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The GLC7 Type 1 Protein Phosphatase Is Required for Glucose Repression in *Saccharomyces cerevisiae*

JIANGLING TU AND MARIAN CARLSON*

Institute of Cancer Research and Departments of Microbiology and Genetics and Development, Columbia University, New York, New York 10032

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We cloned the *GLC7/DIS2S1* gene by complementation of the *cid1-226* mutation, which relieves glucose repression in *Saccharomyces cerevisiae*. *GLC7* encodes the catalytic subunit of type 1 protein phosphatase (PP1). Genetic analysis and sequencing showed that *cid1-226* is an allele of *GLC7*, now designated *glc7-T152K*, which alters threonine 152 to lysine. We also show that the *glc7-1* and *glc7-T152K* alleles cause distinct phenotypes: *glc7-1* causes a severe defect in glycogen accumulation but does not relieve glucose repression, whereas *glc7-T152K* does not prevent glycogen accumulation. These findings are discussed in light of evidence that interaction with different regulatory or targeting subunits directs the participation of PP1 in diverse cellular regulatory mechanisms. Finally, genetic studies suggest that PP1 functions antagonistically to the SNF1 protein kinase in the regulatory response to glucose.

Protein phosphorylation is a major regulatory mechanism in eukaryotic cells. Protein kinases have been shown to play key roles in regulating diverse processes such as the cell cycle, metabolism, transcription, and translation (20). Most phosphorylation events are reversible, and the phosphorylation state of any protein depends on the relative activities of protein kinases and protein phosphatases. It is increasingly clear that protein phosphatases also play important roles in cellular regulation (8, 9, 19).

In the yeast *Saccharomyces cerevisiae*, one of the regulatory mechanisms that involves a protein kinase is glucose repression. Expression of many genes is repressed when glucose is available, and the SNF1 protein-serine/threonine kinase is required for release from glucose repression when glucose is limiting (4). SNF1 is physically associated with the SNF4 protein, which stimulates the kinase activity (6), and several other proteins that interact with SNF1 have been identified, including some that are phosphorylated by SNF1 (24, 46, 47). Genetic evidence suggests that for some glucose-repressed genes, SNF1 functions to relieve transcriptional repression mediated by MIG1, SSN6, TUP1, and other SSN proteins (23, 27, 44). However, the mechanism by which the SNF1 kinase relieves repression has not been elucidated in detail for any glucose-regulated gene.

The critical role of the SNF1 protein kinase in this regulatory pathway invites speculation that an opposing protein phosphatase exists. Various mutations have been identified that relieve glucose repression, consistent with a deficiency in such a protein phosphatase. Some of the cognate genes have been characterized previously, but none encoded a protein phosphatase (for a review, see reference 22). Here we report that the gene corresponding to the *cid1-226* mutation encodes type 1 protein phosphatase (PP1).

The recessive mutation *cid1-226* was identified in a search for mutants resistant to 2-deoxyglucose (2-DG), a glucose analog that causes glucose repression but cannot be metabolized (28). The *cid1* mutation relieves glucose repression of *SUC2* (invertase) and *MAL3* (maltase) gene expression but does not affect glucose repression of the *GAL10* promoter. For *SUC2*, the effect of *cid1* is mediated by upstream regulatory sequences. In addition, homozygous diploids fail to sporulate.

We report here the cloning of the *CID1* gene by complementation. Sequence analysis showed that the cloned gene is the same as *GLC7/DIS2S1*, encoding PP1 (3, 13, 29). Genetic analysis confirmed that *cid1-226* is an allele of *GLC7*, and the sequence of the mutation was determined. We showed that this allele causes a distinct phenotype from the point mutation *glc7-1* (3), consistent with the participation of PP1 in diverse regulatory processes. Finally, we present genetic evidence suggesting that the GLC7 protein phosphatase acts antagonistically to the SNF1 protein kinase in glucose repression.

MATERIALS AND METHODS

Strains and genetic methods. The *S. cerevisiae* strains used are listed in Table 1. The *Escherichia coli* strains used were HB101 and XL1-Blue. Standard methods for yeast genetic analysis (34) and transformation (21) were used. 2-DG resistance was tested on YEP containing 2% sucrose (YPS) and 200 μg of 2-DG (Sigma) per ml under anaerobic conditions with GasPaks (BBL). Control plates lacked 2-DG. Synthetic media were SD (34) or synthetic complete medium (SC). Scoring was performed by spotting cell suspensions onto solid medium. To assess sporulation proficiency, diploid cells were grown on YPD plates overnight, patched on sporulation medium (34), incubated at room temperature for 8 days, and then examined microscopically for the presence of asci.

Cloning of the *CID1/GLC7* gene. Plasmid pYSH (a gift from J. Schultz) contains a centromere, the *TRP1* selectable marker, and the *HIS3* gene under control of the *SUC2* upstream region (Fig. 1). To construct pYSH, the *Eco*RI-*Pst*I fragment (−65 to +680) containing *HIS3* (660 bp) (17) was cloned into the cognate sites of pRS314 (38), and two copies of the *Eco*RI fragment containing the *SUC2* upstream region (nucleotides −900 to −384) (37) were cloned into the *Eco*RI site in the correct orientation (Fig. 1). Yeast YCp50 genomic library plasmids (33) were used to transform MCY2616 (*cid1*) carrying pYSH to uracil prototrophy. Each transformant was patched on supplemented SD lacking tryptophan and uracil

* Corresponding author. Mailing address: 701 W. 168th St., HHSC 922, New York, NY 10032. Phone: (212) 305-6314. Fax: (212) 305-1741.

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype[a]
MCY835	*MATα glc7-T152K lys2-801 ura3-52 SUC2*
MCY1093	*MATa his4-539 lys2-801 ura3-52 SUC2*
MCY1094	*MATα ade2-101 ura3-52 SUC2*
MCY1751	*MATa/MATα ade2-101/+ his4-539/+ lys2-801/+ ura3-52/ura3-52 SUC2/SUC2*
MCY1950	*MATa snf4Δ1 mig1Δ2::LEU2 his(4?) ura3-52 SUC2*
MCY2616	*MATa glc7-T152K his3Δ200 lys2-801 ura3-52 trp1Δ1 SUC2*
MCY2653	*MATa glc7-T152K snf1Δ10 ade2-101 his3Δ200 ura3-52 SUC2*
MCY2656	*MATα glc7-T152K snf4Δ2 lys2-801 ura3-52 SUC2*
MCY2964	*MATα snf1Δ10 mig1Δ2::URA3 ade2-101 lys2-801 ura3-52 trp1Δ1 SUC2*
MCY2966	*MATα glc7-T152K mig1Δ2::URA3 ade2-101 lys2-801 ura3-52 trp1Δ1 SUC2*
MCY2972	*MATα glc7-T152K snf1Δ10 mig1Δ2::URA3 ade2-101 lys2-801 ura3-52 trp1Δ1 SUC2*
MCY3231	*MATa/MATα +/glc7Δ1::URA3 his3Δ200/his3Δ200 leu2-3,112/leu2-3,112 ura3-52/ura3-52 SUC2/SUC2*
JC782.24D[b]	*MATa glc7-1 lys2 leu2 met4 ura3-52*
JC782.26C[b]	*MATα glc7-1 lys2 leu2 met4 ura3-52*
YM3920[c]	*MATα mig1Δ2::URA3 gal80-538 ade2-101 his3Δ200 lys2-801 ura3-52*

[a] All MCY strains have the S288C genetic background; the new designation for the *cid1-226* allele, *glc7-152K*, is used here.
[b] Obtained from J. Cannon; congenic to S288C.
[c] Obtained from M. Johnston; S288C genetic background.

(SD-Trp-Ura), grown overnight, and then replica plated to both SD-Ura-Trp-His and SD-Ura-Trp plates. His$^-$ transformants were tested for 2-DG resistance and assayed for invertase activity under glucose-repressing conditions. Four transformants showed wild-type repressed levels of invertase and 2-DG sensitivity. Plasmid DNA was recovered from these four transformants by passage through bacteria as described previously (43).

Construction of plasmids. pJT16 and pJT17 were derived from pJT15 by deleting the 3.8-kb *Sal*I and 1.9-kb *Sna*BI-*Sph*I fragments, each extending to a vector site, respectively. pJT18 was constructed by subcloning the *Hin*dIII-*Sna*BI fragment into the *Hin*dIII and *Sma*I sites of the centromere-containing vector pRS316 (38), and pJT19 was constructed by subcloning the *Sal*I-*Sac*I (polylinker site) fragment from pJT18 into the cognate sites of pRS316. pUT20 and pJT20 contain the 2.7-kb *Hin*dIII-*Xho*I fragment from pJT18 cloned into the cognate sites of pUC19 and pRS306, respectively (Fig. 2).

To construct *GLC7* fusions, we designed two primers to amplify the entire *GLC7* coding region, from codon 1 to 312, and to introduce *Eco*RI and *Bam*HI sites 5′ and 3′ to the gene, respectively. The 5′ primer was 5′-GGGGAATTCGAAATG GACTCACAAC-3′, and the 3′ primer was 5′-CGGGATC

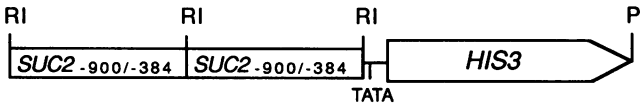

FIG. 1. The *SUC2-HIS3* gene fusion in plasmid pYSH. Two copies of an *Eco*RI fragment containing *SUC2* sequence −900 to −384 are inserted 5′ to the *HIS3* gene at nucleotide −53. A *HIS3* TATA box, located at position −43, controls transcriptional initiation at +12. Restriction sites: *Eco*RI (RI) and *Pst*I (P).

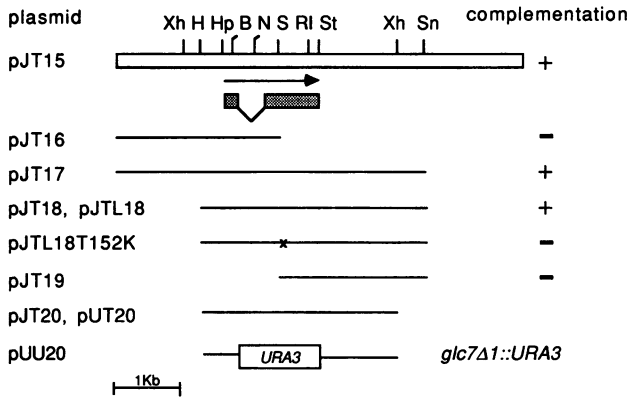

FIG. 2. Restriction maps of *GLC7* plasmids. Yeast genomic DNA cloned in plasmid pJT15 is shown by the bar. The coding region of *GLC7* is shaded, and the direction of transcription is indicated by the arrow. The ability of plasmids to complement *cid1-226* for sensitivity to 2-DG is shown. The position of the mutation in pJTL18T152K is indicated. Restriction sites: *Bgl*II (B), *Eco*RI (RI), *Hin*dIII (H), *Hpa*I (Hp), *Nar*I (N), *Sal*I (S), *Sna*BI(Sn), *Sty*I (St), and *Xho*I (Xh). Only sites referred to in the text are shown.

CTTTTTTCTTTCTACCCCC-3′. Using PCR, we amplified the *GLC7* sequence from pJT18. The *Eco*RI-*Nar*I fragment from the amplified DNA and the *Nar*I-*Bam*HI fragment from pJT18 were subcloned into the *Eco*RI and *Bam*HI sites of pLexA(1-202)+PL (36) and pMA424 (25) to yield pLexA$_{202}$-GLC7 and pG$_{BD}$-GLC7, respectively. pGLC7-lacZ contains the *Hin*dIII-*Sal*I fragment from pJT18 and the *Sal*I-*Bam*HI fragment from the amplified DNA subcloned into the *Hin*dIII and *Bam*HI sites of YEp356R (26). Expression of β-galactosidase activity from this fusion was not regulated by glucose. All of these fusion plasmids were shown to provide *GLC7* function by complementation of a *glc7-T152K* mutant.

Sequence analysis. Restriction fragments were cloned into M13mp18 or M13mp19 (48). The nucleotide sequence was determined using the Sequenase (United States Biochemical) enzyme and a 17-mer sequence primer (Amersham Corp.).

Disruption of chromosomal *GLC7* locus. We first constructed pUU20 by replacing the *Bgl*II-*Sty*I fragment in pUT20 with a 1.3-kb *Bam*HI-*Sma*I *URA3* fragment. The *Hpa*I-*Bam*HI (a polylinker site in the vector) fragment purified from pUU20 was used to transform (35) the wild-type diploid MCY1751. The resulting allele was designated *glc7Δ1::URA3* (Fig. 2).

Cloning and sequencing of the *cid1-226* (*glc7-T152K*) mutation. The gap repair method (30) was used to clone the *cid1-226* mutation. The gapped fragment pJT18-gap (see Fig. 4) was produced by removing the *Bgl*II-*Eco*RI fragment from pJT18 and was used to transform MCY2616 (*cid1-226*). Ura$^+$ transformants were recovered and tested for 2-DG resistance. Two gap-repaired plasmids, designated pJT18-gr12 and pJT18-gr13, were recovered from two 2-DG-resistant transformants. For each gap-repaired plasmid, the *Hin*dIII-*Bgl*II, *Bgl*II-*Eco*RI, and *Eco*RI-*Bam*HI (polylinker site in pJT18) fragments were swapped into pJT18, and the resulting plasmids pJT18S5, pJT18S3, and pJT18SM were tested for complementation of the *cid1-226* defect. Identical results were obtained for swaps with pJT18-gr12 and pJT18-gr13. The *Bgl*II-*Eco*RI fragments from both pJT18-gr plasmids were subcloned into the *Bam*HI and *Eco*RI sites of M13mp18 and M13mp19 for sequencing with universal and synthetic primers (see Fig. 4). Sequence was determined for DNA derived from pJT18-gr12. In addition, the region containing the mutation was sequenced

for pJT18-gr13. The *Bgl*II-*Sal*I fragment from pJT18-gr12 was also subcloned into M13mp18 for sequencing from the *Sal*I site.

pJTL18T152K (see Fig. 4) was constructed by cloning the *Hin*dIII-*Sal*I fragment from pJT18 and the *Sal*I-*Bam*HI fragment from the swapped plasmid pJT18SM between the *Hin*dIII and *Bam*HI sites of pRS315 (38).

Invertase assays. Glucose-repressed cultures were grown to mid-log phase in YEP or selective SC containing 2% glucose; derepressed cultures were prepared by shifting cells to YEP or SC containing 0.05% glucose for 2.5 or 3 h, respectively. Invertase activity was assayed as previously described (43).

Glycogen assay. Levels of glycogen were measured as described previously (15) from a protocol provided by K. Tatchell.

Immunoprecipitation and immune complex kinase assays. Antibodies and procedures were as described previously (46).

RESULTS

Cloning of the *CID1* gene. The centromere-containing plasmid pYSH carries a *SUC2-HIS3* gene fusion in which the *SUC2* upstream region regulates *HIS3* expression in response to glucose availability (Fig. 1). Wild-type cells carrying pYSH are His^- when grown on glucose, whereas *cid1-226* mutants are His^+. To isolate the *CID1* gene, we transformed *cid1-226* mutant cells carrying pYSH with a YCp50 yeast genomic library and identified His^- transformants. Four plasmids which caused a His^- phenotype also complemented the *cid1-226* defect in glucose repression of *SUC2* (invertase) expression. These plasmids carried identical inserts of yeast genomic DNA, and the restriction map of pJT15 is shown in Fig. 2.

To delimit the complementing gene, we constructed subclones of pJT15 (Fig. 2) in centromere-containing vectors and tested for complementation of the His^+ phenotype of a *cid1* mutant carrying pYSH. The results indicated that the *CID1* gene lies within the 3.8-kb region between the *Hin*dIII and *Sna*BI sites, spanning the *Sal*I site.

We next tested the linkage of the cloned DNA to the chromosomal *cid1* locus. Subclone pJT20, carrying the *URA3* marker, was digested with *Sal*I and used to transform wild-type strain MCY1094. Southern blot analysis confirmed that pJT20 had integrated into its cognate chromosomal locus. Two Ura^+ transformants were then crossed to strain MCY2616 (*cid1-226 ura3*). Tetrad analysis of the diploids showed 2+:2− segregation for 2-DG resistance in 21 tetrads, and the *URA3* marker segregated in repulsion. Thus, the cloned gene is tightly linked to the *cid1* locus.

Sequence of *CID1* shows identity to *GLC7/DIS2S1* encoding PP1. Nucleotide sequence analysis from the *Sal*I site in both directions revealed an open reading frame. The inferred protein sequence was compared with the sequences in Protein Identification Resource (release V19.0) of the National Biochemical Research Foundation by using the FASTP program (31). The sequence was the same as that of *GLC7/DIS2S1*, an essential gene encoding the catalytic subunit of PP1 (3, 13, 29). *DIS2S1* was cloned by screening an *S. cerevisiae* genomic library for sequence homology to the PP1 genes *dis2*$^+$ and *sds21*$^+$ from the fission yeast *Schizosaccharomyces pombe* (29). *GLC7* was cloned by complementation of the defect in glycogen accumulation caused by the *glc7-1* mutation (3, 13). The GLC7 protein is very similar to mammalian PP1 (>80% identity).

***glc7-1* and *cid1-226* cause distinct phenotypes.** The *glc7-1* mutation is known to cause pleiotropic phenotypes. First, the *glc7-1* mutant does not accumulate glycogen, apparently because of a defect in dephosphorylation (activation) of glycogen synthase (3, 13, 32). Second, *glc7-1* suppresses the defect in general amino acid control caused by a point mutation in the GCN2 protein kinase, which phosphorylates the α subunit of translation initiation factor 2) (45). Finally, *glc7-1* causes a sporulation defect in homozygous diploids (3).

TABLE 2. Effects of *glc7-1* and *cid1-226* on invertase activity and sporulation

Relevant genotype	Invertase activity[a]		Sporulation
	Repressed	Derepressed	
Wild type	<1	253	+
glc7-1/glc7-1	1	211	−
cid1-226/cid1-226	32	234	−
glc7-1/cid1-226	1	148	−
+/glc7Δ1::URA3	1	246	+
cid1-226/glc7Δ1::URA3	109	238	−

[a] Expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values are averages of three assays, except for the *glc7-1* homozygote (two assays). Standard errors were <10%.

To compare the phenotypic effects of *cid1-226* and *glc7-1*, we first assayed the invertase activity of a *glc7-1* mutant. The *glc7-1* mutant showed wild-type glucose repression and derepression of invertase activity, as did the homozygous mutant diploid (Table 2). Moreover, the *glc7-1* allele complemented *cid1-226* with respect to invertase regulation, and the heteroallelic diploid showed wild-type invertase activity.

We next assayed accumulation of glycogen during growth of *cid1-226* mutant cultures. Wild-type cells accumulate glycogen as they approach stationary phase, whereas *glc7-1* mutants do not (16). The *cid1-226* mutant cells accumulated glycogen, although the levels were somewhat lower than wild-type levels in this and other experiments (Fig. 3). The *cid1-226* cultures began accumulation earlier than wild-type cultures, which may reflect their partial glucose repression defect during log-phase growth. Thus, *cid1-226* and *glc7-1* cause distinct phenotypes with respect to both glucose repression of invertase and glycogen accumulation.

Both *glc7-1* and *cid1-226* cause a sporulation defect in homozygous diploids. The *glc7-1/cid1-226* diploid also failed to sporulate, indicating that the two mutations fail to complement for this shared phenotype.

***cid1-226* is a mutant allele of *GLC7*.** Despite the distinct phenotypes of *cid1-226* and *glc7-1*, considerable evidence suggests allelism: the cloned *GLC7* gene complements *cid1-226* and is tightly linked to the *cid1* locus, and *glc7-1* and *cid1-226* fail to complement for sporulation. To confirm that *cid1-226* is an allele of *GLC7*, we constructed a diploid heterozygous for *cid1-226* and a *glc7* deletion. We reasoned that if *cid1-226* is an allele of *GLC7*, then disruption of the wild-type *GLC7* gene should uncover the *cid1-226* mutant phenotype.

First, we constructed and characterized a mutation, designated *glc7Δ1::URA3*, that deletes codons 42 to 304 and the intron (Fig. 2). The mutation was introduced into the *GLC7* locus on one chromosome of a wild-type diploid (see Materials and Methods). Tetrad analysis of two heterozygous diploid transformants showed 2+:2− segregation for viability in 13 tetrads, and all viable spore clones were Ura^-. In addition, a total of 50 tetrads were dissected from another five Ura^+ transformants, and microscopic examination showed that 44% of the inviable spores generated small buds after incubation at room temperature. These results are consistent with previous studies (3, 7, 13) and show that disruption of the *GLC7* gene causes a defect in mitotic growth.

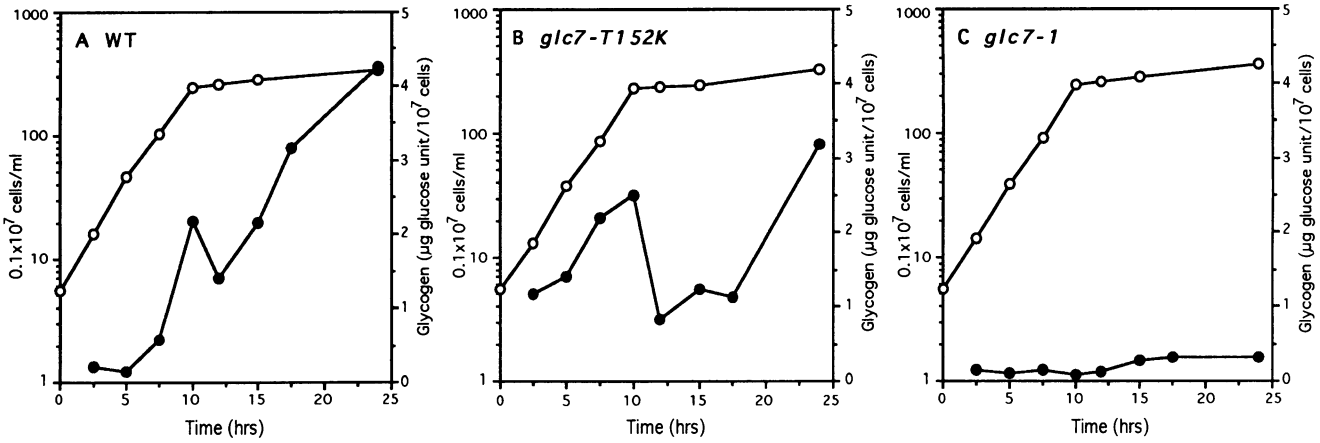

FIG. 3. Glycogen accumulation in wild-type and mutant strains. Growth curves (open circles) and glycogen accumulation (filled circles) are shown for representative cultures of strains MCY1093 (wild type [WT]); A), MCY2616 (*glc7-T152K*); B), and JC782.26C (*glc7-1*); C).

Using the same construct, we then disrupted the *GLC7* locus in a diploid heterozygous for *cid1-226*. Two of 48 Ura^+ transformants were 2-DG resistant. These two *cid1-226/glc7Δ1::URA3* disruptants also showed higher invertase activity under glucose-repressing conditions than a *cid1-226/cid1-226* diploid (Table 2), suggesting that the *cid1-226* mutation does not cause complete loss of function with respect to glucose repression. These two transformants were noticeably unhealthy, which may account for the disproportionate number of +/*glc7*Δ transformants. These findings confirm that *cid1-226* is an allele of *GLC7*.

Mutation alters Thr-152 of PP1. To identify the *cid1-226* mutation, we recovered the sequence from the mutant genomic locus by gap repair (30) of pJT18 cleaved with *Bgl*II plus *Eco*RI (see Materials and Methods). Two gap-repaired plasmids that failed to complement *cid1-226* were analyzed by swapping fragments back into the parent pJT18 and then testing again for complementation. In both cases, the mutation lay in the *Bgl*II-*Eco*RI fragment (Fig. 4). Sequence analysis of both revealed a single base pair change from C to A, resulting in a change from threonine to lysine at codon 152. The *cid1-226* allele will henceforth be called *glc7-T152K*.

PCR was also used to amplify the sequence from *cid1-226* mutant DNA (MCY835 and MCY2616), and similar swap and sequencing experiments were carried out with the PCR fragment. The same C-to-A mutation was found, but the PCR also appeared to introduce other mutations.

To confirm that the *glc7-T152K* mutation is sufficient to

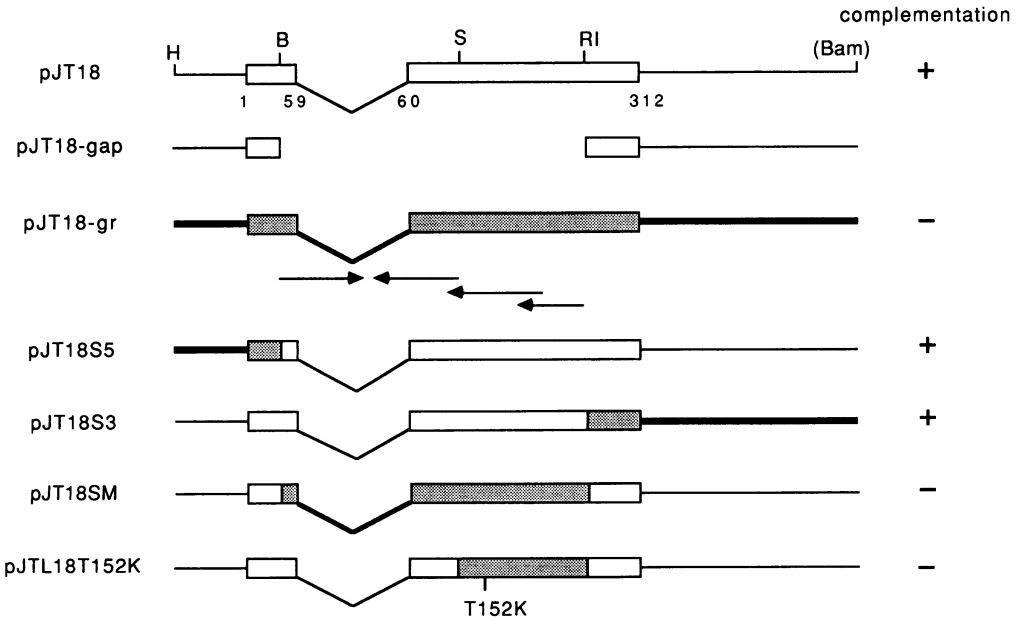

FIG. 4. Cloning and sequencing of the *cid1-226* mutation. The coding region of *GLC7* is represented by bars. The intron is located between codons 59 and 60, as indicated. DNA derived from a gap-repaired plasmid (pJT18-gr) is depicted by a shaded bar and thick line. The ability of plasmids to complement *cid1-226* for sensitivity to 2-DG is shown. Sequenced regions are indicated by arrows. Restriction sites: *Bam*HI (Bam), *Bgl*II (B), *Eco*RI (RI), *Hin*dIII (H), and *Sal*I (S). The *Bam*HI site is from the vector polylinker.

TABLE 3. Effect of increased *GLC7* dosage on *SUC2* expression

Relevant genotype	Plasmid	Invertase activity[a]	
		Repressed	Derepressed
Wild type	YEp24	<1	180
	p1855	<1	190
snf4 mig1	YEp24	1	40
	p1855	2	8

[a] Expressed as in Table 2. Cultures were grown in SC-Ura to select for the plasmids. Alleles were *snf4Δ2* and *mig1Δ2::LEU2*. Values are averages of two assays of two different strains.

relieve glucose repression, we reconstructed the *glc7-T152K* mutant gene from sequenced mutant DNA and wild-type pJT18 DNA. The resulting centromere plasmid pJTL18T152K (Fig. 4) and the vector were used to transform diploid MCY3231 (*+/glc7Δ1::URA3 ura3/ura3 leu2/leu2*). In the vector control, tetrad analysis of a transformant produced two viable Ura^- spores from each of seven tetrads. The diploid carrying pJTL18T152K yielded four tetrads with four viable spores, four tetrads with three viable spores, and three tetrads with two viable spores. All 14 Ura^+ spore clones (*glc7Δ1::URA3*) were Leu^+ and therefore carried the plasmid. All 14 also exhibited the *glc7-T152K* mutant phenotype of 2-DG resistance. Thus, the *glc7-T152K* mutation is sufficient to relieve glucose repression.

Effects of increased *GLC7* gene dosage on invertase expression. The *glc7-T152K* mutant phenotype strongly suggests a role for PP1 in the regulatory response to glucose. To obtain further evidence for such a role, we looked for reciprocal effects of increased *GLC7* gene dosage. We first introduced a multicopy *GLC7* plasmid (p1855, carrying the *Xho*I fragment [3]) into the wild type, but invertase expression was not decreased (Table 3). We then reasoned that a strain with impaired SNF1 protein kinase activity might be more sensitive to increased GLC7 phosphatase activity. A *snf4 mig1* double mutant has reduced SNF1 activity due to loss of the SNF4 activator protein, but the absence of MIG1, a repressor that binds to the *SUC2* promoter (27), allows significant derepression of invertase (44). Increased *GLC7* dosage in *snf4 mig1* double mutants caused a fivefold decrease in derepressed invertase activity (Table 3). These results are consistent with a dosage-dependent regulatory effect of *GLC7*, although we cannot exclude an indirect effect on general health that was not evident in wild type.

The *glc7-T152K* phenotype depends on SNF1 protein kinase activity. The SNF1 protein kinase is required for release from glucose repression (4). To assess the relationship of the GLC7 phosphatase to SNF1, we examined the interaction of *glc7-T152K* with *snf1* and *snf4* mutations. Double mutants were constructed and assayed for invertase activity (Table 4). No activity was detected in strains carrying *snf1*. In contrast, *glc7-T152K* partially restored derepression of invertase activity to a *snf4* mutant. (We note a discrepancy with a previous study [28] reporting no invertase activity in *snf4* double mutants.) In *snf4* mutants, SNF1 activity is impaired but not abolished (5). Thus, these results suggest that phenotypic effects of *glc7-T152K* are dependent on SNF1 kinase activity.

It remained possible that any effect of *glc7-T152K* in a *snf1* mutant escaped detection due to insensitivity of the invertase assay. To address this issue, we introduced a *mig1* mutation, which partially suppresses the *snf1* invertase defect (44). The *glc7-T152K* mutation did not significantly alter invertase expression in a *snf1 mig1* background (Table 4), consistent with

TABLE 4. Interactions of *glc7-T152K* with *snf1*, *snf4*, and *mig1* mutations

Relevant genotype	Invertase activity	
	Repressed	Derepressed
Wild type	<1	217
snf1	<1	<1
snf4	<1	<1
snf1 glc7-T152K	<1	<1
snf4 glc7-T152K	<1	19
mig1 snf1	12	54
mig1 snf1 glc7-T152K	15	72
glc7-T152K	51	279
mig1	48	397
mig1 glc7-T152K	216	381

[a] Expressed as in Table 2. Values are averages of three to six assays, except that assays showing no detectable activity were done in duplicate only. Alleles were *snf1Δ10*, *snf4Δ2*, and *mig1Δ2::URA3*. Standard errors were <21%.

the idea that *glc7-T152K* causes no phenotype in the absence of SNF1. The *mig1* mutation does not appear to obscure a *glc7-T152K* phenotype because glucose-grown *mig1 glc7-T152K* double mutants showed higher expression of invertase than either single mutant parent; thus, the *mig1* and *glc7-T152K* mutations relieve glucose repression of *SUC2* at least in part via different pathways. Together, these results suggest that the GLC7 protein phosphatase acts antagonistically to the SNF1 protein kinase.

Assays for interaction of PP1 with the SNF1 kinase. The genetic data are consistent with various different relationships of the GLC7 protein phosphatase and SNF1. One possible model is that PP1 negatively affects the SNF1 kinase activity by dephosphorylating a key site on SNF1 or on a regulator of the kinase. We used several approaches to examine the possibility that PP1 interacts with the SNF1 kinase complex; SNF1 is known to be associated with SNF4 and other proteins in a large complex (12, 46). We first tested for coimmunoprecipitation of SNF1 with a bifunctional GLC7–β-galactosidase fusion protein (see Materials and Methods) expressed in a *glc7-T152K* mutant. GLC7–β-galactosidase or SNF1 was immunoprecipitated from protein extracts by using antibody specific to β-galactosidase or SNF1, respectively, and precipitated proteins were analyzed by immunoblotting with both antibodies. No coimmunoprecipitation was detected, whereas in control experiments, SNF4–β-galactosidase (6) and SNF1 coprecipitated (data not shown). Thus, this PP1 fusion protein does not appear to be stably associated with the SNF1 kinase complex.

We next tested for protein-protein interaction between PP1 and SNF1 in the two-hybrid system (14). This assay can potentially detect weak or transient interactions in vivo between proteins that would not coimmunoprecipitate. We expressed a hybrid protein, called G_{BD}-GLC7, which contained the GAL4 DNA-binding domain (G_{BD}) fused to GLC7 and provided GLC7 function in a *glc7-T152K* mutant. G_{BD}-GLC7 was tested for interaction with G_{AD}-SNF1 and SNF4-G_{AD} (pEE5 and pNI12 [14]), each containing the GAL4 activation domain (G_{AD}). Interaction of the two hybrid proteins would reconstitute the GAL4 transcriptional activator function, thereby activating expression of β-galactosidase from a target promoter. No β-galactosidase expression was detected either in filter assays (46) for blue colony color or in quantitative assays of enzymatic activity. In addition, no interaction was detected with a LexA-GLC7 protein as the DNA-binding partner.

Finally, we assayed SNF1 activity in glucose-repressed *glc7-*

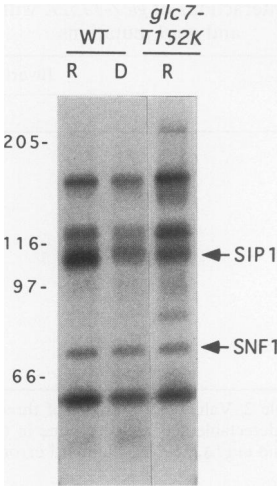

FIG. 5. Immune complex assay of SNF1 kinase activity in the *glc7-T152K* mutant. Cells were grown to exponential phase in rich medium containing 2% glucose (R). Half of the wild-type culture was then derepressed (D) by a shift to 0.05% glucose for 3 h. Lysates were prepared, and proteins were immunoprecipitated with affinity-purified anti-SNF1 as described previously (46). Immunoprecipitates were incubated with [γ-^{32}P]ATP in kinase buffer and subjected to electrophoresis in sodium dodecyl sulfate–7.5% polyacrylamide. Phosphoproteins were visualized by autoradiography. The same immunoprecipitates were analyzed by immunoblotting with an anti-SNF1 serum, and the amount of SNF1 protein appeared a few times higher in the *glc7-T152K* sample. Strains were MCY1094 (wild type [WT]) and MCY2616 (*glc7-T152K*), as indicated above the lanes. Size standards are indicated in kilodaltons. Positions of SIP1 and SNF1 are marked.

T152K mutant extracts, using an immune complex kinase assay (46). The pattern of phosphorylated products was similar to that of the wild type except that the position of the SIP1 band (46) was shifted slightly, as has been noted for derepressed wild-type extracts (12) (Fig. 5). The 85-kDa band visible in the *glc7-T152K* assay is sometimes detected in the wild type. This assay, however, does not show any substantial regulation of SNF1 activity in response to glucose and may not accurately reflect regulation occurring in vivo. No phosphorylated product was detected at the position (36 kDa) predicted for PP1.

Thus, these findings provide no evidence for interaction of PP1 with SNF1 but cannot conclusively exclude an effect of PP1 on the SNF1 kinase activity.

DISCUSSION

Here we present evidence that PP1 of *S. cerevisiae*, encoded by *GLC7*, participates in the regulatory mechanism for glucose repression. We cloned the *GLC7* gene by complementation of a mutation (formerly *cid1-226*) that partially relieves glucose repression of *SUC2* and *MAL3*. We then demonstrated by genetic analysis and sequencing that this mutation is an allele of *GLC7* (renamed *glc7-T152K*). These findings implicate PP1 in the response to glucose availability.

The GLC7 protein phosphatase is closely related to PP1s from other organisms, showing >80% identity with its mammalian counterparts (13, 29). Mammalian PP1 participates in regulation of various cellular processes, including glycogen metabolism, protein synthesis, and muscle contraction (8). In addition, mutations in the PP1 genes *dis2*$^+$/*bws1*$^+$ and *sds21*$^+$ of *S. pombe* (2, 29), *bimG*$^+$ of *Aspergillus nidulans* (10), and *PP1 87B* of *Drosophila melanogaster* (1) cause mitotic defects. Moreover, a human PP1 isoform has been shown to associate with the retinoblastoma protein during mitosis to early G_1 (11). The yeast GLC7 phosphatase is also involved in regulation of diverse processes. *GLC7* is essential for viability and affects control of glycogen metabolism, sporulation, and translation (3, 13, 45). Recent work has implicated *GLC7* in cell cycle progression: a cold-sensitive mutation that alters Cys-170 to Tyr causes defects in the G_2/M transition (18).

The mutation in *GLC7* that relieves glucose repression was found to alter Thr-152 to Lys. This Thr residue is highly conserved among eukaryotic PP1s. The *glc7-T152K* mutation appears to affect predominantly the function of the phosphatase in the glucose response mechanism and does not markedly affect viability, general cell health, or glycogen accumulation. In contrast, the mutation *glc7-1*, which alters Arg-73 to Cys, prevents glycogen accumulation but does not relieve glucose repression (3). However, both mutations impair sporulation, which evidently reflects some overlapping functional effect.

In mammalian systems, regulatory subunits play a major role in the mechanisms governing PP1 function (8, 19). Specific regulatory or targeting subunits direct the PP1 catalytic subunit to particular intracellular locations and/or alter the substrate specificity and regulatory properties. For example, in skeletal muscle, the glycogen-binding (G) regulatory subunit targets the catalytic subunit to glycogen particles (8). Such regulatory subunits are conserved from mammals to yeasts. In *S. pombe*, the sds22 protein associates with PP1 catalytic subunits encoded by *dis2*$^+$ and *sds21*$^+$ and alters the substrate specificity (39). In *S. cerevisiae*, the GAC1 and GLC8 proteins appear to be homologs of mammalian G subunit and inhibitor 2, respectively (3, 16). GAC1 shows sequence similarity to the G subunit (41) and is involved in activation of glycogen synthase and glycogen accumulation (16). Recent evidence indicates that *glc7-1* impairs interaction between the catalytic subunit and GAC1, which could account for the defect in glycogen metabolism caused by this mutation (40).

We suggest, similarly, that *glc7-T152K* may interfere with interaction between the phosphatase catalytic subunit and a targeting subunit. The proposed targeting subunit would specifically direct the GLC7 activity to a target protein(s) controlling expression of *SUC2* and other genes in response to glucose. In this model, the target protein in its dephosphorylated state could negatively regulate gene expression, or alternatively, the phosphorylated protein could positively regulate expression. In either case, the target protein would be inactive in the opposite phosphorylation state.

We favor this model on the grounds of simplicity and precedent. However, a variety of other models are also possible. For example, it is theoretically conceivable that the *glc7-T152K* mutation improves interaction of the phosphatase with an inhibitor of its activity toward targets in the glucose response pathway. The mutation could also partially impair PP1 catalytic activity, reducing it to a level sufficient for viability and glycogen accumulation but insufficient for glucose repression.

The SNF1 protein kinase is required for release from glucose repression, or derepression, whereas the GLC7 protein phosphatase is required to achieve or maintain repression. Thus, PP1 appears to function antagonistically to SNF1 in this regulatory mechanism. In support of this view, genetic analysis showed that the phenotypic effects of the *glc7-T152K* mutation are manifest only in the presence of SNF1 kinase activity. In contrast, SNF1 and GLC7 do not appear to function antagonistically in the control of glycogen metabolism because both *snf1* and *glc7* mutations impair accumulation (3, 42).

Various different relationships of PP1 and SNF1 in the glucose response mechanism can be envisioned. One possible model is that PP1 negatively regulates the activity of the SNF1 kinase toward substrates in the glucose response pathway. We did not detect any major effect of *glc7-T152K* on SNF1 kinase activity in vitro (although phosphorylation of SIP1 was altered slightly) or any interaction between GLC7 and SNF1 proteins by coimmunoprecipitation or the two-hybrid assay. However, in view of the limitations of these assays, this model remains tenable. An alternative model is that PP1 dephosphorylates substrates of the SNF1 kinase, thereby antagonizing the regulatory action of the kinase. Such a model appears to apply in an analogous situation, as both biochemical evidence and genetic evidence indicate that PP1 opposes the action of the GCN2 kinase in modulating phosphorylation of translation initiation factor 2α (45). Finally, it is possible that PP1 opposes the function of another protein kinase that works in concert with SNF1.

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