Isolation of the GSY1 Gene Encoding Yeast Glycogen Synthase and Evidence for the Existence of a Second Gene*

Ilona Farkas$, Thomas A. Hardy§, Anna A. DePaoli-Roach¶, and Peter J. Roach
From the Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46223

Glycogen synthase preparations from Saccharomyces cerevisiae contained two polypeptides of molecular weights 85,000 and 77,000. Oligonucleotides based on protein sequence were utilized to clone a S. cerevisiae glycogen synthase gene, GSY1. The gene would encode a protein of 707 residues, molecular mass 85,601 daltons, with 50% overall identity to mammalian muscle glycogen synthases. The amino-terminal sequence obtained from the 85,000-dalton species matched the NH\textsubscript{2}-terminus predicted by the GSY1 gene. Disruption of the GSY1 gene resulted in a viable haploid with glycogen synthase activity, and purification of glycogen synthase from this mutant strain resulted in an enzyme that contained the 77,000-dalton polypeptide. Southern hybridization of genomic DNA using the GSY1 coding sequence as a probe revealed a second weakly hybridizing fragment, present also in the strain with the GSY1 gene disrupted. However, the sequences of several tryptic peptides derived from the 77,000-dalton polypeptide were identical or similar to the sequence predicted by the GSY1 gene. The data are explained if S. cerevisiae has two glycogen synthase genes encoding proteins with significant sequence similarity. The protein sequence predicted by the GSY1 gene lacks the extreme NH\textsubscript{2}-terminal phosphorylation sites of the mammalian enzymes. The COOH-terminal phosphorylated region of the mammalian enzyme overall displayed low identity to the yeast COOH terminus, but there was homology in the region of the mammalian phosphorylation sites 3 and 4. Three potential cyclic AMP-dependent protein kinase sites are located in this region of the yeast enzyme. The regions of glycogen synthase likely to be involved in covalent regulation are thus more variable than the catalytic center of the molecule.

Though protein phosphorylation is a common and widely distributed mechanism in nature for the regulation of protein function (1, 2), relatively little is known about the evolution of phosphorylation controls. The enzyme glycogen synthase (EC 2.4.1.11) is believed to regulate synthesis of the storage polysaccharide glycogen, and the mammalian glycogen synthase is an enzyme regulated by phosphorylation at multiple sites. The rabbit muscle enzyme contains at least nine sites/~85,000-dalton subunit that are phosphorylated in vivo and which, in vitro, make the enzyme substrate for 10 or so protein kinases (3, 4). The phosphorylation occurs in a hierarchal manner, in which phosphorylation at some sites is the prerequisite for modification of others (5, 6). The enzyme is therefore an interesting candidate for comparative studies of protein phosphorylation.

In Saccharomyces cerevisiae, glycogen is synthesized in response to growth limitation (7-10), and the activity of glycogen synthase increases when, for example, entry into stationary phase is imminent. Work by Cabib and colleagues (11-16) had established that the S. cerevisiae glycogen synthase shared several properties with its mammalian counterpart, including allosteric activation by glucose-6-P (11, 13) and reversible covalent phosphorylation (12, 15). The full details of the phosphorylation control were not worked out, and there was little structural information available for the yeast enzyme. There is biochemical evidence implicating cAMP-dependent protein kinase in the phosphorylation (17), and recent work on cyclic AMP-mediated signaling pathways in yeast also indicated that many mutants defective in the cyclic AMP pathway also have aberrant glycogen accumulation, supporting a role for cyclic AMP in the regulation of glycogen metabolism (18-21). There is also evidence for cyclic AMP-independent control of glycogen accumulation, however (19, 22). We report purification of glycogen synthase from S. cerevisiae, the cloning of a glycogen synthase gene, GSY1, and evidence for the existence of another gene encoding glycogen synthase.

**EXPERIMENTAL PROCEDURES**

Yeast Strains—Strain oe288c (a SUC2 mal mel gal2 CUP1) from the Yeast Genetic Stock Center was used for analysis of growth curves. For large-scale glycogen synthase preparations, we used strain EY-11C (a ciz' pep7 leu2) originating from Dr. E. Young, University of Washington, Seattle. YPH52 (a ura3-52 lys2-801 ade2-101 his3-D200 trpl-D1) originally from Dr. Phil Hieter, Johns Hopkins University, was used for gene disruption experiments.

Growth of Yeast—For initial correlations of growth with glycogen synthase activity, 500-ml cultures (YEFP medium (23)) were inoculated with cells from an overnight 50-ml culture and grown at 30 °C with shaking, and aliquots producing ~150 μl of pelleted cells were removed at various times for analysis. For enzyme purification, 12-liter cultures inoculated with 75 ml of overnight culture were grown at room temperature (20-24 °C) with stirring.

Purification of Glycogen Synthase—The cultures of S. cerevisiae were grown to early stationary phase, and the cells were harvested by centrifugation at 1700 × g. The cells were broken by shaking with glass beads (Bead-Buster) in homogenization buffer containing 50 mM Tris-Cl, 1 mM EDTA, pH 7.5, plus protease inhibitors (3 mM}

20879
dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM TLCK, 1 mM benzamidine, 0.25 μM/ml leupeptin, 0.5 μM/ml aprotinin. All buffers contained these protease inhibitors unless noted otherwise. The slurry was centrifuged for 20 min at 7500 x g, and the supernatant was treated with α-amylase (15 mg, Sigma Type II-A) for 30 min at 30 °C. Two-thirds of the volume of cold (4 °C) 45 mM Tris-Cl, 2 mM dithiothreitol, 5 mM EDTA, 2 mM EGTA, 10% glycerol, pH 7.5, were added before application to a column (10 x 4 cm) of DEAE cellulose equilibrated with the same buffer. The glycosynthase activity was eluted with a 0.0-0.5 M NaCl gradient in buffer containing 50 mM β-glycerol phosphate, 2 mM dithiothreitol, 5 mM EDTA, 2 mM EGTA, 10% glycerol, 0.25% (v/v) shellfish glycosyn (Sigma), pH 7.5. The active fractions were adjusted to 1 mM MnCl₂, 1 mM CaCl₂, and 1 μM pepstatin A (generated as described by the supplier) in 0.1% trifluoroacetic acid using an Applied Biosystems model 130A microbore HPLC apparatus. UV-absorbing or radioactive peaks were collected manually onto trifluoroacetic acid-treated GPC filter discs (Applied Biosystems) which were then submitted for sequence analysis.

Cloning of a S. cerevisiae Glycosynthase Gene—The protein sequence information was used to construct two degenerate oligonucleotide probes. The first, corresponding to the NH²-terminal sequence information was used to construct two degenerate oligonucleotides, one 26-mer (sequence TTACCA/GTAA/GACA/GAAA/C) designed from the sequence KGVN- and another 36-mer designed from searching a database maintained by Dr. Mike Snyder, Yale University School of Medicine. In some cases, the excised polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate-derivatives was performed using Applied Biosystems model 477A. The active fractions were adjusted to 1 mM MnCl₂, 1 mM CaCl₂, and 1 μM pepstatin A (generated as described by the supplier) in 0.1% trifluoroacetic acid using an Applied Biosystems model 130A microbore HPLC apparatus. UV-absorbing or radioactive peaks were collected manually onto trifluoroacetic acid-treated GPC filter discs (Applied Biosystems) which were then submitted for sequence analysis.

Cloning of a S. cerevisiae Glycosynthase Gene—The protein sequence information was used to construct two degenerate oligonucleotide probes. The first, corresponding to the NH²-terminal sequence information was used to construct two degenerate oligonucleotides, one 26-mer (sequence TTACCA/GTAA/GACA/GAAA/C) designed from the sequence KGVN- and another 36-mer designed from searching a database maintained by Dr. Mike Snyder, Yale University School of Medicine. The plasmid was digested with HindIII and BamHI to excise a segment bearing the 5° region of the gene. The fragment was inserted into the corresponding sites in the multiple cloning region of the pRS36 vector DNA. A separate digestion of the pTZ-GSY1 plasmid with HindIII and KpnI was generated and an insert corresponding to a segment of the GSY1 gene which was inserted into the corresponding sites in the modified pRS36 vector. The construct left a unique HindIII site in the plasmid for linearization and was in fact used for disruptions. However, this mutation could potentially allow for the NH²-terminal 300 residues of the protein to be expressed. Thus, the plasmid was digested with SpeI, which had a site 69 bp 5° to the start codon and another site still remaining in the multiple cloning region of the pRS36 vector. Religation generated a construct, pRS36-GSY1Δ1, which would lead ultimately to removal of all but the COOH-terminus 105 bases of the GSY1 coding region (Fig. 1; see also "Results").

Analysis of Yeast Transformants DNA—DNA was isolated (23) from YPH30 or YPH175 using the gta1 mutation, digested with the indicated restriction enzymes, and subjected to electrophoresis on 0.8% agarose. After transfer to nitrocellulose, the filter was hybridized with a 2.3-kb SpeI-NdeI fragment of the GSY1 gene. The fragment was labeled by random priming (U. S. Biochemical Corp. kit) using [α-32P]dCTP and hybridized at 60 °C in 6 X SSPE, 10 X Denhardt's, 0.1% SDS, 0.03% sodium pyrophosphate, and washed with 6 X SSC, 0.1% SDS, 0.05% sodium pyrophosphate at 60 °C, prior to autoradiography.

DNA Sequencing and Sequence Analysis—Nucleotide sequence was determined by the dideoxy chain termination method (29). Restriction fragments were subcloned into M13mp18 or M13mp19 for the production of single-stranded DNA. Both M13 universal primer (15-mer) and synthetic oligonucleotides based on the DNA sequence were used for the sequencing reactions. In a few instances, double-stranded sequencing was performed directly from pTZ vector. Synthetic oligonucleotides were made using an Applied Biosystems model 380B DNA synthesizer. Most manipulations and analyses of DNA and protein sequence information were performed using BIONET; data base searches used a variant of the FASTP algorithm of Pearson and Lipman (30). The alignment of the E. coli and yeast enzymes resulted from searching a data base maintained by Dr. Mark Goebel, Indiana University School of Medicine.

Other Methods—Glycosynthase activity was measured by the method of Thomas et al. (31). A unit of activity transfers 1 μmol of glucose from UDP-glucose to glycogen/min under conditions of the standard assay. The activity ratio, which decreases with an increasing phosphorylation state, is the ratio of activity in the absence of glucose-6-P to that measured in the presence of 0.7 mM sugar phosphate. Protein was quantitated by the method of Bradford (32) using bovine serum albumin as standard. Glycogen measurements utilized ethanol from guest on March 24, 2020http://www.jbc.org/Downloaded from

![Fig. 1. Disruption of the GSY1 gene. A partial restriction map of a 4.2-kb HindIII-I fragment containing the GSY1 gene is shown, with the coding sequence indicated by a filled box. Also shown is the gsy1Δ1 construct (see "Experimental Procedures") in which most of the coding sequence is replaced by pRS306 vector sequences, including the URA3 marker. The parent pRS306-gsy1Δ1 plasmid was linearized at the unique HindIII site (H). HindIII; Sp, SpeI; B, BamHI; K, KpnI.](http://www.jbc.org/)
Different stages of the purification were analyzed by SDS-PAGE and polyacrylamide gel electrophoresis. Enzyme fractions from different eluates were characterized:

- Eluate from DEAE-cellulose: Truck 4, eluate from concanavalin A column: Truck 5, purified enzyme after Mono-Q column.

The arrow indicates a species that contaminated the early glycogen synthase fractions eluting from the Mono-Q column and which was shown to correspond to the Coomassie Blue-stained material in parallel gels (data not shown).

Analysis of a tryptic peptide generated from the 167-kDa species gave the sequence YNHQYHPQDLPSSLDSVLSY which corresponds to residues 782-801 of yeast phosphorylase (38) (P. R. Graves, T. A. Hardy, and P. J. Roach, unpublished results).

**RESULTS**

**Purification of Yeast Glycogen Synthase**—Initial experiments to establish the relationship of glycogen synthase activity eluting from the Mono-Q column with the 85-kDa species showed that the 85-kDa species corresponded to the Coomassie Blue-stained material in parallel gels (data not shown).

In agreement with published reports (35), two major protein species, M, 80,000 and 77,000, whose presence correlated with enzyme activity eluting from the Mono-Q column, was shown to be enzymatically active in the formation of M, 100,000 (Fig. 2), more prominent in the fractions containing M, 100,000 than 80,000 and 77,000, whose presence correlated with enzyme activity eluting earlier from the final column. These two major protein species were found to be active in the formation of M, 100,000 (Fig. 2), more prominent in the fractions containing M, 100,000 than 80,000 and 77,000, whose presence correlated with enzyme activity eluting earlier from the final column.

The purified enzyme, a polypeptide corresponding to the 85-kDa polypeptide, was shown to be glycoamylase (Sigma). Free glucose was then determined with the 85-kDa species. The purified enzyme was shown to be glycoamylase (Sigma). Free glucose was then determined with the 85-kDa species.

**Yeast Glycogen Synthase Gene**—A 4.2-kb HindIII-HindIII fragment containing the coding sequence of the yeast glycogen synthase gene was isolated from a yeast genomic library (see "Experimental Procedures"). The library was constructed with the SalI site of the EMBL3A vector. The 4.2-kb HindIII-HindIII fragment containing the coding sequence was subcloned, and individual sequencing reactions were performed using oligonucleotide primers based on previous sequencing; dotted lines, double-stranded DNA. The sequences are indicated by arrows (dashed lines), and sequencing reactions were performed using oligonucleotide primers. A 4.2-kb HindIII-HindIII fragment containing the coding sequence was isolated from a yeast genomic library (see "Experimental Procedures").

**Initial Protein Sequence Analysis**—Cloning of a S. cerevisiae glycogen synthase cDNA fragment (see "Experimental Procedures") formed the basis for homologous recombination experiments. The protein sequence information was used to design oligonucleotide probes to screen a yeast genomic library (see "Experimental Procedures"). One clone, containing a 17-kb insert, was isolated.

**Disruption of the GSY1 Gene**—The purified enzyme, a polypeptide corresponding to the 85-kDa polypeptide, was shown to be glycoamylase (Sigma). Free glucose was then determined with the 85-kDa species.

**Initial Protein Sequence Analysis**—Cloning of a S. cerevisiae glycogen synthase cDNA fragment (see "Experimental Procedures") formed the basis for homologous recombination experiments. The protein sequence information was used to design oligonucleotide probes to screen a yeast genomic library (see "Experimental Procedures"). One clone, containing a 17-kb insert, was isolated.

**Disruption of the GSY1 Gene**—The purified enzyme, a polypeptide corresponding to the 85-kDa polypeptide, was shown to be glycoamylase (Sigma). Free glucose was then determined with the 85-kDa species.
Yeast Glycogen Synthase Gene

The nucleotide sequence of the GSY1 gene is shown together with the deduced amino acid sequence of S. cerevisiae glycogen synthase. Underlines indicate sequences of amino acids obtained from analysis of protein or peptides during the course of this work (see text). The nucleotide sequence corresponding to the oligonucleotide probe is marked by a bar. A double underline marks two near-consensus polyadenylation signals in the 3'-untranslated region.

(44). The plasmid was linearized by digestion with HindIII (Fig. 1). The resulting DNA contains 5' and 3' segments of the GSY1 gene but with almost all of the intervening coding region replaced by pRS306 sequences, including the URA3 gene. Using the LiAc method (45), we transformed haploid (YPH52) cells with the linearized pRS306-GSY1 DNA, selecting for Ura+ transformants. To confirm that the desired replacement had occurred in the recombinants, DNA from wild-type and mutant strains (Fig. 1) was analyzed by Southern hybridization using a SpeI-Ndel fragment, corresponding to the coding region of the GSY1 gene, as a probe (Fig. 6). The DNA from wild-type cells (Fig. 6, track 1) contained the 4.2-kb HindIII-HindIII fragment predicted by analysis of the GSY1 gene. In the haploid mutant, this fragment was absent (Fig. 6, track 2). Since viable haploids were obtained, the coding region of GSY1 can be isolated, the DNA from wild-type cells (Fig. 6, track 1) contained the 4.2-kb HindIII-HindIII fragment predicted by analysis of the GSY1 gene.
Yeast Glycogen Synthase Gene

3) cells. The presence of hybridizing fragments not due to GSY1 could be detected in cell extracts. Thus, a large-scale culture was grown and subjected to the glycogen synthase purification scheme developed for the wild type (see "Experimental Procedures"). Glycogen synthase activity followed through the purification essentially as had been observed using wild-type yeast (Table I). Recoveries at most steps were comparable to the wild type, except that the yield on the Mono-Q column was somewhat lower for unknown reasons. The overall purification, 1200-fold, was similar to that for wild type. Analysis of the final product by SDS-PAGE revealed a predominant 77-kDa polypeptide species, but the 85-kDa species was absent (Fig. 7). The presence of the 85-kDa species thus correlated with the presence of an intact GSY1 gene.

Protein Sequence Analysis of 77-kDa Polypeptide—We also attempted to explore the relationship between the 77- and 85-kDa polypeptides by analysis of the proteins. In one set of experiments, the 77- and 85-kDa polypeptides were transferred to nitrocellulose paper and subjected separately to trypsin digestion. Analysis of the digests by reverse-phase microbore HPLC indicated similarity but not total identity in the profiles (not shown). Peptides that appeared to be good candidates for protein sequence analysis were collected directly onto sample filters. Three tryptic peptides from the 77-kDa species were found to have sequences consistent with the sequence predicted by the GSY1 gene, and the 77-kDa species was not achieved with various chromatographic methods used or with nondenaturing gel electrophoresis. One peptide derived from the 77-kDa polypeptide had the same N-terminus sequence, S-N-X-T-V-Y-M-X-P-, which could be matched with the GSY1 protein sequence with only 71 identities. Both the 77- and 85-kDa polypeptides were modified to similar extents. Analysis of phospho- aminos acids by partial acid hydrolysis of enzyme phosphorylated by yeast cyclic AMP-dependent protein kinase indicated that only serine residues were phosphorylated (not shown). Enzyme phosphorylated to 0.25 and 0.35 mol of phosphate/mol of polypeptides (in the 85- and 77-kDa species, respectively) was subjected to SDS-PAGE, transferred to polyvinylidene difluoride paper, digested with trypsin, and analyzed by microbore HPLC as described above. Two phosphopeptides from the 77-kDa species, accounting for some 50% of the applied radioactivity, were successfully analyzed. Both peptides had the same NH2-terminal sequences, S-N-X-T-V-Y-D-L-D-, which matched the GSY1 protein sequence with only 71 identities. We were unsuccessful in obtaining a sequence from the wild-type strain of yeast (see "Experimental Procedures") cyclic AMP-dependent protein kinase (data not shown). Both the 77- and 85-kDa polypeptides were modified to similar extents. Analysis of phospho-aminos acids by partial acid hydrolysis of enzyme phosphorylated by yeast cyclic AMP-dependent protein kinase indicated that only serine residues were phosphorylated (not shown). Enzyme phosphorylated to 0.25 and 0.35 mol of phosphate/mol of polypeptides (in the 85- and 77-kDa species, respectively) was subjected to SDS-PAGE, transferred to polyvinylidene difluoride paper, digested with trypsin, and analyzed by microbore HPLC as described above. Two phosphopeptides from the 77-kDa species, accounting for some 50% of the applied radioactivity, were successfully analyzed. Both peptides had the same NH2-terminal sequences, S-N-X-T-V-Y-D-L-D-, which matched the GSY1 protein sequence with only 71 identities.
Yeast Glycogen Synthase Gene

TABLE I

| Step                      | Activity | Protein | Specific activity | Recovery | Purification |
|---------------------------|----------|---------|------------------|----------|--------------|
|                           | units    | mg      | units/mg         | %        | -fold        |
| Wild type                 |          |         |                  |          |              |
| Homogenate                | 223      | 17,154  | 0.013            | 100      | 1            |
| Low speed supernatant     | 218      | 12,111  | 0.018            | 98       | 1.4          |
| DEAE eluate               | 150      | 879     | 0.171            | 67       | 13.2         |
| Concanavalin A eluate     | 26       | 5.1     | 5.1              | 2.1      | 220           |
| Mono-Q eluate             | 46       | 3.5     | 13.1             | 21       | 1,008        |

*Activity was measured in the presence of glucose-6-P.*

1 2 3 4 5

FIG. 7. Analysis of glycogen synthase purified from the gsy1Δ mutant strain. Enzyme fractions from different stages of the purification were analyzed by SDS-PAGE and stained with Coomassie Blue. The two-digit number indicates the molecular mass in kilodaltons. Track 1, low speed supernatant; tracks 2 and 3, eluate from concanavalin A column; tracks 4 and 5, purified enzyme from Mono-Q column.

GSY1 gene. Together with the fact that Southern analysis of yeast genomic DNA revealed a hybridizing fragment not predicted for the GSY1 gene, the data can best be explained if there are two glycogen synthase genes that code for proteins with significant primary structural similarity. This idea would also be consistent with the fact that, although numerous yeast mutants with aberrant glycogen accumulation characteristics have been identified, none of the mutations analyzed has been in a glycogen synthase gene. The chances of isolating glycogen synthase mutants are obviously much reduced if there are multiple copies of the gene. Our hypothesis is that the gene we have cloned, GSY1, would code for the 85-kDa polypeptides while a second gene would code for the 77-kDa polypeptides which may additionally undergo posttranslational modification that blocks the NH₂ terminus. Whether there is a physiological rationale for the existence of two genes will have to await further work.

Amino Acid Sequence of Yeast Glycogen Synthase—The predicted mass of the yeast glycogen synthase coded by GSY1 is 80,501 daltons with a calculated pI of 5.6. The overall acidic character of the protein is similar to that found for mammalian glycogen synthases (40, 41, 46) and reflects a high proportion of Glu and Asp residues (13% in rabbit muscle and 11% in yeast). As observed with the mammalian enzymes, the COOH terminus carries a net negative charge, though aspartic acid predominates over glutamic acid in the yeast protein. The S. cerevisiae glycogen synthase sequence is 50% identical to that of the mammalian muscle enzymes and 47% identical to that of rat liver. Searches of protein sequence data bases did not reveal strong similarities between the yeast glycogen synthase and other proteins. An alignment of yeast glycogen synthase with the NH₂ terminus of E. coli glycogen synthase (47) can be made: 19.2% identity to residues 1-313 of the E. coli enzyme and 31.6% allowing conservative substitutions. This level of similarity is not in itself remarkable except that it includes residues potentially involved in substrate binding. Lys-38 of rabbit muscle glycogen synthase has been implicated in UDP-glucose binding (48, 49) and is in the local sequence -K-V-G-G-. The yeast enzyme has an arginine (Arg-119) in this position with sequence -R-V-G-G-. Lys-15 of the E. coli enzyme was recently reported to be involved in binding its substrate ADP-glucose (50). The local sequence is -K-T-G-G, and Furukawa et al. (50) have suggested that the motif -R/K-X-G-G might be a feature of nucleoside sugar binding sites. Interestingly, the E. coli enzyme also contains a repeat including this motif toward its COOH terminus (-R-T-G-G-L-A-D, residues 378-383); perhaps this is part of another ligand binding site.

Functional Domains of Glycogen Synthase—Definition of the phosphorylation sites in yeast glycogen synthase will require much more work. Our own initial efforts are complicated by the fact that we were successful in obtaining sequence information only from phosphopeptides derived from the 77-kDa polypeptide which we do not think is coded by GSY1. However, the sequence obtained matched a COOH-terminal region of the predicted GSY1 product, starting at Ser-659, a residue preceded by an arginine. Assuming, as seems reason-
able, that the 77-kDa protein is similar in structure to that encoded by GSY1, we would hypothesize that the COOH terminus of the protein is involved in phosphorylation by cyclic AMP-dependent protein kinase. Taking the sequence R/K-X-X-S-S (51) as a minimal recognition motif for cyclic AMP-dependent protein kinase, one can identify six candidate sites in the protein sequence predicted by the GSY1 gene, Ser-158, Ser-362, Ser-559, Ser-650, Ser-659, and Ser-661. Three of these, Ser-650, Ser-659, and Ser-661, have additional NH2-terminal basic residues as are frequently found in sites for this protein kinase (Table II). This is especially true for Ser-659, which aligns with mammalian site 3a and which has a disposition of basic residues very similar to the sites in the yeast ADR1 protein and in the β-subunit of mammalian phosphorylase kinase. Thus, we view Ser-650, Ser-659, and Ser-661 as the most promising candidates for phosphorylation by cyclic AMP-dependent protein kinase.

The similarity between yeast and mammalian glycogen synthases was not uniformly distributed throughout the molecule, and alignment of the sequences allowed the definition of several regions within the molecule (Fig. 5). The extreme NH2-terminal phosphorylated region of the mammalian enzyme is missing in yeast. There are three highly conserved central regions, separated by segments of no identity and insertions in the yeast sequence. Another insertion in the yeast enzyme separates the COOH-terminal segment. The COOH-terminal regions are not similar except in a limited but perhaps important segment around phosphorylation sites 3 and 4 of the mammalian enzyme. Our current view is that the NH2 and COOH termini are involved in covalent control and the central segments are responsible for catalysis and interaction with ligands such as the substrates UDP-glucose and glycogen, and allosteric regulators glucose-6-P and adenine nucleotides.

It is interesting that yeast, muscle, and liver glycogen synthases differ most in sequences at the NH2- and COOH-terminal segments of the molecule, precisely those regions that are likely to be involved in covalent controls. Thus, these segments of the molecule have been subject to different evolutionary constraints than the central conserved portion in which the maintenance of structural and functional integrity must have imposed stricter limitations. In comparing the nearly identical human and rabbit muscle glycogen synthases (97% amino acid identity), the few differences are also concentrated in the NH2- and COOH-terminal sections (40, 41). The COOH-terminal phosphorylated region of yeast glycogen synthase contains multiple sites in the mammalian enzymes. The yeast enzyme lacks sites corresponding to sites 1a and 1b of the mammalian muscle isozyme, as does the mammalian liver isozyme (46, 55, 56). These sites, therefore, must represent a more recent specialization among the mammalian isozymes. Also interesting is the region corresponding to the mammalian sites 3a, 3b, 3c, 4, and 5. This segment, at least around sites 3 and 4, shows conservation between yeast and mammals (Fig. 5) and contains a possible cyclic AMP-dependent protein kinase site in yeast. It is interesting that sites 3a and 4 are slowly phosphorylated by cyclic AMP-dependent protein kinase in rabbit muscle glycogen synthases (57) and that site 3a, together with the preceding Ser, is a site for the same protein kinase in the rabbit liver enzyme (55). Nonetheless, it is most generally believed that in vivo phosphorylation of sites 3a and 4 in mammalian glycogen synthase is catalyzed not by cyclic AMP-dependent protein kinase but by a different enzyme, glycogen synthase kinase 3 (3). It is not yet known if yeast contains an enzyme equivalent to glycogen synthase kinase 3. It will thus be important to define better the mechanisms of phosphorylation of yeast glycogen synthase.

Acknowledgments—We are grateful to Dr. Mark Goebl for his advice during the course of this project and to Cheryl Corbett for synthesizing oligonucleotides.

REFERENCES
1. Hunter, T. (1987) Cell 50, 823-829
2. Cohen, P. (1982) Nature 296, 613-620
3. Cohen, P. (1986) in The Enzymes (Boveri, P. D., and Krebs, E. G., eds) Vol. 17A, pp. 461-497, Academic Press, Orlando, FL
4. Poulter, L., Ang, S.-C., Gibson, B. W., Williams, D. H., Holmes, C. F. B., Caubet, P.-B., Dirich, J., and Cohen, P. (1988) Eur. J. Biochem. 175, 497-510
5. Fiol, C. J., Malureishda, A. M., Weng, Y., Roerske, R. W., and Roach, P. J. (1987) J. Biol. Chem. 262, 14042-14048
6. Flotow, H., and Roach, P. J. (1989) J. Biol. Chem. 264, 9126-9128
7. Lillie, S. H., and Pringle, J. R. (1980) J. Bacteriol. 143, 1384-1394
8. Rothman, L. B., and Cabib, E. (1969) Biochemistry 8, 3332-3341
9. Trevelyan, W. E., and Harrison, J. S. (1966) Biochem. J. 62, 177-183
10. Trevelyan, W. E., and Harrison, J. S. (1966) Biochem. J. 63, 26-33
11. Algranati, I. D., and Cabib, E. (1962) J. Biol. Chem. 237, 1007-1013
12. Huang, K.-P., and Cabib, E. (1974) J. Biol. Chem. 249, 3851-3857
13. Rothman, L. B., and Cabib, E. (1967) Biochemistry 6, 2098-2106
14. Rothman, L. B., and Cabib, E. (1967) Biochemistry 6, 2107-2112
15. Rothman-Denes, L. B., and Cabib, E. (1970) Proc. Natl. Acad. Sci. U. S. A. 66, 967-974
16. Rothman-Denes, L. B., and Cabib, E. (1971) Biochemistry 10, 1236-1242
17. Wingender-Drissen, R. (1983) FEBS Lett. 168, 46-52
18. Brok, D., Samiy, N., Fasano, O., Fujiyama, A., Tamanof, E., and Northrup, J., and Wigler, M. (1985) Cell 41, 763-769
19. Cameron, S., Levin, L., Zoller, M., and Wigler, M. (1988) Cell 59, 555-566
20. Cannon, J. F., and Tatchell, K. (1987) Mol. Cell. Biol. 7, 2653-2663
21. Tatchell, K., Robinson, L. C., and Breitenbach, M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3755-3759
22. François, J., and Hér, G. (1988) Eur. J. Biochem. 174, 561-567
23. Sherman, F., Fink, G. R., and Hicks, J. B. (1986) Methods in Yeast Genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY
24. DePaoli-Roach, A. A., Roach, P. J., and Larner, J. (1979) J. Biol. Chem. 254, 12062-12068
25. Matsudaira, P. (1987) J. Biol. Chem. 262, 10035-10038
26. Pesole, G., Attimonelli, M., and Liuni, S. (1988) Nucleic Acids Res. 16, 1719-1728
27. Snyder, M., Elledge, S., and Davis, R. W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 730-734
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467
29. Pearson, W. R., and Lipman, D. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2444-2448
30. Thomas, J. A., Schleider, K. K., and Larner, J. (1968) Anal. Biochem. 25, 485-495
31. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
32. Bergmeyer, H. U., Bernt, E., Schmidt, F., and Stork, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) 2nd ed., pp. 1196-1201, Academic Press, New York
33. Camus, M., Ahmad, Z., DePaoli-Roach, A. A., and Roach, P. J. (1986) J. Biol. Chem. 261, 2465-2473
34. Algranati, I. D., and Cabib, E. (1962) J. Biol. Chem. 237, 1007-1013
35. DePaoli-Roach, A. A., Ahmad, Z., Camici, M., Lawrence, J. C., Jr, and Roach, P. J. (1983) J. Biol. Chem. 258, 10702-10709
36. Laemmli, U. K. (1970) Nature 227, 680-685
Yeast Glycogen Synthase Gene

37. Francois, J., Villaneuva, M. E., and Hers, H.-G. (1988) Eur. J. Biochem. 174, 551–559
38. Hwang, P. K., and Fletterick, R. J. (1986) Nature 324, 80–84
39. Takeda, Y., Brewer, H. B., Jr., and Larner, J. (1975) J. Biol. Chem. 250, 8043–8050
40. Browner, M. F., Nakano, K., Bang, A. G., and Fletterick, R. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1443–1447
41. Zhang, W., Browner, M. F., Fletterick, R. J., DePaoli-Roach, A. A., and Roach, P. J. (1989) FASEB J. 3, 2532–2536
42. Kozak, M. (1984) Nucleic Acids Res. 12, 857–872
43. Bennetzen, J. L., and Hall, B. D. (1992) J. Biol. Chem. 257, 3026–3031
44. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–211
45. Ito, H., Jukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
46. Bai, G., Zhang, Z., Werner, R., Nuttall, F. Q., Tan, A. W. H., and Lee, E. Y. C. (1990) J. Biol. Chem. 265, 7843–7848
47. Kumar, A., Larsen, C. E., and Preiss, J. (1986) J. Biol. Chem. 261, 16256–16259
48. Mahrenholz, A. M., Wang, Y., and Roach, P. J. (1988) J. Biol. Chem. 263, 10561–10567
49. Tagaya, M., Nakano, K., and Fukui, T. (1985) J. Biol. Chem. 260, 6670–6678
50. Furukawa, K., Tagaya, M., Inouye, M., Preiss, J., and Fukui, T. (1990) J. Biol. Chem. 265, 2086–2090
51. Edelman, A. M., Blumenthal, D. K., and Krebs, E. G. (1987) Annu. Rev. Biochem. 56, 567–613
52. Rittenhouse, J., Harrsch, P. B., Kim, J. N., and Marcus, F. (1986) J. Biol. Chem. 261, 3939–3943
53. Cherry, J. R., Johnson, T. R., Dollard, C., Shuster, J. R., and Denis, C. L. (1982) Cell 56, 409–419
54. Kilimann, M. W., Zander, N. F., Kuhn, C. C., Crabb, J. W., Meyer, H. E., and Heilmeyer, L. M. G., Jr. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9381–9385
55. Wang, Y., Bell, A. W., Hermodson, M. A., and Roach, P. J. (1986) J. Biol. Chem. 261, 16909–16915
56. Wang, Y., Camici, M., Lee, F.-T., DePaoli-Roach, A. A., and Roach, P. J. (1986) Biochim. Biophys. Acta 888, 225–236
57. Sheorain, V. S., Corbin, J. D., and Soderling, T. R. (1985) J. Biol. Chem. 260, 1567–1572
Isolation of the GSY1 gene encoding yeast glycogen synthase and evidence for the existence of a second gene.
I. Farkas, T. A. Hardy, A. A. DePaoli-Roach and P. J. Roach

J. Biol. Chem. 1990, 265:20879-20886.

Access the most updated version of this article at http://www.jbc.org/content/265/34/20879

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/265/34/20879.full.html#ref-list-1