The Yeast GLC7 Gene Required for Glycogen Accumulation Encodes a Type 1 Protein Phosphatase*

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Zhehui Peng‡†‡, Susan E. Wilson‡†, Zao-Yuan Peng‡†‡, Keith K. Schliernder‡, Erwin M. Reimann‡, and Robert J. Trumbly‡†‡
From the Departments of ‡Biochemistry and Molecular Biology and ††Pharmacology, Medical College of Ohio, Toledo, Ohio 43699-0008

The glc7 mutant of the yeast Saccharomyces cerevisiae does not accumulate glycogen due to a defect in glycogen synthase activation (Peng, Z., Trumbly, R. J., and Reimann, E. M. (1990) J. Biol. Chem. 265, 13871–13877) whereas wild-type strains accumulate glycogen as the cell cultures approach stationary phase. We isolated the GLC7 gene by complementation and found that the GLC7 gene is the same as the DIS2S1 gene (Ohkura, H., Kinoshiha, N., Miyatani, S., Toda, T., and Yanagida, M. (1989) Cell 57, 997–1007). The protein product predicted by the GLC7 DNA sequence has a sequence that is 81% identical with rabbit protein phosphatase 1 catalytic subunit. Protein phosphatase 1 activity was greatly diminished in extracts from glc7 mutant cells. Two forms of protein phosphatase 1 were identified after chromatography of extracts on DEAE-cellulose. Both forms were diminished in the glc7 mutant and were partly restored by transformation with a plasmid carrying the GLC7 gene. Southern blots indicate the presence of a single copy of GLC7 in S. cerevisiae, and gene disruption experiments showed that the GLC7 gene is essential for cell viability. The GLC7 mRNA was identified as a 1.4-kilobase RNA that increases 4-fold at the end of exponential growth in wild-type cells, suggesting that activation of glycogen synthase is mediated by increased expression of protein phosphatase 1 as cells reach stationary phase.

The control of glycogen metabolism is in general similar in yeast and mammalian cells. The net rate of glycogen accumulation is determined by the opposing actions of glycogen synthase and glycogen phosphorylase. The activities of these two enzymes are regulated by phosphorylation, with glycogen synthase inhibited (Rothman-Denes and Cabib, 1970) and glycogen phosphorylase activated (Fosset et al., 1971) by phosphorylation. Glycogen synthase in mammalian cells is phosphorylated by a number of protein kinases, acting in a synergistic manner to regulate its activity. The highly phosphorylated forms of glycogen synthase require glucose 6-phosphate for maximal activity, whereas the dephosphorylated forms have activity independent of glucose 6-phosphate (Cohen, 1989). The major protein phosphatases implicated in regulation of mammalian glycogen synthase are protein phosphatases 1 and 2A (Cohen, 1989). Interconversion between phosphorylated and dephosphorylated forms of yeast glycogen synthase has been demonstrated, both in vivo and in vitro (Rothman-Denes and Cabib, 1970; Huang and Cabib, 1974; Francois et al., 1988; Francois and Hers, 1988; Peng et al., 1990), but the protein kinases and phosphatases that regulate glycogen synthase in the yeast cell have not been identified. The cAMP pathway plays an important role in the control of glycogen accumulation in yeast. Mutations that increase cAMP-dependent protein kinase activity, by increasing cAMP levels for example, reduce glycogen accumulation; mutations that reduce cAMP-dependent protein kinase activity, such as ras2 mutations that reduce cAMP levels, induce hyperaccumulation of glycogen (Cannon et al., 1986). Glycogen accumulation in yeast can also be regulated by mechanisms independent of the cAMP pathway (Cameron et al., 1988). We examined glycogen synthase activity in a collection of glycogen-deficient mutants, glc1-glc8 and found the greatest effect in the glc7 strain, where all of the enzyme was highly phosphorylated and dependent on Glc-6-P (Peng et al., 1990). In this report we show, by cloning and sequencing of the GLC7 gene, that GLC7 encodes protein phosphatase 1 and demonstrate that proteolytic activity of protein phosphatase 1 activity is decreased in the glc7 strain.

MATERIALS AND METHODS

Media—YEPD medium is composed of 1% yeast extract, 2% Bactopeptone, and 2% glucose. SD minimal medium is composed of 0.67% Bacto-yeast nitrogen base, 2% glucose with the appropriate supplements (Sherman et al., 1982). Solid media contained 2% agar.

Strains—All yeast strains used in this study are Saccharomyces cerevisiae of the S288c genetic background. A yeast mutant with reduced glycogen accumulation, glc7, was obtained from Dr. J. Pringle (University of Michigan). RTY370, a diploid wild-type strain, is the same as C276 (Lillie and Pringle, 1980). The glc7 mutant strains were derived from the diploid strain 22R1 (glc7/glc7) (Peng et al., 1990). The haploid strain RTY378-1A (MATa glc7) derived from sporulation of 22R1 was crossed with RTY214 (MATa cyc8-20 his4-519 leu2-3 leu2-112 ura3-52). In the tetrads produced by sporulation of this diploid, the glycogen deficiency associated with glc7 segregated 2:2, and a glc7 ura3 segregant, RTY398 (MATa glc7 leu2-3 leu2-112 ura3-52), was chosen to facilitate the isolation of the GLC7 gene. RTY518 is RTY398 transformed with pRS316. RTY520 is RTY398 trans-
formed with pZF135. RTY553 (MATa his4-519) plasmids (Maniatis et al., 1982). The yeast genomic DNA library in the centromere vector YCP50, which contains URA3 as a selectable marker, was a kind gift of Dr. M. D. Rose (Rose et al., 1987). The plasmid pZF2 containing the GLC7 gene isolated from this library had approximately 8.5 kb of insert DNA. The following plasmids (Fig. 1) were derived directly from pZF1. A 7-kb HindIII fragment was subcloned into the HindIII site of YCP50 to produce pZF2; the 2.6-kb XhoI fragment was subcloned into the XhoI site of pRS316, which contains URA3 as a selectable marker (Sikorski and Hieter, 1989), to yield pZF11; and a 3-kb XhoI fragment was ligated into the XhoI site of pRS316, to yield pZF135. pZF135 was created by digestion of pZF1 with EcoRI followed by religation. The 2.1-kb BglII-XhoI fragment of pZF135 was subcloned into the XhoI and BamHI sites of pRS316 to yield pZF141. pZF135 was digested by EcoRI and religated to form pZF144 and digested by SalI and religated to yield pZF145. The 2.7-kb HindIII-XhoI fragment from pZF135 was transferred into the HindIII-XhoI site of pRS316 to give pZF226 and into the HindIII-SalI site of pRS316 to yield pZF1. plasmid pZF238 used for gene disruption was constructed by deleting the EcoRI site in the polylinker of pZF226 by digesting HindIII and XhoI, Kleinow treatment, and then subcloning the 1.5-kb EcoRI-BamHI fragment from YE3p24 (Botstein et al., 1979) containing the URA3 gene into the EcoRI-BglII sites.

To generate colonies for DNA sequencing, the 3-kb XhoI fragment of pZF1 was subcloned into the SalI site of the vector pBS(+) (Strategene) in both orientations to produce pZF29 (+ orientation) and pZF 149 (– orientation). Nested sets of deletions from the ends of insertion were prepared by sequential treatments with exonucleases III and S1 nuclease (Henikoff, 1984).

Iodine Staining—Yeast colonies were patched or replicated from a master plate onto a YEPD plate. The YEPD plate was placed at 30°C overnight, and 10 ml of 0.1% iodine and 1% potassium iodide solution were added to stain colonies for about 10 min at room temperature (Chester, 1967).

Glycogen Synthase Assay—Activity of the glycogen synthase was measured with or without 7.2 mM Glc-6-P (25°C) with 10 ml of plasmid-containing yeast extract for 10 min in an assay mixture that also contained 4.4 mM UDP-[14C]glucose, 50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 100 mg of oyster glycogen/ml, and enzyme in a total volume of 90 ml. Samples were diluted with dilution buffer, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 60 mg of oyster glycogen/ml, and 42 mM UDP-[14C]glucose. Reactions were terminated by spotting 75% of the reaction mixture onto Whatman filter papers that were processed as described previously (Thomas et al., 1968).

Protein Phosphatase Assay—Phosphorylase phosphatase was assayed by measuring the release of 32P from [32P]phosphorylase a (Kildies et al., 1978; Peng et al., 1990). A 100-ml assay contained 50 mM imidazole-HCl, pH 7.4 (et 25°C), 6 mM theophylline, 1.8 mM dithiothreitol, 5 mM Tris-HCl, pH 7.4 (at 25°C), 0.02 mM EDTA, 0.2 μM [32P]phosphorylase a (monomer), protein phosphatase, and unless otherwise indicated, 3 mM okadaic acid to suppress protein phosphatase 2A activity. Co3+/trypsin-stimulated phosphorylase activity was assayed by preincubating the enzymes with 0.2 mM free CoCl2 for 5 min at 30°C, followed by addition of trypsin to a final concentration of 0.02 mg/ml for an additional 10 min (Schlender et al., 1986). Proteolysis was terminated by addition of soybean trypsin inhibitor, and the samples were processed as described above (Thomas et al., 1968).

RESULTS

Cloning the GLC7 Gene—We have found previously that yeast glc7 mutant strains show decreased dephosphorylation of glycogen synthase, causing diminished activation of this enzyme (Peng et al., 1990). A screening method for isolating the GLC7 gene exploited the fact that glc7 mutants do not accumulate glycogen, enabling mutant and wild-type colonies to be distinguished by staining with iodine (Chester, 1967). A yeast glc7 mutant strain RTY370 (MATα glc7 leu2 ura3) was transformed with a genomic DNA library in the centromere vector YCP50. Out of 16,000 UrA+ transformant colonies screened, eight (0.05%) gave a positive staining for glycogen. The plasmids extracted from these eight transformants gave identical restriction maps with an insert of 8.5 kb. One of these plasmids, pZF1, was retransformed into the glc7 mutant strain RTY398. All of the transformant colonies accumulated glycogen to the levels similar to wild-type when assayed by iodine staining. This result confirmed that the complementation of the glc7 mutation was due to plasmid pZF1. One of these transformants was grown to stationary phase in YEPD-rich medium to allow for loss of the plasmid and then plated onto YEPD. When these colonies were replicated onto SD plates without uracil and onto YEPD plates for iodine staining, colonies that failed to grow without uracil also were negative for glycogen after iodine staining. Therefore, complementation of the glc7 defect in glycogen accumulation was due to the presence of the gene on the plasmid.

The GLC7 gene was localized within the 8.5-kb insert by subcloning and testing glycogen accumulation with iodine staining. A restriction map of this region was derived from single and double restriction digests (Fig. 1). Different selected fragments were subcloned into the centromere vectors YCP50 or pRS316 and tested for complementation by trans-
formation of the glc7 mutant RTY398. These experiments localized the GLC7 gene to the 2.7-kb HindIII-XhoI fragment on pZF226.

To demonstrate that we had cloned the GLC7 gene and not an extragenic suppressor, the cloned yeast DNA was used to direct integration of a plasmid into yeast chromosomal DNA. First, the 2.7-kb HindIII-XhoI fragment was subcloned into the integrating vector YIp5. Next, the resulting plasmid pZF239 was cut with SalI, which cuts once within the insert DNA. The diploid yeast strain RTY553, which is heterozygous for the glc7 mutation, was transformed with this DNA. One of the transformants, which had a single copy of pZF239 integrated into the chromosome as determined by Southern blotting (data not shown), was sporulated. Of the 23 tetrads that had regular segregation of the URA3 gene, 22 were parental diploids that did complement the glc7 mutation; + or − means that the transformants accumulated or did not accumulate glycogen, respectively.

**Fig. 1. Localization of the GLC7 gene.** The restriction map of the plasmid pZF1 is shown on the top line. Restriction sites: H, HindIII, E, EcoRI, BglII, S, SalI, XhoI, XbaI, XbaI, XhoI. The line above the restriction map represents the open reading frame of GLC7 gene determined from the DNA sequence. The solid lines represent the exons and the broken line represents the intron. Thin lines, subclones that did complement the glc7 mutation; thick lines, subclones that did not complement the glc7 mutation; + or − means that the transformants accumulated or did not accumulate glycogen, respectively.

The GLC7 gene prevents spore germination or cell proliferation.

**Sequencing the GLC7 Gene**—The nucleotide sequence of the 3-kb XhoI fragment, comprising 3,054 nucleotides, was determined by DNA sequencing (data not shown). The gene contains two long coding regions separated by a region with stop codons in all three reading frames. The open reading frames together code for a protein of 312 amino acids with strong homology to the type 1 protein phosphatases from several species. The putative GLC7 gene product was deduced by aligning the predicted amino acid sequence with those of other type 1 protein phosphatases. The alignment gives consensus sequences for exon-intron splice junctions at the appropriate positions. A defect in protein phosphatase 1 is consistent with the previous finding that glycogen synthase in the glc7 strain is phosphorylated more extensively than normal (Peng et al., 1990). The GLC7 sequence is virtually identical with the S. cerevisiae D1S21 gene, which was isolated by hybridization screening with the Schizosaccharomyces pombe D1S22 gene involved in chromosomal disjunction (Ohkura et al., 1989). The first 204 nucleotides of the sequence are 97% identical with the δ repeat sequence found at the ends of the Ty1-transposable elements (Boeke et al., 1988).

**Protein Phosphatase Activity of Wild-type and Mutant Strains**—Protein phosphatase activity was measured in yeast extracts to determine if the glc7 mutation of the gene for protein phosphatase 1 would be reflected by changes in the activity of protein phosphatase 1. In order to distinguish protein phosphatases 1 and 2A, we made use of two inhibitors: heat-stable protein inhibitor 2 and okadaic acid (Cohen et al., 1989). Protein phosphatase 2A is not inhibited by inhibitor 2 but is almost completely inhibited by low concentrations of okadaic acid (2–5 nM) (Bialojan and Takai, 1988). Treatment of protein phosphatase 1 with Co²⁺ and trypsin can activate "cryptic" forms of protein phosphatase (Tung et al., 1984). These properties were used to optimize the detection of differences in the levels of protein phosphatases 1 and 2A in extracts of different strains of yeast. Phosphorylase α was used as the substrate since other phosphatases do not significantly dephosphorylate this substrate (Cohen, 1989). Initially, we detected no significant difference in protein phosphatase 1 activity between unfractonated cell homogenates from wild-type and glc7 mutant strains (Peng et al., 1990). Subsequent experiments using activation with Co²⁺ and sufficient dilution before assay in the presence of 3 mM okadaic acid, which suppresses phosphatase 2A, indicated that the glc7 mutant has about one-third as much protein phosphatase 1 as the wild-type and that activity is restored to normal after transformation with a plasmid carrying the GLC7 gene (Table 1). The effects on protein phosphatase 1 were similar, irrespective of whether protein phosphatase 1 was defined as the activity sensitive to inhibitor 2 or as the activity remaining in the presence of 3 mM okadaic acid. No major changes in phosphatase 2A were detected with either assay. Since multiple forms of protein phosphatase 1 exist in mammalian cells (Cohen, 1989), these extracts were fractionated on DEAE-cellulose (DE52) to determine if multiple forms of protein phosphatase 1 are present and if these forms are differentially affected by the glc7 mutation. Fractions were treated with Co²⁺ and trypsin and assayed for the ability to dephosphorylate [32P]phosphorylase α in the presence of 3 mM okadaic acid. The data of Fig. 2 show the presence of two forms of protein phosphatase 1, both of which are markedly decreased in the glc7 mutant and restored after transformation. Treatment with Co²⁺ and trypsin increased activity.
Yeast Protein Phosphatase

Glycogen synthase and protein phosphatase activity in yeast extracts

Glycogen synthase was measured as described in the text. Protein phosphatase activity was measured after treatment with Co²⁺ and trypsin.

| Strain          | Glycogen synthase activity ratio* | Protein phosphatase |
|-----------------|----------------------------------|---------------------|
|                 | Expt. 1 | Expt. 2 | Type 1 | Type 2A |
| Wild-type (RTY370) | 0.45  | 0.35  | 630  | 560  | 98  | 109  |
| Mutant (RTY518)   | 0.15  | 0.09  | 255  | 203  | 96  | 148  |
| Transformed (RTY520) | 0.45  | 0.23  | 793  | 848  | 180 | 125  |

*Activity ratio, activity without Glc-6-P/activity with Glc-6-P.

TRYPsin.
eluted with a linear salt gradient from 0 to 0.5 M NaCl. Phosphatase was applied to a 3-ml DEAE-cellulose column, and the phosphatases were remaining in the presence of inhibitor 2.

shown for wild-type strain RTY370 (TR).
treatment with Co²⁺ and trypsin. The phosphatase activities are formed about 5-fold in cell extracts and 3-fold in fractions.

FIG. 2. Separation of two forms of type 1 protein phosphatase by DEAE chromatography. Extracts from 0.5 g of yeast were applied to a 3-ml DEAE-cellulose column, and the phosphatases were eluted with a linear salt gradient from 0 to 0.5 M NaCl. Phosphatase activity was measured in the presence of 3 nM okadaic acid after treatment with Co²⁺ and trypsin. The phosphatase activities are shown for wild-type strain RTY370 (WT); RTY518 (RTY398 glc7 mutant strain transformed with pRS316) (MU), and RTY520 (RTY398 transformed with plasmid pZF135 carrying the GLC7 gene) (TR).

there is a relationship between the amount of GLC7 mRNA and glycogen synthase, the amounts of GLC7 mRNA were determined at different times of batch culture growth. As shown in Fig. 4, a Northern blot probed with a fragment from the 5' end of GLC7 revealed a 1.4 kb band present in poly(A)+ and total RNA. The level of GLC7 mRNA is constant during exponential growth and augments considerably near the end of exponential growth (panel B). The relative amount of GLC7 mRNA increases about 4-fold at this time, as determined by scanning densitometry of the autoradiograph and rRNA stained with ethidium bromide. This increase in mRNA coincides with the increase in glycogen synthase activity ratio noted in previous studies (Rothman-Denes and Cabib, 1970; Peng et al., 1990).

FIG. 3. Southern blot of GLC7 gene in wild-type strain. Chromosomal DNA from wild-type strain RTY370 was prepared by a rapid method (Hoffman and Winston, 1987) and 10-µg portions were digested with different endonucleases and separated by electrophoresis on a 0.8% agarose gel. The DNA was transferred to a nitrocellulose filter and hybridized by standard methods (Maniatis et al., 1982), except the hybridization conditions were 6 x SSC, 55 °C. The probe was a 1.3-kb ClaI-EcoRI fragment comprising most of the second exon of the GLC7 gene. Lane 1, a 7-kb HindIII fragment and a 5.4-kb HindIII-Sall fragment of GLC7 were applied as control DNA (a small amount of the 1.6-kb HindIII-Sall fragment of GLC7 was also present on the gel). Lane 2, chromosomal DNA of RTY370 digested with HindIII. Lane 3, chromosomal DNA of RTY370 digested with XhoI.

TABLE I

| Strain          | Glycogen synthase activity ratio | Protein phosphatase |
|-----------------|---------------------------------|---------------------|
|                 | Expt. 1 | Expt. 2 | Type 1 | Type 2A |
| Wild-type (RTY370) | 0.45  | 0.35  | 630  | 560  | 98  | 109  |
| Mutant (RTY518)   | 0.15  | 0.09  | 255  | 203  | 96  | 148  |
| Transformed (RTY520) | 0.45  | 0.23  | 793  | 848  | 180 | 125  |

activity was measured after treatment with Co²⁺ and trypsin. The phosphatase activities are formed about 5-fold in cell extracts and 3-fold or less in column fractions.

The GLC7 Gene Restores Glycogen Synthase Activity—To confirm that restoration of glycogen synthase activation is associated with increased phosphatase activity in the transformed glc7 strain, extracts of the wild-type, glc7 mutant, and glc7 mutant strains transformed with a plasmid carrying the GLC7 gene were assayed for glycogen synthase activity. As shown in Table I, two separate experiments demonstrated restoration of glycogen synthase activity after transformation.

Southern Blot of Yeast Genomic DNA—Multiple genes encoding type 1 protein phosphatase have been found in fission yeast (Ohkura et al., 1989) and other organisms (Axton et al., 1990; Wadzinski et al., 1990; Sasaki et al., 1990). A Southern blot of S. cerevisiae chromosomal DNA was performed to determine the number of type 1 protein phosphatase genes in this species. A restriction fragment containing most of the GLC7 coding region was hybridized to the blot under conditions of reduced stringency. As shown in Fig. 3, lanes 2 and 3 had a single band, confirming that there is a single gene in the yeast genome encoding a type 1 protein phosphatase.

Regulation of GLC7 mRNA—The level of glycogen synthase activity in yeast cells greatly increases during the transition from exponential growth to stationary phase (Lillie and Pringle, 1980; Cameron et al., 1988; Peng et al., 1990). To see if

DISCUSSION

Until recently, relatively little information has been available regarding the protein phosphatases that participate in the regulation of glycogen metabolism in S. cerevisiae. Peng et al. (1991) showed that phosphatase 2A from yeast could activate yeast glycogen synthase in vitro. The experiments described here demonstrate that the glc7 mutation, which results in glycogen deficiency and excessive phosphorylation of glycogen synthase, is caused by a defect in the GLC7 gene that encodes a type 1 protein phosphatase, showing that protein phosphatase 1 can regulate yeast glycogen synthase in vivo. Under the conditions of the experiments described here, it appears that protein phosphatase 1 is more important than phosphatase 2A for regulating glycogen synthase, since phosphatase 2A activity is normal in the mutant but the glycogen synthase activity ratio is much lower than normal. However, it is possible that phosphatase 2A is also involved in the regulation of the enzyme under other conditions and that glycogen synthase activity would be even lower in the absence of phosphatase 2A activity.

Our finding that a mutation in the yeast gene encoding protein phosphatase 1 decreases protein phosphatase 1 activity and results in glycogen deficiency is consistent with experiments showing that protein phosphatases 1 and 2A are
the major protein phosphatases regulating glycogen synthase in mammalian tissues. In some cells, the catalytic subunit of protein phosphatase 1 is associated with a larger regulatory subunit (G-subunit) which targets the phosphatase activity toward the enzymes of glycogen metabolism. It was shown recently that stimulation of glycogen synthesis by insulin is mediated by the phosphorylation of the G-subunit, which activates the phosphatase activity toward glycogen synthase and phosphorylase kinase (Dent et al., 1990). The protein phosphatase 1 catalytic subunit also associates with inhibitor-2, whose activity may be regulated by phosphorylation by cAMP-dependent protein kinase. It is likely that the yeast protein phosphatase 1 is associated with analogous regulatory subunits, but these have not yet been identified. The presence of two peaks of activity of protein phosphatase 1 when yeast extracts are chromatographed on DEAE-cellulose suggests that at least one of these forms is a complex of the \( GLC7 \) gene product and a regulatory subunit. It is possible that the first of the two peaks, which appears to be increased to a greater extent than the second peak after transformation (Fig. 2), represents the \( GLC7 \) gene product that is not complexed to a regulatory subunit. The increased activity of both peaks after transformation indicates that both contain the \( GLC7 \) gene product. Additional experiments are needed to identify the relationships between the two peaks of phosphatase activity and to determine the exact nature of the defect in the \( GLC7 \) gene. The phosphatase activity is altered in the \( glc7 \) mutant, but the precise nature of the \( glc7 \) mutation has not been determined. The \( GLC7 \) gene is required for cell viability, as shown in this study and recently by Clotet et al. (1991), yet the \( glc7 \) mutant strains grow almost as well as wild-type strains. The \( glc7 \) mutation may reduce the affinity of the catalytic subunit for a G-like subunit, specifically diminishing its role in regulating glycogen metabolism. Alternatively, the catalytic activity or expression level of the catalytic subunit may be reduced so as to affect glycogen metabolism but still be above a threshold for a deleterious effect on vital functions, including cell cycle regulation.

The nucleotide sequence of the \( GLC7 \) gene is virtually identical with that of the \( DIS2SI \) gene that was reported previously (Ohkura et al., 1989). The sole discrepancy in the coding region is at nucleotide 2209 in the \( GLC7 \) sequence, which is a G, whereas the \( DIS2SI \) sequence has a T (position 1343). These would be translated to glycine in the \( GLC7 \) sequence and to valine in the \( DIS2SI \) sequence. A glycine residue is present at this position in all other protein phosphatase 1 sequences reported (Sasaki et al., 1990; Cohen et al., 1990; Shenolikar and Nairn, 1991). The nucleotide sequence reported here also significantly extends the previously reported 5' and 3' sequences. The first 204 bases of the sequence contain part of a \( \Delta \) repeated element, which is also found at the ends of TY1-transposable elements (Boeke et al., 1988). This region is not required for \( GLC7 \) function, since the HindIII-XhoI fragment on pZF226 can complement the \( glc7 \) mutation (Fig. 3).

The complete sequences of many protein phosphatases have recently been derived from their cDNA sequences (Shenolikar and Nairn, 1991). Protein phosphatase 1 sequences from mammals, insects, and yeasts show a remarkable degree of conservation. Type 2A protein phosphatase sequences are also very highly conserved and show about 50% identity with type 1 sequences (Orgad et al., 1990; Cohen et al., 1990). Few type 2B sequences have been determined, and these show a significant but lower degree of similarity to the type 1 and 2A proteins. Several type 2C sequences have been determined, and these show no sequence homology with the other classes of protein phosphatases. Two genes encoding type 1 protein phosphatases have been isolated from the fission yeast \( S. pombe: DIS2 (= BW51) \) and \( SDS21 \) (Ohkura et al., 1989; Booher and Beach, 1989). There are also duplicate genes encoding type 2A phosphatases in fission yeast (Kinoshita et al., 1990). The type 1 and type 2A protein phosphatases have essential but distinct roles in mitosis in fission yeast (Kinoshita et al., 1990). In the budding yeast \( S. cerevisiae \), the protein phosphatase 2A gene \( SIT4 \) was first identified by its effect on transcription (Arndt et al., 1989). Two different protein phosphatase 2A genes, \( PPH21 \) and \( PPH22 \), were isolated from \( S. cerevisiae \) by hybridization with the homologous rabbit cDNA (Sneddon et al., 1990). A single gene encoding a type 1 protein phosphatase, \( DIS2SI \), was isolated from \( S. cerevisiae \) using the fission yeast gene as a hybridization probe (Ohkura et al., 1989).

Glycogen accumulation occurs in yeast cultures as they approach stationary phase (Rothman-Denes and Cabib, 1970; Lillie and Pringle, 1980). At the same time, the percentage of glycogen synthase in the dephosphorylated form and the total amount of glycogen synthase is increased (Rothman-Denes and Cabib, 1970; Peng et al., 1990). We have found that the \( GLC7 \) mRNA increases at the end of logarithmic growth, at the same time that glycogen synthase activity increases. An increase in protein phosphatase activity at this time may be responsible for the increase in the glycogen synthase activity. It is also possible that the expression of the glycogen synthase and protein phosphatase genes are coregulated by the same metabolic signals to coordinate glycogen metabolism.
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