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Regulation of Yeast Trehalase by a Monocyclic, Cyclic AMP-Dependent Phosphorylation-Dephosphorylation Cascade System

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Mutation at the *GLC1* locus in *Saccharomyces cerevisiae* resulted in simultaneous deficiencies in glycogen and trehalose accumulation. Extracts of yeast cells containing the *glc1* mutation exhibited an abnormally high trehalase activity. This elevated activity was associated with a defective cyclic AMP (cAMP)-dependent monocyclic cascade which, in normal cells, regulates trehalase activity by means of protein phosphorylation and dephosphorylation. Trehalase in extracts of normal cells was largely in a cryptic form which could be activated *in vitro* by ATP · Mg in the presence of cAMP. Normal extracts also exhibited a correlated cAMP-dependent protein kinase which catalyzed incorporation of label from [γ-^{32}P]ATP into protamine. In contrast, cAMP had little or no additional activating effect on trehalase or on protamine phosphorylation in extracts of *glc1* cells. Similar, unregulated activation of cryptic trehalase was also found in glycogen-deficient strains bearing a second, independently isolated mutant allele, *glc1-2*. Since trehalase activity was not directly affected by cAMP, the results indicate that the *glc1* mutation results in an abnormally active protein kinase which has lost its normal dependence on cAMP. Trehalase in extracts of either normal or mutant cells underwent conversion to a cryptic form in an Mg^{2+}-dependent, fluoride-sensitive reaction. Rates of this reversible reduction of activity were similar in extracts of mutant and normal cells. This same, unregulated protein kinase would act on glycogen synthase, maintaining it in the phosphorylated low-activity D-form. The *glc1* mutants provide a novel model system for investigating the *in vivo* metabolic functions of a specific, cAMP-dependent protein kinase.

The yeast reserve carbohydrate trehalose can be mobilized as an endogenous source of glucose under a variety of physiological conditions through action of the hydrolytic enzyme trehalase (EC 3.2.1.28) (11, 12, 15).

A glycogen-deficient yeast mutant, bearing the *glc1* gene, was shown by Rothman-Denes and Cabib (19) to prevent the in vivo conversion of low-activity D-glycogen synthase into the high-activity I-form (independent) of the enzyme. Although they considered the possibility that this defect might involve a protein kinase-protein phosphatase, they were unable to demonstrate an abnormality in such a system (20).

In recent studies (1) it has been demonstrated that mutations at the *GLC1* locus result in simultaneous deficiencies in both glycogen and trehalose, which are inherited together, indicating that they have a common molecular basis. The present paper explores this relationship further and presents evidence indicating that both traits could be related to an abnormal protein kinase reaction which has lost its dependence on cyclic AMP (cAMP), as was suggested by preliminary results (14).

Yeast cells contain two forms of the hydrolytic enzyme trehalase, a highly active form, trehalase-*a*, and an inactive "cryptic" form designated trehalase-*c* (26). Cryptic trehalase appears to be converted to active trehalase by action of a cAMP-dependent protein kinase. Both trehalase-*c* and protein kinase activities have been partially purified (27).

The present study demonstrates that the abnormally high trehalase activity associated with the *glc1* mutation can be related to an elevated protein kinase activity which can incorporate label from [γ-^{32}P]ATP into protamine. In extracts of normal cells, both protein kinase activity and cryptic trehalase activation are stimulated by cAMP in the presence of ATP · Mg. In contrast, cAMP has little or no effect on either

activity in mutant extracts. Evidence is also presented that active trehalase is converted to its cryptic form by a protein phosphatase present in extracts and that in normal *Saccharomyces cerevisiae* cells trehalase activity is regulated by a cAMP-dependent, monocyclic cascade system (3).

MATERIALS AND METHODS

Yeast cultures. The genotypes of the various strains used in this study are listed in Table 1.

Growth conditions. Cells were grown in a medium containing 1% yeast extract–1% glucose. Cultures were grown in 400 ml of medium in 2-liter Erlenmeyer flasks, and incubation was at 28°C on a rotary shaker operated at 160 rpm. Harvested cells were washed twice with distilled water. Growth of cultures was followed by turbidity measurements at 570 nm. Glucose was determined with glucose oxidase (18).

Trehalase preparation. Cells were grown in 1% yeast extract-1% glucose, harvested at the onset of diauxie, and washed twice in cold water. The cell pellet, containing 0.6 to 0.9 g (dry weight), was suspended in 4.0 ml of 0.1 M acetate buffer, pH 5.6, and disrupted in a Braun shaker with 5 g of glass beads (0.45-mm diameter) for two 1-min periods. The homogenate was centrifuged at 1,000 × *g* for 10 min. The resulting supernatant solution was centrifuged again at 10,000 × *g* for 25 min, and the precipitate was discarded. The supernatant fluid was cleared of metabolites by passage through a Sephadex G-25 column (1.0 by 20.0 cm) equilibrated with 0.05 M phosphate buffer, pH 7.5.

Activation of cryptic trehalase. To 0.4 ml of the column effluent, 0.1 ml of an activation mixture containing 4 mM ATP, 9 mM $MgSO_4$, 50 μM cAMP, 50 mM NaF, and 5 mM theophylline was added (27). This mixture was prepared in 0.05 M phosphate buffer, pH 7.5. To the control, 0.1 ml of pure buffer was added. The reaction mixture was incubated at 30°C for 10 min and interrupted by adding 1.5 ml of 0.07 M maleate buffer, pH 6.0, containing 10 mM EDTA. The mixture was then centrifuged at 100,000 × *g* for 30 min to eliminate the microsomal fraction, and the supernatant solution was used for trehalase activity determination.

Trehalase activity determination. For *GLC* strains, 0.2 mg of protein was used in the assay, whereas for *glc1* strains 0.05 to 0.1 mg of protein was normally sufficient. The reaction was run in 0.2 ml of 0.07 M maleate buffer, pH 6.0, containing 20 μmol of trehalose and incubated at 30°C for 5 to 20 min. The reaction was interrupted by heating in a water bath at 100°C for 3 min. Before proceeding with glucose determination, 0.3 ml of 0.1 M acetate buffer, pH 5.6, was added, and glucose was determined by a modification of the glucose oxidase-peroxidase method (28). To the reaction mixture 0.1 ml of *o*-dianisidine in 0.03 N HCl was added followed by 0.4 ml of a mixture of glucose-oxidase (9.9 U/ml) and peroxidase (11.1 U/ml) in 0.1 M acetate buffer, pH 5.6.

Protein kinase determination. For protein kinase activity, 10 to 20 μl of the 10,000 × *g* supernatant (10 to 15 μg of protein) was added to 100 μl of a reaction mixture containing 50 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP (50 to 200 cpm/pmol), 15 μg of phenylmethylsulfonyl fluoride, and 100 μg of protamine (13). When necessary, 10 μM cAMP was added. After incubation at 30°C for 10 min, the reaction was interrupted by addition of 10 μl of a solution containing 10 mM ATP, 20 mM sodium pyrophosphate, and 50 mM sodium phosphate. Samples of 55 μl were applied on Whatman 3 MM disks, soaked in 15% trichloroacetic acid for 15 min, and washed twice with 5% trichloroacetic acid. The samples were then immersed in 95% ethanol and dried at room temperature, and the radioactivity was determined in a scintillation counter.

TABLE 1. Yeast strains used: genotypes and sources

Strain	Genotype	Source[a]
S288C	*GLC mal gal2*	1
GS1-36	α *glc1-1 mal gal2*	1
BR15-7C	a *glc1-1 MAL4*^{c} *his1 ura3*	2
BR15-4C	a *GLC MAL4*^{c} *ura3*	2
D311-3A	a *GLC mal his lys2 trp2*	3
20Q1-3A	α *GLC mal gal2*	4
20Q1-3B	a *glc1-2 mal gal2*	4
20Q1-3C	α *glc1-2 mal gal2*	4
20Q1-3D	a *GLC mal gal2*	4

[a] Sources: 1, E. Cabib, National Institutes of Health, Bethesda, Md.; 2, prepared at the Federal University of Rio de Janeiro; 3, F. Sherman, University of Rochester, Rochester, N.Y.; 4, J. R. Pringle, Institute of Microbiology, Swiss Federal Institute of Technology, Zürich, Switzerland.

Genetic analysis. Isolation of diploids, induction of sporulation, dissections, and tetrad analysis were performed as described by Hawthorne and Mortimer (9) and Sherman (21). Maltose fermentation was indicated by indicator color change on agar medium containing 0.33% bromocresol purple. Glycogen synthesis in intact cells was indicated by direct staining of yeast colonies with a single drop of an iodine solution (0.4% KI, 0.2% I_2). Normal cells stained brown, whereas deficient *glc1* cells acquired only a yellow shade after staining.

Special chemicals. Trehalose, theophylline, ATP, cAMP, NaF, protamine, phenylmethylsulfonyl fluoride and the enzymes for glucose determination were purchased from Sigma Chemical Co. Sephadex G-25 (superfine) was from Pharmacia Fine Chemicals, Inc. [γ-^{32}P]ATP was prepared according to Cashel et al. (2).

RESULTS

The hypothesis that the *glc1-1* mutant contained an abnormally active trehalase (14) was tested by measuring trehalase activity in extracts prepared from the original isogenic strains, S288C and GS1-36, harvested near the onset of the diauxic transition phase. Trehalase

activity in normal extracts could be increased severalfold by incubation with ATP · Mg and cAMP, indicating that most of the enzyme was in a latent or cryptic form. In contrast, most of the trehalase activity in mutant extracts was already activated (Table 2).

When the mutant allele was introduced into a normal wild-type strain, the highly active trehalase characteristic was inherited together with the deficiency in glycogen accumulation (strain BR15-7C).

An independently isolated glycogen-deficient mutant which contains a defective gene, allelic to *glc1-1*, has also been shown to be deficient in trehalose accumulation (4). The data in Table 2 demonstrate that whenever the *glc1-2* allele is present cAMP causes little or no activation of trehalase. A complete tetrad isolated from diploid strain 20Q1 is shown. High levels of cryptic trehalase are found only in normal segregants.

It is well known that the relative activities of a number of yeast enzymes can vary widely at different stages of growth in batch culture. Changes in enzyme concentration or enzyme activation or both may occur. Trehalose accumulation capacity is also subject to variations with the physiological state of the cells (16). It was, therefore, necessary to demonstrate that the apparent abnormal trehalase-*c* activation in extracts of the *glc1-1* mutant was not merely a consequence of harvesting mutant and parental strains in different physiological states. Figure 1 shows that, even though total trehalase activity varies during growth, the extremely low proportion of trehalase-*c* in the mutant was a constant characteristic.

The low trehalase activity seen in the normal parental strain (S288C) can be increased dramatically by treatment with ATP · Mg in the presence of cAMP (Table 3). Therefore, although the extract from the parental strain does contain trehalase activity, most of the enzyme (81%) is in the inactive or cryptic state. Similar cAMP-dependent activation could be seen in extracts of another normal strain, D311-3A. In that case cryptic trehalase was activated from 20.7 to 96.9 nmol of glucose min^{-1} mg of $protein^{-1}$.

In contrast, trehalase in extracts prepared from the *glc1-1* mutant is almost entirely in its active form irrespective of any additions. The data in Table 3 show that activation of the cryptic trehalase in extracts of the normal strain, S288C, requires both ATP and cAMP, whereas neither nucleotide causes an appreciable change in the trehalase activity obtained from cells of the *glc1-1* mutant. Apparently, then, the trehalase from the mutant is already almost completely activated.

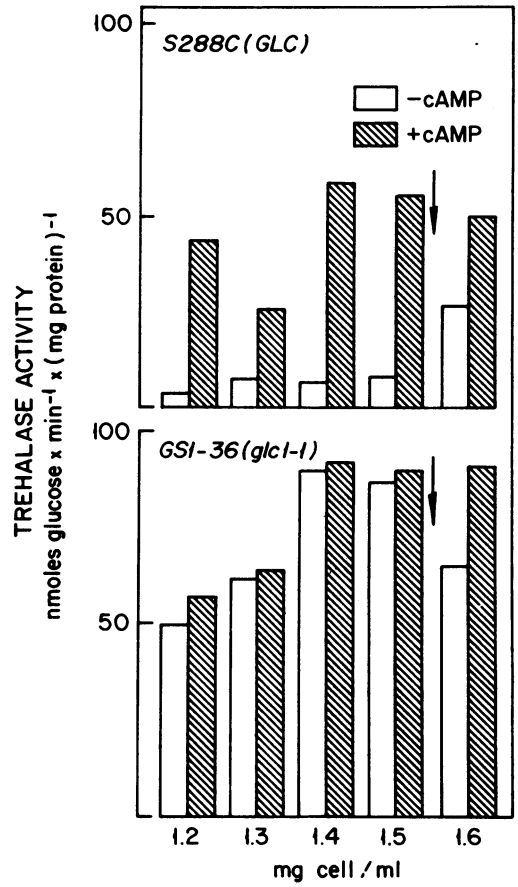

FIG. 1. Changes in trehalase activity during growth. Cells were harvested (0.6 to 0.9 g, dry weight) at different stages of growth in 1% glucose–1% yeast extract medium. Arrow indicates onset of the diauxic phase. Cell-free extracts, activation, and determination of trehalase activity were performed as described in the text.

TABLE 2. Effects of *glc1* mutation on trehalase activity in vitro[a]

Strain	Genotype	Trehalase activity (nmol of glucose min^{-1} mg of $protein^{-1}$		Activation ratio
		Control	+ cAMP	
S288C	*GLC*	9.8	52.5	5.40
GS1-36	*glc1-1*	75.0	84.0	1.12
BR15-4C	*GLC*	7.9		
BR15-7C	*glc1-1*	81.0		
20Q1-3A	*GLC*	10.3	25.8	2.50
20Q1-3B	*glc1-2*	48.6	49.5	1.02
20Q1-3C	*glc1-2*	49.4	49.3	0.99
20Q1-3D	*GLC*	11.3	27.3	2.40

[a] Cells were harvested 30 min before total glucose uptake. The values correspond to averages taken from two separate experiments run in duplicate. The activation mixture contained 2.2 mM ATP · Mg, 50 mM NaF, 5 mM theophylline, and 50 μM cAMP.

TABLE 3. Dependence of trehalase activation on ATP and cAMP[a]

Omission(s) from reaction mixture	Trehalase activity (nmol of glucose min^{-1} mg of $protein^{-1}$)	
	S288C (*GLC*)	GS1-36 (*glc1-1*)
None	53.80 ± 2.94	78.02 ± 1.76
ATP, cAMP	10.16 ± 0.38	73.85 ± 4.12
ATP	9.38 ± 0.52	72.93 ± 4.50
cAMP	9.73 ± 0.42	72.86 ± 3.44

[a] The activation mixture contained 2.2 mM ATP · Mg, 50 mM NaF, 5 mM theophylline, and 50 μM cAMP. For activation, 8 mg of protein was used. When the enzyme was in the active form 0.05 mg of protein was used for trehalase determination, whereas 0.2 mg was required when the enzyme was in its less active form. The data presented are the means ± standard deviations from six different experiments run in duplicate.

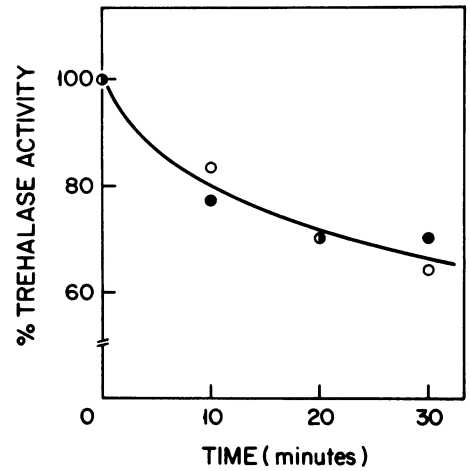

FIG. 2. Time course of Mg^{2+}-dependent trehalase inactivation in cell-free extracts. The 10,000 × *g* supernatant fluid was passed through G-25 Sephadex and treated with 13 mM EDTA. The enzyme preparation was incubated at 30°C in the presence of 4 mM Mg^{2+} and 50 mM NaF. Symbols: ○, strain S288C (*GLC*); ●, *glc1-1* mutant GS1-36.

Table 4 shows that trehalase in both mutant (GS1-36) and normal (S288C) extracts can undergo a reversible, Mg^{2+}-dependent, partial inactivation which is completely inhibited by 50 mM fluoride. Reactivation of trehalase by ATP · Mg after its conversion to the cryptic form by Mg^{2+} is completely dependent on added cAMP in the extracts of normal cells, whereas a substantial reactivation of trehalase occurs in mutant extracts even in the absence of cAMP. These observations strongly suggest that the high trehalase activity of mutant extracts may result from an abnormally elevated cAMP-independent protein kinase activity which converts cryptic trehalase into the active, phosphorylated form. The high cAMP-independent phosphorylation seen in mutant extracts is indicative of an alteration in the regulatory capacity of the regulatory (R) subunit.

Figure 2 shows that the rate of trehalase inactivation by a putative protein phosphatase is similar in mutant and normal extracts. Therefore, it is unlikely that the abnormally high trehalase activity in strain GS1-36 results from a defect in an inactivating enzyme (protein phosphatase).

Direct measurements of protein phosphorylation in the two types of extracts were made with [γ-^{32}P]ATP and protamine as substrates (Table 5). Although both types of extract catalyzed substantial protamine phosphorylation, only the reaction catalyzed by an extract of normal cells was increased by adding cAMP. This result is indicative of the existence, in the mutant preparation, of an unusually active protein kinase with very limited sensitivity to cAMP.

Figure 3 depicts the relationship between trehalase activity and the concentration of the protein kinase effector, cAMP. Activation by the parental cell extract is clearly sigmoidal. This behavior is presumed to indicate that cAMP acts as a positive effector in a forward, regulatory cascade (3).

Figure 4 shows that data calculated from the lower curve in Fig. 3 can be used to construct a linear sensitivity index plot (analogous to a Hill

TABLE 4. Reversible conversion of trehalase[a]

Strain	Trehalase activity (nmol of glucose min^{-1} mg of $protein^{-1}$)				
	Control	Mg^{2+}, NaF	Mg^{2+}	ATP·Mg	ATP·Mg, cAMP
S288C (*GLC*)	9.38 ± 0.52	9.73 ± 0.42	5.96 ± 0.23	5.71 ± 0.79	57.92 ± 3.66
GS1-36 (*glc1-1*)	60.94 ± 5.25	64.39 ± 0.63	35.64 ± 7.63	73.86 ± 3.56	93.12 ± 3.78

[a] The 10,000 × *g* supernatant fluid was passed through a G-25 Sephadex column and treated with 13 mM EDTA. Thereafter the enzyme preparation was incubated at 30°C for 20 min in the presence of 5 mM Mg^{2+} and 50 mM NaF to show the inhibitory effect of NaF on putative phosphatase activity. Incubation in the presence of 5.0 mM Mg^{2+} at 30°C for 10 min reduced trehalase activity. For activation, the preparation treated with Mg^{2+} was used. The results are averages ± standard deviations from four separate experiments run in duplicate.

TABLE 5. Protamine phosphorylation by cell-free extracts[a]

Strain	Protein kinase activity (pmol of $^{32}P_i$ min^{-1} mg of $protein^{-1}$)		
	Control	cAMP (10 μM)	Activation ratio
S288C (*GLC*)	54.97 ± 10.08	118.31 ± 13.96	2.2
GS1-36 (*glc1-1*)	150.54 ± 55.66	143.50 ± 47.92	1.0

[a] The assay mixture (100 μl) contained 50 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 15 μg of protamine, [γ^{-32}P]ATP (50 to 200 cpm/pmol), and 10 to 20 μl of the 10,000 × *g* supernatant fluid (10 to 15 μg of protein). Incubation was at 30°C for 10 min. The results are the means ± standard deviations of three experiments.

plot), as described by Chock et al. (3). The symbol I refers to the total amount (activity) of interconvertible enzyme (trehalase) and m-I refers to the modified (activated, phosphorylated) trehalase. The sensitivity index is analogous to the Hill number for an allosteric enzyme. The sensitivity index determined from this plot was 4.4, which according to calculations made by Stadtman and Chock (22) corresponds to a Hill number of 2.3, indicating that trehalase is converted by a monocyclic cascade.

The data plotted in Fig. 5 show the dependence of trehalase activation on ATP · Mg concentration. In the presence of cAMP, activation of trehalase in extracts of normal cells is almost completely dependent upon added ATP · Mg, whereas the highly active trehalase of mutant cell extracts undergoes only a small additional activation by ATP · Mg. The concentration of ATP · Mg required to attain maximum activation is very close to the physiological range of 1.5 ± 0.1 mM in yeast cells grown on glucose, as determined by Gancedo and Gancedo (7). Since Mg^{2+} is probably present at nonlimiting concentrations in vivo, it could be expected that trehalase activities in mutant cells should be at or near the maximum value, as we observed.

The direct effect of cAMP in extracts of normal cells is confined to its action on the converter enzyme (the protein kinase). That it has no direct effect on the catalytic activity of trehalase is illustrated by the double-reciprocal plots depicted in Fig. 6. The apparent K_m for trehalose was determined for trehalase in normal extracts before and after activation (upper and lower curves, respectively). Although V_{max} was increased threefold by addition of 10 μM cAMP (in the presence of ATP · Mg), the value of K_m was unchanged. Moreover, the K_m of the enzyme in mutant extracts was also identical, indicating that the *glc1* mutation has no detectable effect on trehalase itself, but only on its activation. The estimated mean (± standard error) of the K_m calculated with a Sharp PC-1211 pocket computer was $5.62 \times 10^{-3} \pm 0.73 \times 10^{-3}$ M.

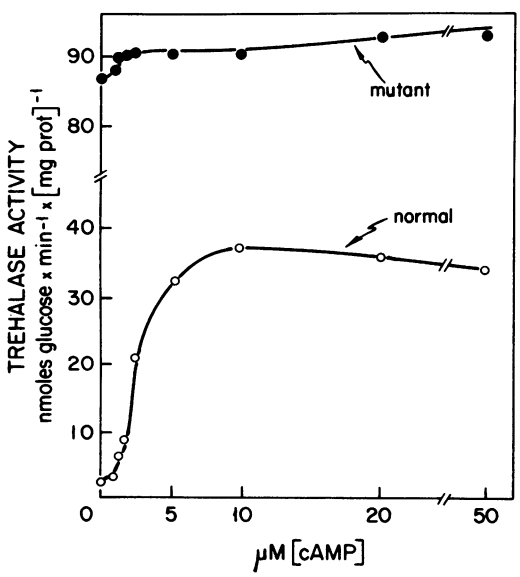

FIG. 3. Effect of cAMP on trehalase activity. The activation mixture contained 2.2 mM ATP · Mg, 50 mM NaF, 5 mM teophylline, and 0.4 ml of the 10,000 × *g* supernatant fluid in a final volume of 0.5 ml. Incubation was at 30°C for 10 min.

DISCUSSION

The scheme in Fig. 7 presents a mechanism consistent with the observations presented in

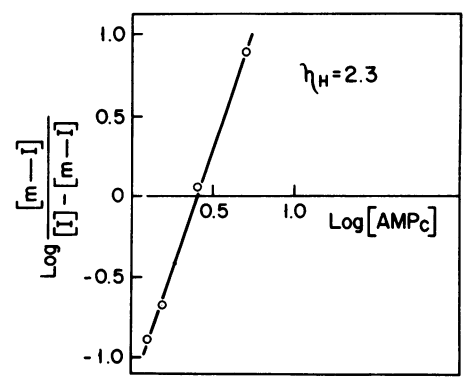

FIG. 4. Sensitivity index plot for activation of trehalase by a cAMP-dependent protein kinase. [I] Concentration of interconvertible enzyme; [m-I] concentration of modified form of the interconvertible enzyme. Values correspond to those for the normal strain (S288C) taken from Fig. 2.

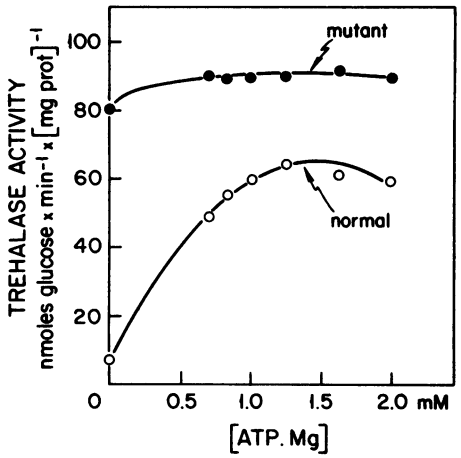

FIG. 5. Effect of ATP · Mg on trehalase activity. The activation mixture contained 50 mM NaF, 5 mM theophylline, 50 μM cAMP, and 0.4 ml of the 10,000 × *g* supernatant fluid in a final volume of 0.5 ml. Incubation was at 30°C for 10 min. Values for [ATP · Mg] were based on the association constant for $MgSO_4$, according to Emara et al. (6).

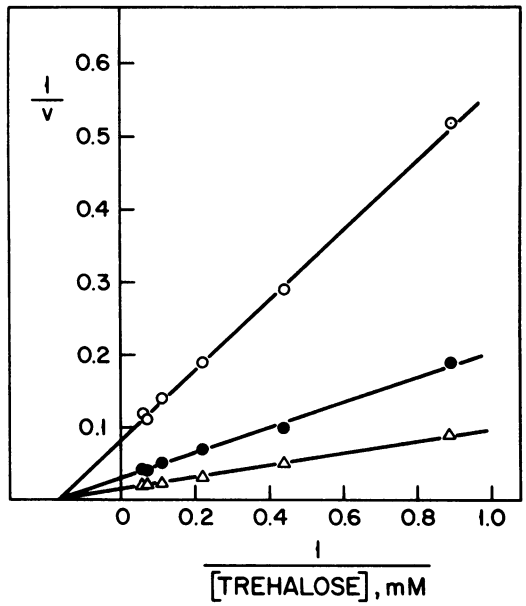

FIG. 6. Double-reciprocal plots for cryptic trehalase (○) and for the activated enzyme by 10 μM cAMP (●) from cell-free extracts of strain S288C (*GLC*) as compared with the untreated preparation (△) from the mutant GS1-36 (*glc1-1*).

this and other studies. Investigations of yeast protein kinase by Takai et al. (25) and of the R subunit purified from *S. cerevisiae* (10, 23) indicate that this organism contains a cAMP-dependent enzyme with properties similar to protein kinase (type II) found in various animal tissues. The finding that the modulation system which regulates the reversible interconversion of cryptic and active trehalase has the properties of a monocyclic cascade (sensitivity index, 4.4) indicates that the cAMP-dependent converter enzyme, in extracts of the normal strain (S288C), acts directly on trehalase-*c*, a natural substrate. It is proposed that the *glc1* mutation alters a subunit of this enzyme in such a way that the dissociation constant of the putative R_2C_2 complex is greatly increased. This could result from a structural change in the R subunit. Alternatively, the mutation could lead to deficiency in the amount of R produced relative to C. Irrespective of the molecular change caused by *glc1*, there is, in fact, some residual effect of cAMP on the protein kinase activity of mutant extracts. In either case, the *glc1* system provides a novel vehicle for investigation of protein kinase function both in vivo and in vitro.

The second regulatory component of the monocyclic system which modifies the interconvertible enzyme, trehalase, is most probably a protein phosphatase which is activated by Mg^{2+} and inhibited by fluoride ions. At present, we have no information on the specificity of this enzyme or on possible allosteric effectors which might regulate its action in vivo. Since extracts of *glc1* cells exhibit protein phosphatase activities (reduction of trehalase activity) similar to those found in normal extracts, it is unlikely that the trehalose accumulation deficiency can be attributed to an alteration in the reverse cascade, that is, to a noneffective inactivation of trehalase.

The pleiotropic nature of the *glc1* phenotype served as an important clue in this investigation. In yeasts, trehalose accumulation and glycogen accumulation vary with the physiological state of the cells. When normal yeast cells are grown

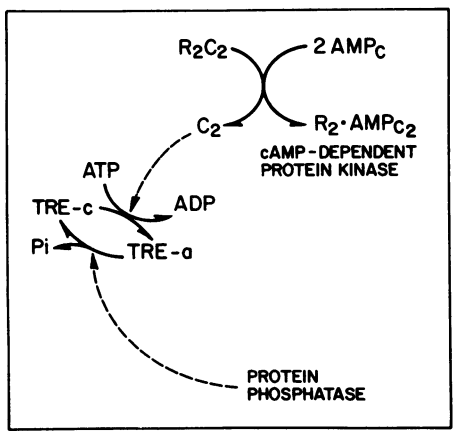

FIG. 7. Scheme for the interconversion of trehalase (TRE) in *S. cerevisiae*.

in glucose medium, trehalose accumulation capacity increases (17) and substantial glycogen synthesis occurs (19) near the end of the logarithmic growth phase. Moreover, in normal strains both carbohydrates accumulate in nonproliferating cells incubated in glucose buffered with phosphate (17, 19). It has been demonstrated (20) that the *glc1-1* mutation resulted in a deficiency in converting the low-activity D-form of glycogen synthase to the high-activity I-form of the enzyme. The authors also demonstrated an ATP-dependent I-to-D transformation and a Mg^{2+}-stimulated D-to-I transforming activity in cell-free extracts. However, because they found these transforming activities in both mutant and normal extracts, and since they were unable to demonstrate a cAMP effect, they failed to reach a conclusion concerning the enzymatic defect associated with *glc1*.

The protein kinase responsible for the I-to-D transformation of yeast glycogen synthase may be a form of the cAMP-dependent enzyme described by Takai and colleagues (24, 25). These investigators showed that their enzyme preparation could inactivate muscle glycogen synthase. Hixson and Krebs (10) purified the R subunit of a cAMP-dependent yeast protein kinase, using the proteinase inhibitor phenylmethylsulfonyl fluoride to minimize proteolytic effects. This protein is much larger than the analogous R subunit reported by Takai et al. (25), who did not use proteolytic inhibitors.

It seems quite likely that the "activating factor protein" (27) which activates trehalase is the same cAMP-dependent protein kinase which transforms active glycogen synthase to its less active D-form. The simultaneous losses of both glycogen and trehalose accumulation resulting from independent *glc1* mutations and the inheritance of these deficiencies, together with the lack of trehalase "crypticity," argue strongly that a single polypeptide, controlled by the nuclear gene *GLC1*, is involved in regulating both glycogen synthase and trehalase activity in vivo.

It is pertinent to note that the trehalase activating factor protein tended to lose dependence on cAMP during purification (27). The authors did not use proteinase inhibitors. The failure to find cAMP effects on glycogen synthase I-to-D transformation (20) might have been caused by action of endogenous proteolytic enzymes on the R subunit of the native kinase.

Since the presence of a *glc1* allele in any strain results in glycogen deficiency as well as in trehalose deficiency (1, 4), it is apparent that the unregulated protein kinase described here is also acting on glycogen synthase, maintaining it in the low-activity D-form. It seems likely that, in yeasts, a single cAMP-dependent protein kinase acts as a converter enzyme for both the trehalase and the glycogen synthase cascades. However, since glycogen synthase from animal tissues contains multiple phosphorylation sites which can act as phosphate acceptors from different protein kinases in vitro (22), we cannot be completely certain that glycogen synthase is a "normal" physiological substrate of the trehalase phosphorylation kinase described in this study.

Notwithstanding the possibility of "misdirected" protein phosphorylation artifacts, the demonstration that the *glc1* mutation is intimately associated with a protein kinase activity is a significant advance in the investigation of the physiological role of this important class of regulatory enzymes. An altered regulation of a cAMP-dependent protein kinase in a hepatoma cell line deficient in the activity of the cAMP binding protein has been described (8). The results of ^{32}P incorporation into histone, in the presence and absence of the cyclic nucleotide, when hepatocytes and hepatoma cells are compared are similar to those we report for the parental strain and the *glc1* mutant of *S. cerevisiae*. We have shown elsewhere (4) that mutations at the *GLC1* locus also result in a severe deficiency in synthesis of yeast catalase T. In this case, the protein kinase is implicated in regulating the actual synthesis of the enzyme rather than its activity. Conceivably, the translation of yeast catalase T is subject to heme-dependent protein kinase action such as that which modulates eIF-2 in hemoglobin synthesis (5). Moreover, it was demonstrated that cyclic nucleotide binding to the regulatory subunit of bakers' yeast protein kinase is prevented by heme (10).

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