Deletion of the Gene Encoding the Cyclin-Dependent Protein Kinase Pho85 Alters Glycogen Metabolism in Saccharomyces cerevisiae

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ABSTRACT

Pho85, a protein kinase with significant homology to the cyclin-dependent kinase, Cdc28, has been shown to function in repression of transcription of acid phosphatase (APase, encoded by PHO5) in high phosphate (Pi) medium, as well as in regulation of the cell cycle at G1/S. We describe several unique phenotypes associated with the deletion of the PHO85 gene including growth defects on a variety of carbon sources and hyperaccumulation of glycogen in rich medium high in Pi. Hyperaccumulation of glycogen in the pho85 strains is independent of other APase regulatory molecules and is not signaled through Snf1 kinase. However, constitutive activation of cAPK suppresses the hyperaccumulation of glycogen in a pho85 mutant. Mutation of the type-I protein phosphatase encoded by GLC7 only partially suppresses the glycogen phenotype of the pho85 mutant. Additionally, strains containing a deletion of the PHO85 gene show an increase in expression of GS12. This work provides evidence that Pho85 has functions in addition to transcriptional regulation of APase and cell-cycle progression including the regulation of glycogen levels in the cell and may provide a link between the nutritional state of the cell and these growth related responses.

NUTRIENT limitation or depletion results in numerous phenotypes in the yeast Saccharomyces cerevisiae. These phenotypes range from arrest in the G1 phase of the cell cycle (PRINGLE and HARTWELL 1981) to accumulation of the storage carbohydrates glycogen and trehalose (LILLIE and PRINGLE 1980). Pathways signaling changes in nutrient levels such as glucose, nitrogen and phosphate and initiation of these stress responses are not well characterized. Glycogen accumulation and degradation is regulated in part by changes in the phosphorylation state of glycogen synthase and glycogen phosphorylase (ROTHMAN-DENES and CARB 1970; FRANCOIS and HERS 1988; FRANCOIS et al. 1988; PENG et al. 1990). Glycogen synthase (GS) in yeast is encoded by two genes, GSY1 and GSY2, which encode glycogen synthase-1 (GS-1) and glycogen synthase-2 (GS-2), respectively (FARKAS et al. 1990, 1991). GSY2 expression is increased before entry into stationary phase in response to the depletion of the carbon source, and the encoded protein GS-2 is additionally regulated by phosphorylation/dephosphorylation of three sites within the carboxy-terminus (FARKAS et al. 1990, 1991; HARDY and ROACH 1993; NI and LAPORTE 1995). Two protein kinases, Snf1 and cAMP-dependent protein kinase (cAPK), have been implicated in the regulation of glycogen biosynthesis (BROEK et al. 1985; TATCHELL et al. 1985; CANNON and TATCHELL 1987; CAMERON et al. 1988; THOMPSON-JAEGER et al. 1991; HARDY et al. 1994). Null SNF1 mutants are unable to initiate the dephosphorylation of the carboxy-terminus in response to nutritional deprivation, rendering the cell deficient in glycogen accumulation (HARDY et al. 1994). It is likely that Snf1 functions to signal PP-1, a type-I protein phosphatase encoded by GLC7 (FENG et al. 1991). PP-1, in association with the Gac1 regulatory subunit, is most likely involved in the direct dephosphorylation of GS-2 (FENG et al. 1991; FRANCOIS et al. 1992). Mutations in GLC7 (Glc7-1) and deletion of GAC1 result in a decrease in glycogen levels in nutritionally stressed cells (FENG et al. 1990; FRANCOIS et al. 1992). Truncation of the carboxy-terminus of GS-2 suppresses the glycogen deficiency of a snf1 mutant, as well as the glc7-1 and gac1 mutants (HARDY and ROACH 1993; HARDY et al. 1994).

Glycogen synthesis and degradation is also regulated through the cAPK in which the functionally overlapping catalytic subunits are encoded by TPK1, TPK2, and TPK3 (CAMERON et al. 1988). During periods of nutritional abundance, cAPK is in its active form, which results in an increase in activity of the glycogen phosphorylase and a decrease in glycogen synthase activity (BROEK et al. 1985; TATCHELL et al. 1985; CANNON and TATCHELL 1987; CAMERON et al. 1988). Upon nutrient depletion, cAPK is inhibited by the binding of its regulatory subunit, encoded by BCY1 (TODA et al. 1987; CANNON et al. 1990). Deletion of BCY1 conversely acti-
vates the kinase, which results in the cell's inability to respond properly to nutritional stress including accumulation of glycogen (Toda et al. 1987). **Byl** mutants show a dramatic decrease in the amount of GS2 mRNA present in nutritionally stressed cells (Hardy et al. 1994). Additionally, **byl** mutants that constitutively express GS2 from a ADH1 promoter still show a decrease in GS-2 activity suggesting a posttranslational regulatory role for cAPK. **Byl** mutants can respond normally to nutritional stress upon attenuation of the cAPK catalytic subunits, suggesting that a regulatory pathway independent of cAPK exists (Cameron et al. 1988). This pathway has yet to be characterized.

This laboratory is interested in the biochemical changes within the yeast cell after depletion of phosphate from the exocellular environment. These changes include an increase in the expression of several acid and alkaline phosphatases (Boer and Stenn-Farve 1966; Onish et al. 1979), accumulation of storage carbohydrates (Lillie and Pringle 1980) and sporulation of a/α diploids (Freese et al. 1982). Little is known about the signaling pathway between changes in the phosphate level and initiation of these stress responses, but these processes are known to be regulated by protein kinases and protein phosphatases (reviewed by Roach et al. 1991; Johnston and Carlson 1992; Mitchell, 1994). A well-studied regulatory system focuses on the transcriptional regulation of the **Phos** gene in response to inorganic phosphate (Pi) levels (Bostian et al. 1980; Kramer and Andersen 1980; Le- mire et al. 1985). During periods of high Pi, **Phos** expression is repressed and derepression occurs upon the depletion of environmental phosphate (Thill et al. 1983; Bajwa et al. 1984). Five factors have been genetically defined that regulate APase expression. The positive regulatory factors are encoded by **PHO4**, **PHO2**, and **PHO81** genes and deletion of any of these genes results in a nonderepressible phenotype (Oshima 1981; Tamai et al. 1985). Deletion of either of the two negative factors, encoded by **PHO85** and **PHO80**, results in constitutive expression of APase in high Pi medium (Ueda et al. 1975).

**PHO85** encodes a protein kinase that has 51% identity to the cyclin dependent protein kinase Cdc28 (Useno et al. 1987; Toh-e et al. 1988). Recently, it has been shown that Pho85 functions in conjunction with the cyclin Pho80 to regulate APase expression (Kaffman et al. 1994), as well as with two additional cyclins, Pcl1 and Pcl2, to function in regulating progression of the cell cycle (Espinoza et al. 1994; Measday et al. 1994). What specific role Pho85 is playing in cell-cycle regulation is not known at this time. In this paper, we have identified a function for Pho85 outside of APase regulation and cell-cycle control. Disruption of the **PHO85** gene results in several phenotypes in addition to the constitutive synthesis of APase. These include slower growth on rich media with glucose as the carbon source, failure to grow on carbon sources such as glycerol/lactate, suggesting a mitochondrial defect, inability of a/α homozygous diploids to sporulate and hyperaccumulation of glycogen in rich medium high in phosphate. Hyperaccumulation of glycogen is the result of the increased activity of glycogen synthase. Signaling for this increase in glycogen levels does not directly involve the Snf1 protein kinase, but hyperaccumulation of glycogen is suppressed by a constitutively active cAPK. Mutations in the type-1 protein phosphatase encoded by **GLC7** (gle-71) only partially suppress the hyperaccumulation of glycogen in a pho85 strain. Additionally, strains containing a deletion of the **PHO85** gene show an increase in expression of **GS2**. The data we present here indicate that Pho85 is involved in the regulation of glycogen synthesis at least in part at the level of transcription.

**MATERIALS AND METHODS**

**Strains and media:** All yeast strains used in this study are listed in Table 1. Yeast strains were grown in rich medium (YEPA, 1.0% yeast extract, 2.0% peptone) or synthetic medium supplemented with 2.0% glucose, 2.0% galactose or 3.0% glycerol/2.0% lactate, where indicated. Transformations of yeast cells were performed by the lithium acetate procedure (Ito et al. 1985) or by the lithium acetate/DMSO procedure (Gietz et al. 1989). Sporulation of various strains was tested in sporulation media (2.0% potassium acetate 0.1% glucose, 0.25% yeast extract, pH 7.0).

**Plasmid construction:** The **PHO85** gene was cloned from the genome of yeast strain **YP98** by amplification of a 1.27-kb fragment using a 5′ oligonucleotide containing a **SalI** site and a 3′ oligonucleotide containing a **XbaI** site corresponding to a Sacl site and a Sacl site corresponding to the **PHO85** sequences reported previously (Useno et al. 1987). The amplified product corresponds to sequences −144 (relative to 1 of the ATG of the protein produced from the spliced mRNA) to 1114 (95 bp present 3′ of the termination codon). This fragment will complement a pho85::**Trp1** allele when introduced on a centromeric vector (data not shown).

A partial deletion of the **PHO85** gene was constructed by digestion of the resulting plasmid with **BglII** and EcoRI to remove 89 bp internal to the **PHO85** coding region (bases 257 to 346). An 860-bp EcoRI-BglII fragment containing the **Trp1** gene was ligated into this plasmid and the identity of the resulting plasmid, p85-**Trp1**, was confirmed by restriction analysis. A 1.4-kb **XhoI-BstII** fragment (clavage sites are located at base pairs 101 and 751, respectively) from p85-**Trp1** was purified and used to transform strain **YP98** to tryptophan prototrophy. Tryptophan transmutsants were screened for constitutive synthesis of APase on complete synthetic high Pi medium plates containing 50 μg/ml 5-bromo-4-chloro-3-indoyl-phosphate (X-P) and supplemented with 2% glucose. Genomic DNA was isolated from strain **YP98** and a putative **pho85 deletion** strain (85-**Trp1**-A) and Southern analysis performed to confirm the partial deletion of the **PHO85** gene. This construction removes amino acids 53–83 of Pho85 with the insertion of the **Trp1** fragment. Subsequently, strain 85-**Trp1-A** was mated to **YP202** and sporulated to yield strain 85T1C. A pho85::**His3** gene disruption was constructed by ligating a 1782 bp **BamHI** fragment containing the **His3** gene into the **BglI** site of **PHO85** resulting in plasmid p18-85SX::**His3** A 3.1-kb **SalI-BamHI** fragment from p18-85SX::**His3** was purified and used to transform strains **YP102**, KT1113, KT1114, and CV130 to histidine prototrophy. The **SalI** site was generated
TABLE 1

Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP98</td>
<td>MATa ura3-52, by2-801, ade2-101, trpl-D1, leu2-D1</td>
<td>P. HETEr</td>
</tr>
<tr>
<td>YP102</td>
<td>MATa ura3-52, by2-801, ade2-101, his3-D200, leu2-D1</td>
<td>P. HETEr</td>
</tr>
<tr>
<td>YP198</td>
<td>MATa ura3-52, by2-801, ade2-101, his3-D200, leu2-D1, trpl-D1</td>
<td>L. W. BERGMAN</td>
</tr>
<tr>
<td>YP202</td>
<td>MATa ura3-52, by2-801, ade2-101, his3-D200, leu2-D1, trpl-D1</td>
<td>L. W. BERGMAN</td>
</tr>
<tr>
<td>85-TRP-A</td>
<td>MATa ura3-52, by2-801, ade2-101, leu2-D1, trpl-D1, pho85::TRP1</td>
<td>L. W. BERGMAN</td>
</tr>
<tr>
<td>85T-1C</td>
<td>MATa ura3-52, by2-801, ade2-101, his3-D200, leu2-D1, trpl-D1, pho85::TRP1</td>
<td>L. W. BERGMAN</td>
</tr>
</tbody>
</table>

in the original amplification and the BanHI site is located 3' of the Xhol (from the initial amplification) in the pUC18 vector. His' transformants were screened for constitutive synthesis of APase on complete synthetic high Pi medium X-P plates supplemented with 2% glucose plates. Genomic DNA was isolated from His' strains and Southern analysis performed to confirm disruption of PHO85.

The snf1::URA3 and bcl::URA3 alleles were constructed by replacement of the entire coding region of each gene with URA3. Briefly, fragments from the 5' flanking region of each gene were amplified using the PCR and primers containing a Xhol site at the 5' end and a HindIII site at the 3' end. The resulting clones were digested with Xhol and BamHI and used to transform various strains. The BCR1 gene was disrupted in wild-type strain YPT102 to yield strain YBT168. Deletion of the BCR1 gene was confirmed by Southern analysis. Strain YBT168 was subsequently disrupted for the PHO85 gene (pho85::HIS3) as described above. The SNF1 gene was disrupted in the heterozygous pho85::HIS3 diploid YBT110. Ura+ colonies were obtained, DNA isolated and deletion of SNF1 confirmed by Southern analysis. Heterozygous snf1::URA3 diploids were subjected to tetrad analysis to yield strains YBT166 and YBT167.

A PHO85 "kinase dead" (pho85-K36R) mutation was constructed by site directed mutagenesis (SANTOS et al. 1995). A 1.3-kb SalI-XhoI fragment from either the wild type or pho85-K36R mutant of PHO85 was subcloned into pRS316 (SOKORSKI and HETEr 1989). The resulting plasmids were digested with NotI (within the URA3 gene) to target the integration to ura3-52 in strain 85T-1C and Ura+ colonies screened for APase expression. Southern analysis was performed to confirm URA3 targeting and integration of only a single copy of the plasmid (data not shown). A GSY2-lacZ fusion was constructed by fusing a SalI-BamHI fragment of the lacZ structural gene in plasmid YEp367R (MYERS et al. 1986). The 830-bp SalI-BamHI fragment (~821-9 relative to 1 of the ATG) was obtained by PCR amplification in which the BamHI restriction site was created within the oligonucleotide with the fusion containing only the first three amino acids of GS. The resulting plasmid was digested with Xhol and targeted the integration to GSY2, which is used to transform strain YBT110. Southern analysis was performed to confirm GSY2 targeting and subsequently a Leu+ diploid was subjected to tetrad analysis.

Iodine staining: Yeast cells were replica plated to a rich medium plate, grown for 48 hr at 30° and stained with 10 ml of the iodine solution (0.1% iodine and 1% potassium iodide) for 3 min at room temperature (FENG et al. 1991).
Quantitative analysis of glycogen content: Yeast strains were inoculated to an OD$_{600}$ of 0.1 into 50 ml of rich medium supplemented with 2.0% glucose and grown to an OD$_{600}$ of ~0.8 at 30°C with shaking. A 30-ml sample was collected by filtration onto cellulose acetate filters (0.45 μm HA Millipore) and stored at −20°C until glycogen content was analyzed (GUNJA-SMITH et al. 1977) with modifications by LILLIE and PRINGLE (1980).

Enzyme assays: Strains were grown in rich medium supplemented with 2% glucose to an OD$_{600}$ of ~0.8. To assay glyco-
gen synthase, a 30-ml sample was collected by filtration, flash frozen in a dry-ice ethanol bath and stored at −80°C until the enzyme assay was performed. Samples were washed in 1 ml of extraction buffer (50.0 mM TrisCl, pH 7.5, 2.0 mM EDTA, 2.0 mM DTT, 1.0 μg/ml aprotinin, 2.0 mM PMSF, 1.0 mM benzamidine, 0.5 μg/ml leupeptin, and 1.0 μl/ml pepstatin) and pellets were resuspended in 300 μl cold extraction buffer and lysed by vortexing in the presence of glass beads. Homog-
enates were spun through a 3-μl syringe/glass wool spin col-

RESULTS

Disruption of the PHO85 gene results in marked growth defects: Previous studies have shown that the deletion of the PHO85 gene results in constitutive synthesis of APase (MADDEN et al. 1990). In addition, we have observed altered growth properties of the pho85 mutant cell, therefore, we have examined the growth rates of strain 85-TRP-A (pho85::TRPI) on a variety of carbon sources. Growth of the pho85 strain was significantly slower with glucose as a carbon source as com-
pared with the isogenic wild-type strain W98 (Figure 1A). Furthermore, strain 85-TRP-A fails to grow in galactose or in glycerol/lactate as a carbon source (Figure 1, B and C, respectively) as well as in other nonfer-
mentable carbon sources (data not shown). The slow growth or lack of growth is complemented by transforma-
tion of strain 85-TRP-A with a centromere plasmid containing the PHO85 gene (data not shown). Genetic analysis reveals the Gal− phenotype, but not the Gly/ Lac− phenotype is strain specific and results from an

In addition to these growth defects, diploid strains that are homozygous for the deletion of the PHO85 gene fail to sporulate as there are no ascii detected under several conditions that induce sporulation for either a wild-type strain or a strain heterozygous for the pho85 deletion (data not shown). The inability of the mutant to sporulate or to use glycerol/lactate as a car-

Deletion of PHO85 gene results in the hyperaccumu-

![Graph A](image-url)

![Graph B](image-url)

![Graph C](image-url)

**FIGURE 1.**—Deletion of the PHO85 gene results in growth defects in a variety of carbon sources. Growth curves were performed for the strains YP98 (PHO85) (△) and 85-TRP-A (pho85::TRPI) (●) in rich media supplemented with either 2.0% glucose (A), 2.0% galactose (B) or 3.0% glycerol/2.0% lactate (C).

In a screen for high copy suppressors of the Gal− growth defect resulting from the disruption of the PHO85 gene, we have isolated the GLC7 and TPK2 genes (data not shown). Since PP-1 and cAPK are both involved in regulation of glycogen accumulation and degradation (BROEK et al. 1985; TATCHELL et al. 1985; CANNON and TATCHELL 1987; CAMERON et al. 1988; FENG et al. 1991; FRANÇOIS et al. 1992; HARDY et al. 1994), we have examined glycogen levels in the pho85::TRPI strain. Cells were grown in rich medium supplemented with 2.0% glucose to mid-logarithmic phase (an OD$_{600}$ of ~0.8), and glycogen content was analyzed. Figure 2A shows that wild-type strain YP202 (PHO85) contains a minimal level of glycogen when grown in a rich medium and deletion of the PHO85 gene results in an ~3.6-fold increase in the level of glycogen (as compared with the isogenic wild-type strain) when grown under identical conditions. Growth of the wild-type strain in low Pi media also results in a significant increase in glycogen levels (data not shown). Similar levels of glycogen were seen in a pho85::HIS3 (strain 85-HIS-A) mutant as compared with the pho85::TRPI mutant (strain 85-TRP-A; data not shown).
positive transcription factor PHO4 (strain CY71) (OGAWA and OSHIMA 1990) or in the mediator PHO81 (strain CY81) (CREASY et al. 1993) resulting in constitutive APase synthesis show essentially equivalent levels of glycogen when grown in rich medium supplemented with 2.0% glucose and high Pi (Figure 2A) as compared with the wild-type strain. This suggests that the increase in APase levels is not triggering the hyperaccumulation of glycogen in the pho85 mutant.

Figure 2A also shows that deletion of the gene encoding the negative regulatory factor PHO80 (strain h-A), which also results in constitutive expression of APase, does not result in the hyperaccumulation of glycogen when cells are grown in rich medium supplemented with 2.0% glucose and high Pi. This suggests the Pho80 cyclin in association with Pho85 does not regulate glycogen synthesis. Interestingly, strain h-A (pho80::LEU2) fails to grow in medium when glycerol/lactate is used as the sole carbon source (data not shown) suggesting additional regulatory roles of the Pho80/Pho85 complex.

The glycogen level in a pho1::URA3, pho85::TRPI mutant (strain CY38) was examined to ensure production of APase is not required for initiation of glycogen synthesis. Deletion of PHO4 results in a nonderepressible phenotype for PHO5 expression even in a pho85::TRPI background (OSHIMA 1981). As seen in Figure 2A, strain CY38 shows an increase in glycogen levels when grown in a high phosphate rich medium, indicating the synthesis of APase is not required in glycogen accumulation in the pho85 background.

To ensure that it is the kinase function of Pho85 that is required for glycogen regulation, we have used a “kinase dead” pho85-K36R mutation within the putative ATP-binding site of Pho85 (SANTOS et al. 1995). This mutation or the wild-type gene was subcloned into pRS306 and integrated into strain 85T-1C at the ura3-52 locus, and glycogen levels were determined. As seen in Figure 2A, the glycogen levels seen in the “kinase dead” mutant are similar to that seen in a pho85 deletion strain. Glycogen levels are significantly lower upon integration of the wild-type PHO85 gene into strain 85T-1C. This indicates that the Pho85 kinase activity is essential for the negative regulation of glycogen synthesis.

**Pho85 does not function through Snf1 in glycogen regulation:** The Snf1 protein kinase is involved in activation of glycogen synthase in response to depletion of glucose or phosphate in the medium (THOMPSON-JAEGGER et al. 1991). To examine if Pho85 is involved in the regulation of Snf1 or vice versa, we deleted SNFI by replacement of the coding region of SNFI with the URA3 gene in a pho85 heterozygous diploid (strain YBT110) as described in MATERIALS AND METHODS. Disruption of SNFI was confirmed by Southern analysis (data not shown). After sporulation and dissection of the snf1, pho85 heterozygous diploid (strain YBT165), four spore tetrads were screened for auxotrophic re-
requirements and several tetra-types were selected and screened initially for glycogen content by iodine vapor stain. Darker staining cells were shown to be His\(^+\) Ura\(^+\) (pho85::HIS3, snfl::URA3) (data not shown) suggesting deletion of SNFI does not suppress the hyperaccumulation phenotype of a pho85 strain. Quantitative analysis of glycogen levels of strains resulting from a tetra-type ascus show the pho85::HIS3 and pho85::HIS3, snfl::URA3 mutants hyperaccumulated glycogen in rich medium high in phosphate to 2.4- or 2.8-fold, respectively, over that of the wild-type strain and 6.3- or 7.5-fold, respectively, over that of the wild-type strain YBT199. Wild-type strain KT1113 and strains with either the glc7-1 mutation (CV130) or disrupted for glc7-1 (YP102) and for gk7-I (KT1149) accumulate low levels of glycogen when grown in high Pi rich media (PENG et al. 1990; FRANCOIS et al. 1992). Quantitative analysis of glycogen levels show the deletion of the PHO85 gene in the wild-type strain KT1113 increases the glycogen level of the cell by 4.8-fold (Figure 4). In addition, both strain CV130 (glc7-1) and strain KT1149 (gk7-I, ura3) contain higher levels of glycogen when the pho85::HIS3 deletions (strains YBT201 and YBT200, respectively) are also present (2.5- and 1.7-fold, respectively). However, glycogen levels of strain YBT201 (glc7-1, pho85::HIS3) are approximately twofold lower when compared with strain YBT199 (pho85::HIS3). These results suggest the glc7-1 mutation can partially suppress the hyperaccumulation of glycogen of a pho85 mutant. There is still a significant increase in the glycogen levels of the strain YBT201 (glc7-1, pho85::HIS3) over that of the wild-type strain KT1113 and of strain CV130 (glc7-1) suggesting that Pho85 does not function in a epistatic manner to PP-1, but that the glc7-1 mutation may be masking the hyperaccumulation of glycogen phenotype.
of the pho85 mutant strain. This could indicate that Pho85 may be directly regulating glycogen synthase in a posttranslational manner.

**Glycogen synthase activity is increased upon deletion of the PHO85 gene:** Since mutations within PP-1 (glc7-1) fail to completely suppress the hyperaccumulation phenotype of a pho85 mutant, we examined whether the deletion of the PHO85 gene affected the enzymatic activity of glycogen synthase and whether deletions of the snfl or beyl genes resulted in a change in this activity. As seen in Figure 5, deletion of the PHO85 gene (pho85::HIS3) increases both the level of active glycogen synthase (activity measured in the absence of glucose-6-phosphate) and the total glycogen synthase activity in the cell (activity measured in the absence of glucose-6-phosphate). Consistent with the observation that the beyl, pho85 double mutant failed to accumulate glycogen, glycogen synthase activity is low in the pho85, beyl mutant strain (strain YBT169). However, in contrast, there is a significant increase in both active and total glycogen synthase activity in the snfl, pho85 mutant (strain YBT167) as compared with either the wild-type or pho85 strains (Figure 5). At present, the mechanism responsible for this observation is not clear.

**Deletion of the PHO85 gene results in the increase of expression of GSY2:** We have investigated whether Pho85 is regulating the expression of the inducible glycogen synthase gene GSY2 by examining the expression of a GSY2::lacZ fusion gene, which contains 820 bp upstream of the translational start site and only the first 3 bp of the coding region. This fusion gene was integrated into the genome of a pho85 heterozygous diploid (YBT110) as described in MATERIALS AND METHODS. After sporulation and dissection of a Leu' diploid, pho85::HIS3 haploids and wild-type haploids that all contained the GSY2-lacZ fusion gene were grown in rich medium until an OD_{600} of ~0.8 and β-galactosidase activity was measured (Figure 6). β-galactosidase activity of the pho85 deletion strains were ~4.1-fold greater than that of the wild-type strains. This result suggests that deletion of the PHO85 gene results in increased expression of GSY2 under these growth conditions.

**DISCUSSION**

Adequate glycogen stores are essential to the survival of the yeast cell during periods of nutrient deprivation. Glycogen levels increase when the cell senses the depletion of essential nutrients such as glucose, phosphate, sulfate or nitrogen (Lillie and Pringle 1980). Previously, the Pho85 kinase has been shown to be involved in repression of acid phosphatase production, which is subsequently derepressed upon phosphate deprivation (reviewed by Johnston and Carlson 1992). GSY2 encodes for the majority of the glycogen synthase (GS-2) induced upon nutrient depletion in the cell (Farrak et al. 1990, 1991). Regulation of glycogen synthase is achieved both at the level of GSY2 transcription and by posttranslational phosphorylation/dephosphorylation signaled through antagonistic pathways that involve, in part, the Snf1 and cAPK kinases (Hardy et al. 1994).
This report provides evidence that the cyclin-dependent protein kinase Pho85 is involved in regulation of glycogen biosynthesis. Deletion of the PHO85 gene results in the inappropriate synthesis and accumulation of glycogen in rapidly dividing cells. Using a GSY2-lacZ fusion, we have demonstrated that this phenotype is mediated, at least in part, by increased expression of the GSY2 gene. Strains containing the GSY2-lacZ fusion in both a pho85::HIS3 and a PHO85 background were isolated and examined for β-galactosidase activity during exponential growth (OD₆₀₀ of ~0.8) as described previously (CREASY et al. 1993). The activities presented are the average of four isolates assayed in duplicate.

![Figure 6](image)

**Figure 6.**—Deletion of the PHO85 gene results in the increase of expression of the GSY2 gene. A GSY2-lacZ fusion was integrated into a pho85 heterozygous diploid (YBT110) at the GSY2 locus. Leu⁺ diploids were subjected to tetrad analysis. Strains containing the GSY2-lacZ fusion in both a pho85::HIS3 and a PHO85 background were isolated and examined for β-galactosidase activity during exponential growth (OD₆₀₀ of ~0.8) as described previously (CREASY et al. 1993). The activities presented are the average of four isolates assayed in duplicate.

Pho85 is a cyclin-dependent protein kinase (CDK), and thus it is assumed that its role in regulating glycogen biosynthesis is in association with a cyclin. Surprisingly, deletion of the PHO80 gene, which encodes the cyclin associated with Pho85 in repressing acid phosphatase expression, does not result in the hyperaccumulation of glycogen (Figure 2A). This indicates that the substrate specificity for the Pho85/Pho80 complex does not potentially include those substrates involved in regulation of glycogen levels. It is possible there is a redundancy in cyclin function in regulating glycogen metabolism that would essentially mask the glycogen accumulation phenotype of a pho80 mutant. However, we can not rule out that Pho85 acts to reduce GSY2 expression in the absence of an associated cyclin subunit. Experiments are under way to examine the role that Pho80 and the additional Pho85 associated cyclins (such as Pcl1 and Pcl2) may play in glycogen metabolism.

Transcriptional control of GSY2 is in part mediated through cAPK. A bcyl deletion, resulting in constitutively active cAPK, inhibited the hyperaccumulation of glycogen in a pho85 mutant (Figure 2B). This is supported by the glycogen synthase activity data, which shows minimal total activity levels in a pho85, bcyl strain (as measured in the presence of glucose-6-phosphate). These results suggest that Pho85 functions epistatic to cAPK in signaling nutritional conditions. However, it is possible the decrease in the glycogen synthase activity of the pho85, bcyl strain may be due to the posttranslational function of the constitutively active cAPK (HARDY et al. 1994) as constitutively expressed GSY2 (using an ADH1 promoter) fails to suppress the glycogen accumulation defect observed in the bcyl strain. This theory is supported by the finding that a bcyl strain can respond to nutritional stress by accumulating glycogen if the strain is additionally attenuated for the catalytic subunits of cAPK (CAMERON et al. 1988). This is indicative of a signaling pathway independent of cAPK that to date has not been defined and may in part be signaled through the Pho85 kinase.

It is difficult from the experiments presented in this report, to rule out a role for Pho85 in the posttranslational modification of GS-2. The snf1, gac1 deletions and the gca7-1 mutation all result in cells that fail to accumulate glycogen and are involved in signaling the dephosphorylation and activation of GS-2 (PENG et al. 1990; FENG et al. 1991; FRANCOIS et al. 1992). These mutations either fail to or only partially suppress the glycogen accumulation phenotype observed in strains lacking the Pho85 kinase. Although there is an increase in total GS activity in the pho85 strains (presumably due to increased GSY2 expression), there is also an increase in the amount of active GS (Figure 5, measured in the absence of glucose-6-phosphate). It should be noted that these experiments were performed using logarithmically growing cells, a condition where both GSY2 expression and GS activity are low. We are currently examining whether GS-2 is also a substrate for Pho85 phosphorylation and whether the phosphorylation is biochemically relevant since the in vivo sites of GS phosphorylation have been identified (HARDY and ROACH 1993).

In conclusion, the loss of Pho85 kinase activity results in the activation of two major metabolic pathways both of which aid in the cell's response to phosphate depletion. These pathways are the derepression of acid phosphatase production and increased storage carbohydrate synthesis. Furthermore, recent evidence has demon-
strated that Pho85 plays a role in regulating cell cycle progression (Kaffman et al. 1994). These findings further support the critical role of the CDKs in responding to various environmental stimuli not only in yeast cells, but also as observed in mammalian cells (reviewed by Roach et al. 1991).

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