Multiple Positive and Negative Elements Involved in the Regulation of Expression of GSY1 in *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* is exposed to a wide variety of environmental stressors, such as growth into stationary phase, heat shock, and osmotic shock. Cells respond to these stressors by modifying many metabolic processes, presumably making cells more resistant to these stressors. Among these changes are increased activities of the glycogen metabolic enzymes and the deposition of glycogen up to 23% of the dry weight of the cell (see below).

Glycogen metabolism is highly conserved from yeast to mammals. The regulation of glycogen metabolism in *S. cerevisiae* closely parallels the more extensively studied counterparts in mammals (1, 2). Regulation is mediated primarily by effects on the activities of glycogen synthase and glycogen phosphorylase. These enzymes are regulated, in part, by protein phosphorylation. Cyclic AMP appears to play a central role in the regulation of these enzymes in *S. cerevisiae*, as it does in mammals, although the precise mechanisms remain to be identified. The parallels between glycogen metabolism in *S. cerevisiae* and mammals extends to the level of protein sequence. Glycogen synthase and glycogen phosphorylase from this yeast and mammals are 50 and 49% identical, respectively (3–6).

Glycogen metabolism is also regulated at the level of gene expression in *S. cerevisiae*. The protein levels of the enzymes involved in glycogen metabolism increase in parallel with glycogen accumulation as cells approach stationary phase or when nutrients are depleted (7, 8). This increase in the level of glycogen metabolic enzyme activity appears to result, in part, from the regulation at the level of transcription. Northern blot analysis has shown that the levels of mRNA expressed from *GPH1* (encoding glycogen phosphorylase), *GLC3* (glycogen branching enzyme), *GAC1* (glycogen-binding subunit of protein phosphatase 1), and *GSY2* (glycogen synthase) increase as cells progress from log phase to stationary phase (9–12). The simultaneous increases in mRNA levels of the proteins involved in glycogen metabolism suggest that the expression of these genes may be coordinately regulated.

Our long term goal is to understand how glycogen metabolism is regulated at the level of gene expression and how these regulatory processes are coordinated with post-translational control of the glycogen metabolic enzymes. The first step toward this goal is to characterize the promoters for these genes. In this paper, we report a characterization of the promoter of *GSY1* (glycogen synthase). This is a surprisingly complex promoter that allows transcription to respond to a wide variety of cellular stressors.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction enzymes, other DNA modification enzymes, and oligonucleotides linkers were purchased from Invitrogen or New England Biolabs. Radiochemicals were obtained from PerkinElmer Life Sciences and Amersham. AmpliTaq polymerase for PCR from PerkinElmer Life Sciences, and sequencing reagents from U. S. Biochemical Corp. Oligonucleotide primers were products of National BioSciences, Inc. or Invitrogen. The frozen E-Z transformation kit was purchased from Zymo Research, Orange, CA. All other reagents were of the purest grades available.

**Isolation of the GSY1 Clone—GSY1**, the gene encoding yeast glycogen synthase, was isolated as described by Meinke in 1993 (13). Briefly, glycogen synthase was purified from strain S288C cells grown on YPD medium. The purified protein was reduced, carboxymethylated, and digested with trypsin. Two peptides were purified by reverse phase high performance liquid chromatography, their sequences were determined, and oligonucleotide probes were prepared. *GSY1* was isolated following screening of a yeast genomic library in a lambda (Stratagene Cloning Systems) by plaque hybridization. The identities of the clones were confirmed by nucleotide sequence analysis and they were recloned as *SalI* fragments into plasmid vector pSEY18 (14).

**Yeast Methods**—Yeast strains were manipulated by standard methods unless indicated otherwise (15). Transformation of linear DNA fragments was done by either the lithium acetate transformation method (16) or using the E-Z yeast transformation kit supplied by Zymo Research, Inc. Northern analysis was performed by standard procedures (17). Constructs were transferred to the CAN1 locus of the chromosome.
The sequence of oligonucleotides are shown 5' to 3' on the coding sequence of GSY1 upstream. The mutated bases are in lowercase. The sequence of the unique restriction site that is created by the mutagenesis is shown in bold. At the end of the sequence, the name of the newly created restriction site is given in parentheses.

| Oligonucleotide | Purpose          |
|-----------------|------------------|
| CGGAATTCATTCTCAAGCACATTTCCCG (XbaI) | Mutates STRE-1   |
| CCCACAAGTAAAGGGAGATTATTTCAG (SalI) | Mutates STRE-2   |
| GAAAGCGAAGTCCTAGCTTGTC (XbaI) | Mutates ROX1     |
| GTCTCTGGACACGCAAGATATAC (SalI) | Mutates MIG1     |
| CCGCAGCGGTACAGAGCAGTTAGCTGTC (Neill) | Mutates N1     |
| GGGGATGTGCTGCAAGGCG (LacZ primer) | Primer extension |
| CGCACAATTTCCCG (XbaI) | N1m2     |

FIG. 1. Induction of GSY1 expression in stationary phase. Strain YUL5 (GSY1::lacZ) was grown in YPD media in a gyratory incubator at 30 °C. Growth was monitored by light scattering at 600 nm. Cells were collected at different points along the growth curve and β-galactosidase activity was determined as described under “Experimental Procedures.”

unless otherwise noted. Transfer was accomplished using the integrating plasmid pRL95 (12, 18). This vector includes two fragments of the CAN1 gene. Digestion with a restriction enzyme that cuts between these segments produces a linear fragment that will integrate into the CAN1 locus, replacing the resident sequences and producing a stable, single-copy integrant. Integrants were selected as uracil prototrophs (because of the URA3 gene carried by the vector) and then confirmed by resistance to canavanine plates and sensitivity to 5-fluoroorotic acid.

Culture Conditions—Yeast cells were grown either in YPD (2% glucose, 2% peptone, and 1% yeast extract) or in SD media (2% glucose, 0.5% ammonium sulfate, and 0.17% yeast nitrogen base minus the amino acids) supplemented with the appropriate nutritional requirements (15). 5-Fluoroorotic acid plates were prepared as described (19). SD + CAN plates contained SD supplemented with canavanine at a concentration of 60 μg/ml (20).

GSY1 expression was routinely induced by growth into early stationary phase. Growth was monitored by light scattering at 600 nm. Cultures were grown at 30 °C and samples were taken at early log phase (A₆₀₀ between 0.05 and 0.15) or in early stationary phase (14–18 h later).

For heat shock experiments, 250-ml cultures were grown at 21 °C to an A₆₀₀ of 0.1 to 0.2. Then 100 ml was transferred into each of the two flasks. One flask was shaken at 21 °C and the other at 37 °C. The remainder was harvested. The control and the heat-shocked samples were collected 1 h later. Induction was assayed by measuring β-galactosidase activity (see below).

Construction of the GSY1::lacZ Fusion—The coding sequence of GSY1 was digested with BstBI (which cleaves at base +31 relative to translational start); the ends were filled with Klenow fragment of DNA polymerase I and BamHI linkers were attached. The EcoRI-BamHI fragment, which includes the 5' end of GSY1, was inserted into plasmid YCP50 (21). A lacZ gene fusion cassette, derived from pMC1871 (22) was then inserted into the BamHI site. The resulting plasmid carries a potential cis-elements was performed with the Clontech Transformer

FIG. 2. GSY1 transcription start sites and mutation of the TATA element. A, primer extension analysis was performed to identify the transcriptional start sites. Lanes 1 and 2 are primer extension products from stationary phase and log phase cultures of cells transformed with the multicopy plasmid overexpressing a GSY1::lacZ fusion, respectively. Lane 3 is primer extension product from a stationary phase culture of the strain containing the control empty vector. One major transcription start site (indicated by two stars) and multiple minor transcription start sites (the most prominent minor sites are indicated by a single star) were identified. B, a TATA element is required for the optimal expression of the GSY1 gene. GSY1::lacZ fusion genes were prepared that carried wild-type and mutant TATA elements (see text). The constructs were introduced into the CAN1 locus of strain YRL40. Cells were grown to stationary phase and assayed for β-galactosidase activity. The error bars for the TATA mutant were too low to be seen on the graph.

GSY1::lacZ fusion gene that includes the first 31 bp of the GSY1 structural gene fused in-frame with the lacZ gene and has 1700 bp of GSY1 upstream sequences. This fusion was subcloned into the integration vector, pRL95, to give pUL5.

Construction of Deletions and Point Mutations—Plasmid pUL5 was used for constructing deletions or mutations in GSY1. Deletions were made using available restriction sites. Site-directed mutagenesis of the potential cis-elements was performed with the Clontech Transformer
Fig. 3. Stress response elements and the MSN2 and MSN4 genes are involved in the induction of GSY1. The consensus sequence for stress response element and two STRE-like sequences (designated as STRE-1 and STRE-2) in the GSY1 promoter are shown. STRE-1 and STRE-2 were mutated in a GSY1::lacZ construct as described under "Experimental Procedures." These constructs were tested in a wild-type or the congenic msn2 msn4 deletion strain. Plus signs indicate the presence and minus signs indicate the absence of the respective genes or intact binding sites. Cells were collected in the log phase over a period of 3 h and activity was measured in early log phase cultures and in stationary phase cultures and this allowed rapid screening for the mutants, which were then verified by sequencing with Sequenase kit (U. S. Biochemical Corp.). The products were analyzed on an 8% polyacrylamide-urea gel. The start sites of GSY1 were determined by comparison with a DNA-sequencing ladder that was run alongside the products of primer extension. The sequencing reactions were carried out using the same primer as that for primer extension.

Mutagenesis Kit. Escherichia coli strain BMH17–81 was used for the initial amplification after mutagenesis. A 1.7-kb GSY1 upstream sequence from pUL5 was subcloned into a pT3/T7α-18 vector. The strategy employed for mutagenesis resulted in the conversion of the putative cis-element to a unique restriction site (absent in the original vector) and this allowed rapid screening for the mutants, which were then verified by sequencing with Sequenase kit (U. S. Biochemical Corp.).

For generating the STRE-1 sequence, the XbaI–NheI fragment was ligated into restriction sites either 34 bp downstream of the HAP 2/3/4 site (354 bp) were subcloned upstream of a basal CYC1 promoter and activity was measured in early log phase cultures and asayed for β-galactosidase activity. The expected mutated element is indicated by a star.

β-Galactosidase Assay—Yeast cell samples were collected by rapid filtration and quick-frozen on dry ice. To carry out the β-galactosidase assay, the pellet was thawed and resuspended in Z-buffer (100 mM sodium phosphate, pH 7.5, 10 mM KC1, 1 mM MgSO4, and 50 mM β-mercaptoethanol) and 1 mM phenylmethylsulfonyl fluoride. Cells were disrupted by vortexing in the presence of glass beads and the protein extract was obtained by centrifugation. The extract was then incubated at 30 °C with o-nitrophenyl-β-galactopyranoside and absorbance was measured at 420 nm (25). Protein concentration was determined by the method of Lowry et al. (26), using bovine serum albumin as the standard.

β-Galactosidase activities are normalized for the protein concentration in the cell extract. Specific activities reported are the average of three or more independent experiments. At least two independent isolates were used for each construct. In all cases, S.E. ± mean was below 15% of the value shown.

Mobility Shift Assay—Yeast strain YRL40 was grown in 100 ml of YPD medium to an A590 of 1.0. Cells were harvested by centrifugation, washed in extraction buffer (0.2 mM Tris-Cl, pH 8.0, 400 mM ammonium sulfate, 10 mM MgCl2, 1 mM EDTA, 10% glycerol, 7 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride, and 1 µg/ml leupeptin), and then resuspended in 200 µl of extraction buffer. Samples were transferred to 1.5-ml Eppendorf tubes containing 75 µl of glass beads and frozen in a dry ice/ethanol bath. After thawing the tubes, they were vortexed in the cold room for 20 min. 100 µl of extraction buffer was added and the samples were centrifuged to remove glass beads and larger cell debris. The supernatant was isolated and clarified by centrifugation at 14,000 rpm for 1 h at 4 °C. Protein was precipitated by adding ammonium sulfate to 70%. The precipitate was collected by centrifugation and resuspended in 300 µl of 10 mM Heps, pH 8, 5 mM EDTA, 7 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and 1 µg/ml leupeptin), and then resuspended in 200 µl of extraction buffer. Samples were dialyzed twice against 500 ml of the same buffer for 2 h each. The dialyzed extracts were centrifuged, and supernatants were aliquoted and stored at −70 °C.

The double stranded N1 oligonucleotide was end-labeled using T4-poly(nucleotide) kinase and [γ-32P]ATP. The sample was then passed through a G-25 column to remove excess labeled ATP. The binding reactions were carried out in a final volume of 20 µl containing 2 µl of 10× DNA binding buffer (0.2 mM Heps, pH 7.6, 1% Nonidet P-40, and 0.5 mM KC1), 10 µl β-mercaptoethanol, 5 µl of 80% glycerol, and 0.1 µg of poly(dI-dC). A typical reaction contained 120,000 cpm (0.8-9 nmo l or 0.2 ng) of end-labeled probe and 30 µg of yeast extract. Following incubation at room temperature for 30 min, the samples were electrophoresed on a 5% non-denaturing polyacrylamide gel containing 0.25× TBE (Tris borate/EDTA) and 5% glycerol, for 3.5 h at 4 °C. Competition experiments were performed using unlabeled N1, unlabeled N1m2 (a version

Fig. 4. Induction of GSY1 in response to heat shock. Strains expressing GSY1::lacZ were grown at 21 °C to early log phase (A590 < 0.2). Cultures were then split and either returned to 21 °C or were shifted to 37 °C. Growth was continued for 1 h, cells were collected from control and heat-shocked cultures and assayed for β-galactosidase activity. The mutated element is indicated by a star. 

EDTA, 5'-end labeled probe (7 × 106 cpm) and the final reaction volume was adjusted to 25 µl with water. This sample was heated to 90 °C, cooled to 50 °C and 25 µl of 2X RT mixture (0.1 µl Tris-HCl, pH 8.2, 12 mM MgCl2, 20 mM dithiothreitol, and 1 mM each dNTP) and 20 units of avian myeloblastosis virus reverse transcriptase were added to the mixture. The sample was then allowed to incubate at 42 °C for 90 min. The products were analyzed on an 8% polyacrylamide-urea gel. The start sites of GSY1 were determined by comparison with a DNA-sequencing ladder that was run alongside the products of primer extension. The sequencing reactions were carried out using the same primer as that for primer extension.
MSN2 and MSN4 Genes

In previous studies, we found that the state of MSN2 and MSN4 genes greatly reduced GSY1: lacZ activity. However, the fold induction was still comparable with that of the wild-type. It appears that this effect of heme on GSY1: lacZ expression.

The major STRE-specific binding factor in yeast is the product of the MSN2 gene. MSN4p, a close structural homologue of MSN2p, was also shown to be capable of binding the STRE sequence. Mutation of either or both STRE-1 or STRE-2 within the center of the glycogen synthase in S. cerevisiae, employed STRE to induce expression in response to stationary phase and heat shock (12, 29). Sequences that appear to match the consensus for STREs were also found in the promoter of GSY1, centered at −374 and −236. These elements were designated as STRE-1 and STRE-2, respectively (Fig. 3).

Mutation of either or both STRE-1 or STRE-2 within the 1700 bp of upstream sequences resulted in a striking decrease in expression of GSY1 in both log and stationary phase (Fig. 3). Thus, these elements appear to be functional and are required for expression of GSY1. These elements may act synergistically, because the sum of the activities observed with either element alone was less than that observed when both were intact.

The major STRE-specific binding factor in yeast is the product of the MSN2 gene. MSN4p, a close structural homologue of MSN2p, was also shown to be capable of binding the STRE sequence. Mutation of msn2 and msn4 genes greatly reduced GSY1: lacZ activity (Fig. 3), consistent with the suggestion that they act on GSY1 through the STRE.

STRE mediate induction of a number of genes in response to

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a variety of stressors in addition to stationary phase (32). As a further test of the roles of STRE-1 and STRE-2, we examined the response of GSY1 to heat shock. Shifting a culture from 21 to 37 °C caused a 6-fold induction of GSY1 promoter activity within an hour following the shift (Fig. 4). Mutation of either element greatly reduced this response and the double mutant failed to respond at all. As with the effect of growth into stationary phase, STRE-1 and STRE-2 appeared to act synergistically.

**Rox1 Represses GSY1 Expression**—During a deletion analysis of the GSY1 promoter, we found a region between −209 and −154 bp that appeared to include a negative element (data not shown). This deletion resulted in a striking increase in promoter activity in both log (19-fold) and stationary phase (7-fold) of growth. Examination of this region identified a sequence that matched the consensus binding site for Rox1 (Fig. 5), a repressor protein that is induced in response to oxygen (33). To test the role of this element in GSY1 promoter function, we mutated this sequence (−210 to −199 bp) in the GSY1::lacZ reporter. Mutation of the ROXI gene increased expression of a GSY1::lacZ fusion in both log phase and stationary phase (Fig. 5A), consistent with the suggestion that the Rox1 repressor controls GSY1. Similarly, mutation of the ROXI binding site in a ROXI background increased GSY1::lacZ expression. In contrast, mutation of this site had no effect in a roxl strain. Mutation of the ROXI gene had a greater effect on GSY1::lacZ expression than did mutation of the ROXI binding site (Fig. 5A). This probably did not result from the presence of a second Rox1 site in the GSY1 promoter, because we could find no other sequence that matched the binding site consensus. The greater effect of the ROXI gene mutation may have been a secondary consequence of a general effect on cellular metabolism, although that remains to be proven.

The ROXI gene is known to be transcriptionally activated by heme (34). If GSY1 is, indeed, repressed by Rox1, then a heme deficiency would be expected to derepress GSY1 expression because of a reduction in Rox1. We tested this prediction by inserting the GSY1::lacZ fusion genes into isogenic strains that carried wild-type or deleted alleles of HEM1, a gene required for heme biosynthesis (35). Deletion of HEM1 did, indeed, derepress GSY1::lacZ, consistent with the prediction (Fig. 5B). The cis-mutation in the Rox1 element had a somewhat greater effect, perhaps because Rox1 was not completely eliminated by the HEM1 deletion. Mutation of both HEM1 and the Rox1 element were not additive. It thus appears that Rox1 represses GSY1 and may mediate an effect of heme.

It might be noted that the wild-type strains in panels A and B of Fig. 5 exhibit quite different levels of β-galactosidase activities. This difference is most likely explained by the fact that these strains have different genetic backgrounds.

**Mig1 Also Represses GSY1 Expression**—Examination of the GSY1 promoter revealed a second possible repressor binding site at −250 bp. This sequence matches the consensus binding site of Mig1 (36, 37), a protein that participates in
Regulation of Expression of GSY1 in S. cerevisiae

To determine whether N1 was sufficient to repress expression from an STRE-linked promoter, we synthesized an N1 oligonucleotide that included the sequence from 322 to 314 bp of the N1 element as N1. This construct showed high binding activities in cell-free extracts. The double stranded N1 oligonucleotide that included the sequence from 322 to 314 bp yielded a 2-fold increase in lacZ expression (Fig. 7). When present upstream of STRE-1, a single copy of N1 in either orientation yielded a modest (2.5-fold) reduction in expression and also did not block the induction observed when these cells enter stationary phase (data not shown). Thus, the strong repression appears to be specific to the novel N1 sequence.

When present upstream of STRE-1, a single copy of N1 in either orientation yielded a modest decrease in CYC1: lacZ expression. In contrast to its effect downstream of STRE, it caused a modest (2.5-fold) reduction in expression and also did not block the induction observed when these cells enter stationary phase (data not shown). Thus, the strong repression appears to be specific to the novel N1 sequence.

We next tested the effects of N1 on a heterologous promoter, the UAS from CYC1. The effect of N1 on this UAS was qualitatively similar to its effect on STRE-1, although the repression was less pronounced (Fig. 9). N1 repressed expression both upstream and downstream of this UAS, although the latter was more effective. Repression occurred with the element in both orientations. Two copies of the element were more effective than one.

Gel mobility shift assays were carried out to test for N1 binding activities in cell-free extracts. The double stranded N1 oligonucleotide was end-labeled and incubated with the yeast-glucose repression. Mutation of this site yielded a 3-fold increase in GSY1: lacZ expression (Fig. 6). A similar increase was observed when the MIG1 gene was mutated. These effects were not additive: mutation of the MIG1 gene and the Mig1 site had the same effect as either mutation alone. These results indicate that GSY1 is repressed by Mig1 when grown on glucose.

The observation that Mig1 appears to repress GSY1 expression suggested that this gene should respond specifically to glucose as a carbon source. Consistent with this prediction, the levels of GSY1 fusion protein were extremely low on glucose (3 ± 0.3 β-galactosidase units) compared with growth on raffinose (122 ± 7), glycerol (117 ± 4), or lactate (125 ± 7) media.

Identification of a Novel Repressor Element in the GSY1 Promoter—Experiments mapping the GSY1 promoter revealed a third negative element. Mutations between −322 and −316 bp yielded a 2-fold increase in GSY1: lacZ expression (Fig. 7). Deletion analysis indicated that the negative element lay, at least in part, between −328 to −314 bp and effects up to 5-fold were observed when the full element was deleted.

We have narrowed the location of this negative element by oligonucleotide-directed mutagenesis (Fig. 7). Mutation or deletions outside of the region from −324 to −314 bp had no effect on expression, suggesting that the negative element lay within this region. A literature search revealed no published sequence in the yeast literature that resembles this region, suggesting that this element may be novel. We refer to this negative element as N1.

To determine whether N1 was sufficient to repress expression from an STRE-linked promoter, we synthesized an N1 oligonucleotide that included the sequence from −324 to −314 bp. This oligonucleotide was inserted downstream of STRE-1 in the basal promoter (containing only the TATA element and no regulatory elements) of CYC1: lacZ. This construct showed high promoter activity in the log phase in the absence of any negative elements and an induction in β-galactosidase levels as cells enter stationary phase. A single copy of N1 was sufficient to repress expression (12–24-fold) from STRE-1 in both log and stationary phases (Fig. 8). Perhaps more importantly, the residual activity was not induced when the cells entered stationary phase, indicating that induction had been blocked.
Regulation of Expression of GSY1 in S. cerevisiae

Fig. 11. Overview of GSY1 regulation. Expression of GSY1 depends on two STRE, elements that mediate cellular responses to a wide variety of stressors. Msn2p and Msn4p have been shown to act through these elements. We also identified the binding sites for the negative factors: Mig1 (response to glucose) and Rox1 (response to heme and oxygen). A third negative cis element, N1, was identified and appears to be a novel element.

The ability of Rox1 to repress GSY1 suggests that expression of this gene should respond to heme and oxygen (33, 34). Consistent with this suggestion, anaerobic growth and mutation of HEM1 (a gene required for heme biosynthesis) each increased GSY1: lacZ expression 3–4-fold. However, these results should be interpreted with caution because anaerobic growth and the inability to synthesize heme would be expected to produce pleiotropic effects.

GSY1 expression may respond to glucose levels through the Mig1 repressor. Mig1 has been shown to play a central role in glucose repression of a variety of genes. Mutation of the MIG1 gene or the Mig1 site within the GSY1 promoter increased expression of a GSY1: lacZ fusion, indicating a role for this protein in the control of GSY1. Also, growth on glucose yields a 50-fold reduction in GSY1: lacZ expression compared with growth on raffinose, glycerol, or lactate, which also indicates that GSY1 responds to glucose levels. However, as with anaerobic growth, the response to the carbon source result must be interpreted with caution because of the complexity of the effects produced by these different growth conditions.

Mig1 has been shown to be phosphorylated by Snf1, the yeast homologue of the mammalian AMP-sensitive kinase (42–45). Increasing AMP levels signal a lack of glucose in the medium, triggering the activation of Snf1 kinase and derepression of the glucose-repressed genes (46). Snf1 mutants have reduced glycogen levels (47), consistent with a role for Snf1 in controlling the glycogen synthase genes.

The GSY1 promoter also contains a novel negative element, which we refer to as N1. This element represses transcription when present either upstream or downstream of a UAS, although the latter position is more effective. N1 is functional in either orientation and is more repressive when multiple copies are present. It is not specific to STREs, repressing at least the UAS of CYC1 as well. The role of N1 in regulating GSY1 expression has yet to be determined. However, it is intriguing that this element can block induction from a single copy of an STRE even when it does not completely repress expression. We are unaware of any negative element in yeast with a similar sequence, suggesting that N1 is a new element.

Why is the promoter of GSY1 so complex? Glycogen accumulates rapidly in response to a wide variety of stressors, such as entry into stationary phase, starvation, heat shock, and osmotic shock (41, 48, 49). Rapid accumulation is probably advantageous, because it increases the amount of glycogen that is available to the cell during the metabolic crisis. The response of GSY1 to oxygen that appears to be mediated by Rox1 is also likely to be advantageous, because accumulated glycogen can


distinguishable. One major and three minor DNA-protein complexes were observed (Fig. 10, lane 2). Inclusion of unlabeled N1 oligonucleotide yielded a striking decrease in the intensity of the major band (lane 3-5). However, even in the presence of 250-fold excess of the cold competitor, the reduction in binding of the hot oligonucleotide was less than proportional. This result might be observed if the binding activity was present in excess of the labeled oligonucleotide. In contrast, the mutated oligonucleotide (N1m2) and the salmon sperm DNA had no effect on the intensity of this band (lane 7-10). Two of the minor bands were subject to competition by both unlabeled probe and salmon sperm DNA. A third minor band paralleled the behavior of the major band and may be related to it. Thus, it appears that the major band represents a specific N1 binding activity.

This binding activity may mediate the repressor activity of N1, although this remains to be demonstrated.

DISCUSSION

Glycogen synthase is a major control point for glycogen metabolism in S. cerevisiae, as it is in mammals (1). In addition to post-translational control mediated by protein phosphorylation and allosteric mechanisms (38), glycogen synthase in yeast is regulated at the level of gene expression. The promoter of GSY1, one of the two genes encoding glycogen synthase, is surprisingly complex (Fig. 11). Expression is dependent on two STRE. Binding sites for the transcriptional repressors Rox1 and Mig1 were identified. GSY1 may be the only gene whose promoter contains sites for both of these repressors. A novel negative element was also detected.

STREs have been identified in a number of stress inducible genes in S. cerevisiae. These elements respond to a variety of stressors, such as stationary phase, heat shock, and osmotic shock (39). Msn2 and Msn4 proteins have been shown to be required for transcriptional induction through the STREs in S. cerevisiae (30, 31, 40). GSY1 has two of the STRE that act synergistically with each other. No induction of GSY1 expression through these STREs was observed in msn2 msn4 double mutants supporting a role for these genes in the stress response of GSY1.

Rox1 is a repressor protein that was first identified during studies of CYC1. Expression of Rox1 responds to the levels of oxygen and heme. Under conditions of low oxygen or heme, Rox1 levels are reduced, inducing expression of Rox1-regulated genes. Rox1 also appears to control GSY1 expression. Mutation of either the ROX1 gene or of the Rox1 site within the GSY1 promoter increased the expression of a GSY1: lacZ reporter. The ability of Rox1 to repress GSY1 suggests that expression of this gene should respond to heme and oxygen (33, 34).

extract.
be fermented under anaerobic conditions. The complexity of the GSY1 promoter may ensure that glycogen synthase is induced quickly in response to a wide variety of stressors. Yeast glycogen synthase is encoded by two genes: GSY1 and GSY2. Both promoters have STRE that contribute to the induction of the gene upon entry into stationary phase. The GSY1 promoter also includes a number of negative elements, whereas no such elements have been found in the GSY2 promoter (12). GSY2 has been found to express glycogen synthase at a higher level than does GSY1 and so is thought to be the major contributor to glycogen synthesis under normal growth conditions. However, the presence of negative elements in GSY2, but not GSY1, suggests that the product of GSY1 may become the dominant form of glycogen synthase under conditions that relieve repression from these elements. These negative elements would afford a much broader range of expression levels and the ability to respond to a wider array of metabolic conditions than would be obtained with STRE alone.

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