system in this organism (15).

Effect of glycerol on the rate of 3-0-MG uptake. The experiment shown in Fig. 6 was designed to determine whether system II was subject to feedback inhibition by catabolites of glycerol. Previous studies showed that feedback inhibition of metabolite uptake systems may exhibit competitive or noncompetitive kinetics (4). In *Salmonella typhimurium*, the kinetic pattern of feedback inhibition of α-methyl glucoside uptake is mixed, i.e., V_{max} is decreased and K_m is increased (2). In the experiment described in Fig. 6, cells were grown for 16 hr on glucose, transferred to medium containing 2% glycerol, and incubated for 3 hr. Incubation in glycerol medium for 3 hr was previously shown to be suffi-

cient time to adapt cells to growth on glycerol and to derepress system II. The initial rates of 3-0-MG uptake were measured in the presence and absence of 2% glycerol. Figure 6 shows a typical hyperbolic substrate saturation curve for system II (3-0-MG substrate) in the presence and absence of glycerol. The difference in the two curves is slight, suggesting that no significant competitive or noncompetitive inhibition occurred. Similarly, the fact that V_{max} for glucose uptake is higher than that observed for 3-0-MG uptake (13) suggests that catabolites formed from glucose metabolism do not feed back and inhibit the activity of system II in the manner described for glucoside uptake in *S. typhimurium* (2). Further, the activity of system II is unaffected by the pool size (unaltered sugar) since preloading cells with unlabeled 3-0-MG increased the rate of labeled 3-0-MG uptake.

DISCUSSION

The increased activity of a low K_m system for glucose uptake during growth of *Neurospora* on casein hydrolysate and tryptone and by sugar deprivation (13) is the result of derepression of synthesis of this system. The low K_m system was completely repressed by growth of the cells on glucose and sucrose and about 80 to 90% by growth on fructose. Thus, the pattern of regulation of sugar uptake in *Neurospora* is by repression and derepression of synthesis of the low K_m system (system II). This differs from inductive synthesis observed in bacteria and yeast. Regulation of sugar uptake in *Neurospora* is similar to control of amino acid transport in bacteria (4) and *Neurospora* (15); these systems are synthesized constitutively and are subject to repression.

Our data show that germination and growth of conidia for 16 hr on 0.1 M fructose or incubation of germinated conidia for 90 min in 1 mM glucose results in repression of system II activity. Scarborough (10, 11), on the other hand, reported that growth on fructose or 1 mM glucose derepresses the low K_m system. There is no clear explanation for the differences in our results and those of Scarborough (11); however, it is possible that the shorter germination time used by Scarborough was not sufficient to decrease system II activity developed during adaptation to growth on fructose. We found that derepression of system II occurred during the adaptation to growth on fructose (starved cells). In addition, in an experiment in which the cells were grown on 1 mM glucose for 90 min, the cells used 20% of the glucose present in the medium. It is possible that in the experiments reported by Scarborough the cells reduced the concentration of glucose to levels that permitted derepression. Strain differ-
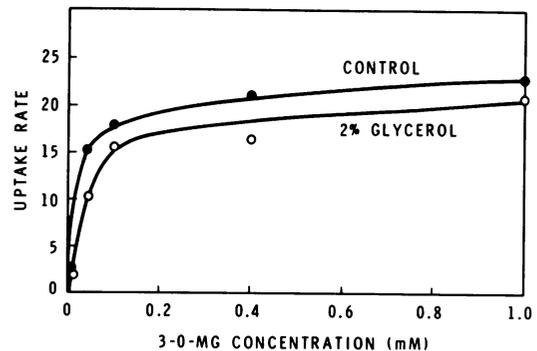

FIG. 6. *Effect of glycerol on the kinetics of 3-0-MG uptake by system II. Cells were grown on glycerol for 3 hr, harvested, and washed; rates of 3-0-MG uptake were measured in the presence and absence of 2% glycerol. The initial rates of uptake of various concentrations were measured by the procedure described for Fig. 1.*

ences may have also contributed to the discrepancies in our results.

Regulation of uptake systems by specific protein degradation is apparently a widespread phenomenon in microorganisms. Wiley and Matchett (15) showed that the tryptophan uptake system of *Neurospora* turns over continuously, and that tryptophan uptake decays with a half-time of about 15 min after addition of cyclohexeximide or repressive concentrations of tryptophan to the incubation medium. Robertson and Halvorson (8) reported that glucose stimulated inactivation of sugar uptake in yeast. Uptake of α-methyl glucose was inactivated with a half-time of about 50 min after addition of glucose to cultures adapted to growth on maltose. The time course of inactivation was similar to that described in this investigation.

Controlled inactivation or degradation of the uptake systems provides an excellent means for regulation of the rates of metabolite flow and avoids the accumulation of toxic concentrations of intermediates. The fact that feedback inhibition of system II activity was not observed during glucose metabolism suggests that specific degradation of sugar transport systems may play a physiologically important role in the fine control of sugar uptake in *Neurospora*.

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