JOURNAL OF BACTERIOLOGY, June 1989, p. 3545–3548
0021-9193/89/063545-04$02.00/0

Vol. 171, No. 6

NOTES

Role of Cyclic-AMP-Dependent Protein Kinase in Catabolite Inactivation of the Glucose and Galactose Transporters in *Saccharomyces cerevisiae*

JOSÉ RAMOS† AND VINCENT P. CIRILLO*

Department of Biochemistry, State University of New York, Stony Brook, New York 11794

Received 2 September 1988/Accepted 6 March 1989

The derepressed high-affinity glucose transport system and the induced galactose transport system are catabolite inactivated when cells with these transport systems are incubated with glucose. The role of the cyclic AMP cascade in the catabolite inactivation of these transport systems was shown by using mutants affected in the activity of cyclic-AMP-dependent protein kinase (cAPK). In *tpk1(w)* mutants with reduced cAPK activity, the sugar transport systems were expressed but were not catabolite inactivated. In *bcy1* mutants with unbridled cAPK activity resulting from a defective regulatory subunit, the transport systems were absent or present at low levels.

Yeast cells can adapt to changing environmental and physiological conditions. Cells growing in an aerated glucose medium initially transport glucose by a constitutive, low-affinity system (1, 20) and metabolize it primarily by ethanol oxidative phosphorylation, and mitochondrial biogenesis are all repressed. As the glucose concentration falls and the ethanol concentration rises, the cells derepress all of these systems. Upon inoculation into a high-glucose medium,

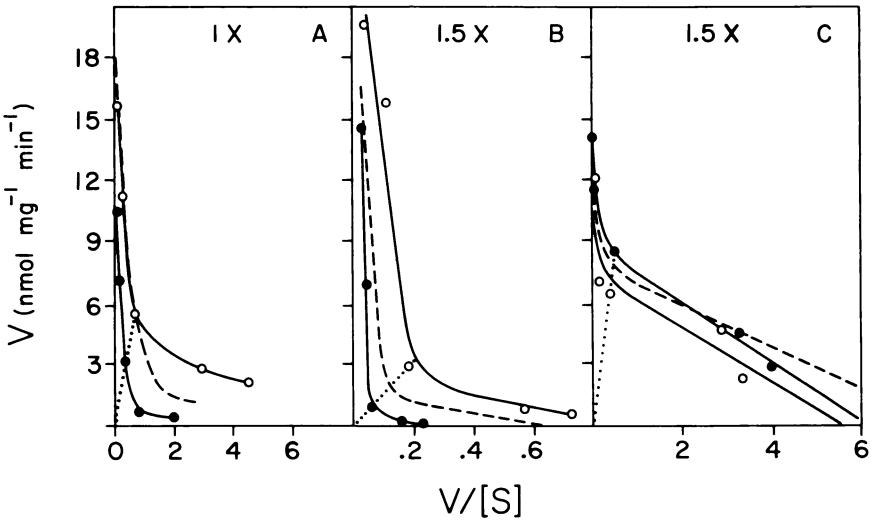

FIG. 1. Catabolite inactivation of glucose transport as shown by Eadie-Hofstee plots of glucose uptake by wild-type (A), *bcy1* (B), and *bcy1 tpk1(w11)* (C) cells. Glucose uptake rates were calculated from 1-min uptake levels by 2.5 mg (wet weight) of cells at 30°C in glucose concentrations of 0.5 to 300 mM. Uptake was measured in cells used without further treatment (○) and in cells suspended in SD medium containing 10% glucose for 2 (— — — — [to avoid crowding, no datum points are included]) or 4 (●), h, respectively. The dotted line passing through the origin of the coordinates connects datum points representing glucose uptake rates for 10 mM glucose. The ordinate scales for panels B and C are reduced by a factor of 1.5 relative to that of panel A.

fermentation. The transport system for high-affinity glucose transport (1, 20), the enzymes for gluconeogenesis and these adaptations are reversed, some by carbon catabolite repression (i.e., inhibition of synthesis) and others by catabolite inactivation (proteolytic degradation). The high-affinity glucose transport system is catabolite inactivated (17). The inducible galactose (10) and maltose (19) transport systems are also catabolite inactivated when induced cells are trans-

* Corresponding author.
† Permanent address: Department of Microbiology, University of Cordoba, Cordoba, Spain.

TABLE 1. Relevant genotypes of yeast strains used[a]

Strain	Genotype
HSC	Wild type
T162-1A	*BCY1 TPK1 tpk2::HIS3 tpk3::TRP1 ura3 gal2*
S13-58A	*bcy1::LEU2 TPK1 tpk2::HIS3 tpk3::TRP1 ura3 gal2*
TFS13-58A	*bcy1::LEU2 TPK1 tpk2::HIS3 tpk3::TRP1 URA3 GAL2*[b]
RS13-58A-1	*bcy1::HIS3 tpk1 (w1) tpk2::HIS3 tpk3::TRP1 ura3 gal2*
RS13-58A-11	*bcy1::HIS3 tpk1 (w11) tpk2::HIS3 tpk3::TRP1 ura3 gal2*
TFRS13-58A-11	*bcy1::HIS3 tpk1(w11) tpk2::HIS3 tpk3::TRP1 URA3 GAL2*[b]
TF14.1	*bcy1::HIS3 tpk1(w1) tpk2::HIS3 tpk3::TRP1 cyr1::URA3*

[a] Strain HSC was obtained from P. Maitra; strains T162-1A, S13-58A, RS13-58A-1, RS13-58A-11, and TF14.1 were obtained from S. Cameron, M. Zoller, and M. Wigler (3).
[b] Transformed with pTUG4.

ferred to glucose. Interestingly, the enzymes induced with the transporters for the utilization of these sugars are only catabolite repressed (2, 9, 18). Catabolite inactivation of derepressible and inducible sugar transporters is observed in most if not all yeasts (1, 2, 6, 8, 12, 17, 21, 24). Extensive studies with the gluconeogenic enzyme, fructose-1,6-bisphosphatase, have shown that catabolite inactivation in yeast cells depends on the activity of cyclic-AMP-dependent protein kinase (cAPK) (14–16, 22). In this preliminary report, we present evidence that catabolite inactivation of the high-affinity glucose transport and the galactose transport system of *Saccharomyces cerevisiae* also depend on cAPK activity.

The sources and relevant genotypes of the *S. cerevisiae* strains used are presented in Table 1. The yeast cells were grown in shake flasks at 30°C in YP (1% yeast extract, 2% peptone) or SD (yeast nitrogen base; Difco Laboratories, Detroit, Mich.) medium containing the sugars indicated. Sugar uptake by whole cells was measured as previously described (11, 17). The V_{max} and K_m for the high- and low-affinity components of glucose and galactose transport were determined from Eadie-Hofstee plots (17). Catabolite inactivation was initiated by incubating cells with glucose in SD medium. We have shown that catabolite inhibition occurs under nongrowing conditions, i.e., in SD medium without amino acid or growth factor supplements and in distilled water.

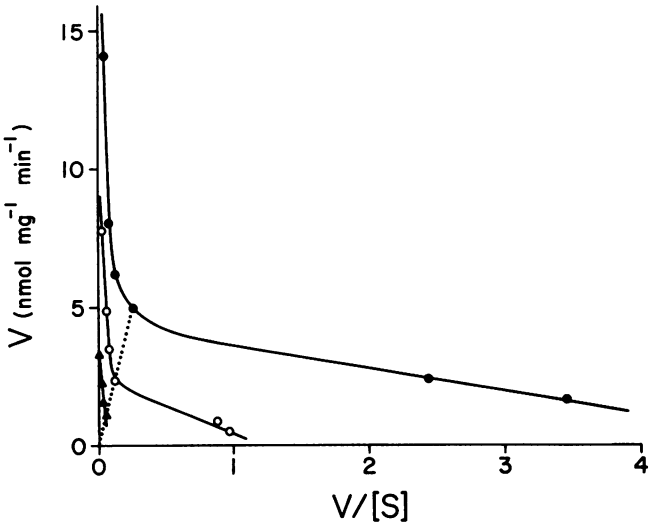

FIG. 2. Catabolite inactivation of galactose transport as shown by Eadie-Hofstee plots of galactose uptake by wild-type cells. Catabolite inactivation and uptake conditions were as described in the legend to Fig. 1 except for the substitution of galactose for glucose and the use of SD medium containing 2% glucose. Uptake was measured in cells suspended in SD medium without further treatment (●) and in cells suspended in SD medium containing 2% glucose for 2 (○) or 4 (▲) h, respectively.

Figure 1A shows the phenomenon of catabolite inactivation of the high-affinity glucose transport system in the wild-type strain HSC. The Eadie-Hofstee plot of glucose uptake by these cells showed biphasic kinetics, with a high- and a low-affinity component; the V_{max} and K_m for the high-affinity component were 4 nmol/mg of cells per min and 0.5 mM, respectively; for the low-affinity component, they were 14 nmol/mg of cells per min and 20 mM. When the cells were incubated in a 10% glucose solution (at a cell density of 2.5% (wet wt/vol) in a rotary shaker at 30°C), there was a progressive decrease in the V_{max} of the high-affinity component but very little change in the low-affinity component. After 2 and 4 h of incubation in glucose, the V_{max} of the high-affinity transport process fell to 50 and 90% of the initial values, whereas the corresponding values for the low-affinity process were 114 and 86%, respectively. The K_ms did not change.

The role of cAPK (4, 7) in catabolite inactivation of the high-affinity glucose transport system was tested with mutations that affect cAPK activity. Two classes of mutants were

TABLE 2. Effects of cAPK mutations on expression and catabolite inactivation of glucose and galactose transport

cAPK genotype	cAPK phenotype	Glucose transport				Galactose transport			
		High affinity		Low affinity		High affinity		Low affinity	
		Expression[a]	Catabolite inactivation[b]	Expression	Catabolite inactivation	Expression	Catabolite inactivation	Expression	Catabolite inactivation
Wild type	Wild type	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
bcy1	Unbridled	Low	Yes	Yes	No	No		No	
tpk1(w)	Defective	Yes	No	Yes	No	Yes	No	Yes	No

[a] V_{max} measured in Eadie-Hofstee plots. Yes, Level of activity comparable to that of the wild type; Low, ca. 20% of the wild-type level; No, no detectable activity.
[b] Loss of activity after 4 h of exposure to glucose in comparison with the wild-type level. Yes, At least 50% loss of activity; No, less than 10% loss of activity.

TABLE 3. Catabolite inactivation of low-affinity galactose transport activity in APK-deficient cells

Strain	Velocity (nmol/mg of cells per min) after given time (h) of incubation in glucose[a]		
	0	2	4
HSC	14.1 (100)	7.8 (55)	3.3 (23)
tpk1(w1)	8.2 (100)	8.9 (108)	9.8 (119)

[a] Velocity of uptake was measured at a galactose concentration of 300 mM for 1 min at 30°C. Catabolite inactivation was induced by incubating galactose-grown cells in SD medium containing 2% glucose at 30°C. Numbers in parentheses are percentages of initial velocity.

tested: (i) *bcy1* mutants with unbridled cAPK activity resulting from a defect in the regulatory subunit and (ii) *tpk1(w)* mutants with markedly reduced cAPK activity (3, 13). The *bcy1* mutants showed a greatly reduced level (ca. 20 to 25%) of high-affinity activity (V_{max}, 1 nmol/mg of cells per min) and normal catabolite inactivation of the residual activity (Fig. 1B). The *bcy1 tpk1(w1)* and *bcy1 tpk1(w11)* (i.e., cAPK-deficient) strains showed a normal level of the high-affinity transporter (V_{max}, 5 nmol/mg of cells per min) that was not catabolite inactivated after 4 h of incubation (Fig. 1C). The combination of the *bcy1 tpk1(w1)* mutants with the *cyr1* gene (defective for adenylate cyclase) did not change from the results obtained with the former alone (data not shown). The wild-type parental strain, *BCY1 TPK1 CYR1*, showed a pattern of catabolite inactivation similar to that of strain HSC (Fig. 1A) (data not shown). The results of these mutations on the presence or absence of high- or low-affinity glucose transport activity and their susceptibilities to catabolite inactivation are summarized in Table 2. Note that none of the mutations had any effect on the low-affinity glucose transport process, which is not susceptible to catabolite inactivation (1, 17).

Eadie-Hofstee plots of galactose transport by induced cells were invariably biphasic, reflecting the presence of high-affinity and low-affinity components (Fig. 2, time zero). Catabolite inactivation of wild-type HSC cells affected both components (Fig. 2). Both components of galactose transport require the galactose permease (i.e., the product of the *GAL2* gene), but the high-affinity process requires, in addition, galactokinase (i.e., the product of the *GAL1* gene [18]). Since the mutants used to test for the role of cAPK in catabolite inactivation of galactose transport were *GAL1 gal2* (3), it was necessary to transform them with plasmid pTUG4, which contains the cloned *GAL2* gene (23). By using *GAL2*-transformed *bcy1* and *bcy1 tpk1(w11)* mutants, it was found that cAPK was required for catabolite inactivation of both components of galactose transport. *bcy1* mutants (i.e., with unbridled cAPK) showed no detectable level of either component of galactose transport (Table 2). *bcy1 tpk1(w11)* (i.e., with reduced cAPK) mutants produced normal levels of both components, and neither was catabolite inactivated (Table 3).

These preliminary data are consistent with a role for cAPK in catabolite inactivation of the sugar transporters. Although recent sequence information for three yeast sugar transporters subject to catabolite inactivation (i.e., glucose [5], galactose [K. Szkutnicka, J. Tschopp, L. Andrews, and V. P. Cirillo, unpublished data], and maltose [C. Michels and Q. Cheng, unpublished data] show that these transporters contain potential cAPK substrate sites, it is not certain that the sugar transporters themselves are targets of cAPK phosphorylation. Another cell or membrane component, such as protease, may be the target; the effect on the transporters may be indirect.

We are grateful to the following for yeast strains and the plasmid used in this study and for many useful discussions: Pabitra Maitra for the wild-type HSC strain, S. Cameron and M. Wigler for the *bcy1*-, *bcy1 tpk1(w)*-, and *cyr1*-containing mutants, Jurg Tschopp and Randy Schekman for plasmid pTUG4. We also thank C. Michels for providing the unpublished sequence of the maltose transporter.

This research was supported by National Science Foundation grant DMB-8702085 to V.P.C. and by grant PB86-0545-C02-00 from the Comisión Asesora para la Investigación Científica y Técnica to J.R.

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