Genetic and Physical Interactions Between Yeast RGR1 and SIN4 in Chromatin Organization and Transcriptional Regulation

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ABSTRACT

The SIN4 and RGR1 genes of Saccharomyces cerevisiae were identified by mutations in quite different genetic screens. We have shown that the SIN4 gene product is required for proper transcriptional regulation of many genes and that a sin4 mutation can affect either activation or repression of specific genes. We have suggested that this dual nature of SIN4 in transcriptional regulation is due to its involvement in chromatin organization. We now report that the role of RGR1 in gene regulation is similar to that of SIN4. SIN4 and RGR1 both function as negative transcriptional regulators of HO and IME1, and mutations in either gene lead to decreased expression of other genes including CTS1. Strains with sin4 or rgr1 mutations both have phenotypes similar to those caused by histone mutations, including suppression of δ insertion into promoters (Spt− phenotype), activation of promoters lacking UAS elements, and decreased superhelical density of plasmid DNA molecules. Overexpression of RGR1 suppresses the temperature sensitivity due to a sin4 mutation. Finally, we use yeast strains expressing GST fusion proteins to demonstrate that the Sin4p and Rgrlp proteins are physically associated in vivo. These results indicate that Sin4p and Rgrlp act together in vivo to organize chromatin structure and thus regulate transcription.

The study of transcriptional regulation of HO gene has revealed its complex pattern and involvement of multiple regulators (Herskowitz et al. 1992; Nasmuth 1993). SIN4 is a negative regulator of HO, since a sin4 mutation causes the expression of HO in the absence of transcriptional activators like SWI5 (Jiang and Stillman 1992). SIN4 is the same as TFS3 and is required for transcriptional repression of the GAL1-GAL10 promoter (Chen et al. 1993). SIN4 is required for the transcriptional repression of the IME1 gene (Covitz et al. 1994). In contrast to these results indicating that SIN4 functions as a negative transcriptional regulator, sin4 mutants are defective for the transcriptional activation of a number of yeast genes, including CTS1, HIS4, and MATa2 (Jiang and Stillman 1992, 1995).

We have suggested that SIN4 functions by organizing the structure of chromatin, and thus a sin4 mutation can have defects in either transcriptional activation or transcriptional repression, depending upon the promoter (Jiang and Stillman 1992). A sin4 mutant has phenotypes also seen in strains with mutations in histone genes, supporting the chromatin hypothesis. These phenotypes include expression of promoters that lack UAS elements, suppression of the promoter inactivation caused by insertion of a Ty δ transposable element (Spt− phenotype), and altered superhelical density of circular DNA molecules (Jiang and Stillman 1992). RGR1 was first identified by a mutation that allowed oversecretion of a mouse a-amylase protein expressed in yeast (Sakai et al. 1988). The RGR1 gene encodes a 123-kD protein with no significant homology to proteins in the databases. The gene is essential, but the carboxyl-terminal one-third of the protein can be deleted, resulting in the viable rgr1Δ2:URA3 truncation mutant (Sakai et al. 1990). This mutant displayed a number of phenotypes, including resistance to glucose repression, sporulation deficiency, temperature sensitive lethality, and abnormal cellular morphology.

We have recently demonstrated that RGR1, like SIN4, functions as a negative regulator of HO gene expression (Stillman et al. 1994). Epistasis analysis of sia4 and rgr1 mutants indicate that SIN4 and RGR1 act in the same genetic pathway for HO regulation. Covitz et al. (1994) recently demonstrated that transcriptional repression of the IME1 gene requires both SIN4 and RGR1. In this communication we report that sin4 and rgr1 mutants have the same spectrum of phenotypes. Both mutants have an Spt− phenotype, allow expression of UAS-less promoters, show an altered linking number of circular DNA molecules, and show defects in transcriptional regulation. Finally, we show that the Sin4p and Rgrlp proteins are physically associated in vivo.

MATERIALS AND METHODS

Strains and media: Yeast strains used in this study are listed in Table 1. Strain FY56 was obtained from Fred Winston and
TABLE 1

Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>DY150</td>
<td>MATa ade2 can1 his3 leu2 trp1 ura3</td>
</tr>
<tr>
<td>DY881</td>
<td>MATa ade2 his3 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY1717</td>
<td>MATa sin4::TRP1 ade2 his3 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY2010</td>
<td>MATa rgr1-Δ2::TRP1 can1 leu2 trp1 ura3</td>
</tr>
<tr>
<td>DY2081</td>
<td>MATa his4-9126 lys2-1286 sin4::URA3 ura3</td>
</tr>
<tr>
<td>DY2273</td>
<td>MATa ade2 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY2278</td>
<td>MATa sin4::TRP1 ade2 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY2578</td>
<td>MATa rgr1-Δ2::URA3 his4-9126 lys2-1286 ura3</td>
</tr>
<tr>
<td>DY2610</td>
<td>MATa URA3::CTS1-lacZ ade2 his3 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY2643</td>
<td>MATa URA3::CTS1-lacZ sin4::LEU2 ade2 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY2665</td>
<td>MATa URA3::CTS1-lacZ rgr1-Δ2::TRP1 ade2 his3 leu2 lys2 trp1 ura3</td>
</tr>
<tr>
<td>DY2689</td>
<td>MATa/MATa HO::lacZ HMRa HMLa +/swi5::URA3 ade2 ade6 can1 his3 leu2 trp1 ura3</td>
</tr>
<tr>
<td>DY2736</td>
<td>MATa/MATa HO::lacZ HMRa HMLa +/swi5::URA3 +/rgr1-Δ2::TRP1 ade2 ade6 can1 his3 leu2 trp1 ura3</td>
</tr>
<tr>
<td>FY56</td>
<td>MATa his4-9126 lys2-1286 ura3-32</td>
</tr>
<tr>
<td>A488</td>
<td>MATa ara7 can1 gal2 trp1 ura3</td>
</tr>
<tr>
<td>A479</td>
<td>MATa rgr1-Δ2::URA3 can1 leu2 trp1 ura3</td>
</tr>
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</table>

strains A479 and A488 were obtained from A. SAKAI. All strains are in the S288C background except DY150, which is a W303 strain, and DY2699 and DY2736 that are in the K1107 background, which contains a chromosomal HO::lacZ reporter. Strains DY881, DY1717, DY2273, and DY2278 have been described previously (Jiang and Stillman 1992, 1995). Plasmids M1977 and M1978 digested with KpnI were used to replace the RGR1 gene with the rgr1-Δ2::TRP1 and rgr1-Δ2::URA3 truncation alleles, respectively, by the one step gene replacement method (Rothstein 1991). Gene replacements were confirmed by Southern blotting. Plasmid M1593 was cleaved with Stul and used to construct strains with CTS1-lacZ reporters integrated at the URA3 locus. Strains were derived using standard genetic techniques (Sherman et al. 1986). Media and cell cultivation conditions are indicated in the text or as described previously (Jiang and Stillman 1992, 1995).

Plasmids: Plasmids M1977 and M1978 were designed for gamma disruption (Sikorski and Hieter 1989) of RGR1 and are derived from plasmids Yplac204 and Yplac211 (Gietz and Sugino 1988), with TRP1 and URA3 markers, respectively. These plasmids create rgr1-Δ2 truncation alleles by replacing the 1.3-kb C-terminal XbaI fragment of RGR1 gene with the TRP1 or URA3 genetic markers. The Ylp-CTS1::lacZ-integrating reporter plasmid M1593 is derived from the Ylp553 (Myers et al. 1996) and contains 1.3 kb of the CTS1 promoter-driving expression of the lacZ gene. Plasmid pLL53, containing a HIS4-lacZ fusion gene under control of the his4-912d promoter (with a Ty 6 insert), and plasmid pLGA-178, which has a UAS-less CYC1 promoter driving lacZ expression, have been described (Jiang and Stillman 1992).

Plasmid pRD56, which is plasmid pRS516 (URA3 marker) containing a GAL1 promoter-driving expression of glutathione-S-transferase (GST) followed by a polylinker (Park et al. 1993), was obtained from Ray Deshaies. Plasmid M1967, which expresses a GST-Rgrlp fusion protein from the GAL1 promoter, was constructed by inserting a 4.2-kb BamHI-SalI RGR1 fragment into pRD56. Similarly, plasmid M1968, which expresses a GST-Sin4p fusion protein from the GAL1 promoter, was constructed by inserting a 3.8-kb EcoRI-SalI SIN4 fragment from clone M1389 into pRD56. The 5′ end of clone M1389 was generated by EcoRI (for DNA sequencing), and the EcoRI and XbaI sites are from the plasmid polylinker. Plasmid M1391 (LEU2 marker) that expresses an HA epitope-tagged version of Sin4p from the ADH1 promoter was constructed by inserting a Sall-SalI fragment containing the SIN4 coding region into plasmid pAD5 (Field et al. 1988), obtained from Michael Wigler. Plasmid M1981 (HIS3 marker), which expresses a LexA-Rgrlp fusion protein from the GAL1 promoter, was constructed by inserting a 4.2-kb BamHI-SalI RGR1 fragment into a pSH2-1 (Hanes and Brent 1989) derivative where the polylinker had been modified to change the reading frame. Although the GST-Rgrlp and LexA-Rgrlp fusion proteins lack the first five amino acids of Rgrlp, each fusion protein can complement the rgr1 defect. The entire SIN4 coding region is expressed in the GST-Sin4p and HA epitope-Sin4p fusion proteins, and they complement a sin4 mutation.

Antisera to Sin4p: A GST-Sin4p fusion protein was expressed in Escherichia coli from plasmid M2309, which was constructed by inserting the region encoding the first 458 amino acids of Sin4p into the pGEX-3X vector (Pharmacia). Thus, the antisera recognizes Sin4p in addition to epitopes in GST. The fusion protein was purified by glutathione-agarose chromatography (Smith and Johnson 1988), dialyzed against phosphate-buffered saline, and used to immunize rabbits (HRP Inc., Denver, PA).

GST copurifications: Strain DY150 (wild type) containing the indicated plasmids was used for this experiment. Cells were grown in 1 liter of synthetic complete media with 2% galactose as a carbon source; the media lacked either uracil and leucine or uracil and histidine for plasmid selection. Protein extracts were prepared as described (Brazas and Stillman 1993). Glutathione-agarose (1.5 ml bed volume, Sigma) was added to 20 mg of yeast protein, and the mixture was incubated at 4°C with continual rotation. The slurry was poured into a column, the flow-through was collected by washing with 100 mM NaCl, 20 mM Tris pH 8.0, 10% glycerol, 0.5 mM EDTA, and 0.5 mM DTT; the bound proteins were eluted with 100 mM NaCl, 50 mM Tris pH 8.0, 10% glycerol, 10 mM reduced glutathione. Fifty microliters each of the flowthrough and the bound fractions and 12 μg of the total protein were electrophoresed on SDS-polyacrylamide gels and electroblotted. The Western blots were incubated either with the anti-GST-Sin4p serum, anti-LexA serum (obtained from Roger Brent), or anti-Sp12p (SPT2 = SIN1) serum (obtained from Warren Krueger and Ira Herskowitz) and visualized with the ECL (Amersham) enhanced chemiluminescence kit.

Other methods: RNA blot hybridization was performed using CTS1 and Actin probes as described previously (Dohrmann et al. 1988).
MANN et al. 1992). Transformation of yeast was carried out using the Hi Lithium transformation protocol (JIANG and STILLMAN 1995). Two-dimensional electrophoresis of DNA in the presence of chloroquine was performed as described previously (JIANG and STILLMAN 1992). Quantitative determinations of β-galactosidase activity were performed in triplicate as described (BREEDEN and NASMYTH 1987).

RESULTS

Effects of rgr1 and sin4 mutations in different strain backgrounds: The sin4 mutation was first identified by its ability to permit expression of HO in the absence of the Swi5p transcriptional activator (NASMYTH et al. 1987; STERNBERG et al. 1987). We demonstrated previously that an rgr1 mutation also suppresses the swi5 defect for expression of HO (STILLMAN et al. 1994). Epistasis analysis indicated that SIN4 and RGR1 act in the same pathway for HO expression. In these experiments, however, the strains bearing a rgr1-D2::URA3 truncation allele were not isogenic with the parent strain and the sin4 single mutant. The rgr1-D2 truncation allele was originally constructed in an S288C strain (SAKAI et al. 1990). We therefore introduced a rgr1-D2 truncation mutation into the K1107 strain background that contains a chromosomal HO::lacZ reporter. When this diploid DY2699 containing the rgr1-D2::TRP1 truncation mutation was sporulated, no viable Trp+ progeny were recovered, suggesting that K1107 strains bearing the rgr1-D2 truncation allele are not viable. Alternatively, the rgr1-D2 mutation may cause a germination defect in K1107 strains.

We investigated the phenotype of the rgr1-D2 truncation mutation in two other commonly used strain backgrounds, W303 and YPH499/YPH500 (S288C). The rgr1-D2 truncation mutation was introduced into diploids, and we were able to isolate haploid rgr1-D2 segregants that grow extremely slowly and are inviable at 37°. We also noted that the viability of rgr1 mutants drastically decreases when liquid cultures enter stationary phase. It has been previously noted that both sin4 and rgr1 mutants are clumpy, due to an apparent defect in cell separation (SAKAI et al. 1990; JIANG and STILLMAN 1992). Interestingly, we found that the sin4 mutation causes an additional phenotype in the S288C background. These strains are flocculent, in that liquid cultures cells aggregate in large clumps, and this flocculence can be reversed by addition of EDTA. The sin4 mutation does not cause a flocculent phenotype in W303 strains. All of the experiments reported here were conducted with S288C strains. The sin4 mutations used are null alleles with complete deletion of the SIN4 gene, and the rgr1-D2 allele used is a truncation allele expressing the first two-thirds of the Rgrlp protein.

Similar phenotypes in rgr1 and sin4 mutants: It has been shown that expression of the CTSl gene is reduced in sin4 mutants (JIANG and STILLMAN 1992, 1995), and we therefore decided to determine whether

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>CTSl-lacZ</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>DY2610</td>
<td>wildtype</td>
<td>2900 ± 290</td>
<td>100 %</td>
</tr>
<tr>
<td>DY2643</td>
<td>sin4</td>
<td>1000 ± 20</td>
<td>34 %</td>
</tr>
<tr>
<td>DY2645</td>
<td>rgr1</td>
<td>520 ± 30</td>
<td>18 %</td>
</tr>
</tbody>
</table>

FIGURE 1.—CTSI expression is decreased in an rgr1 mutant. (A) Quantitative measurements of β-galactosidase activity were made from extracts prepared from strains DY2610 (wild type), DY2643 (sin4), and DY2645 (rgr1), which contain an integrated CTSl-lacZ reporter. Extracts for β-galactosidase assay were prepared from cells growing logarithmically in YEPD media. (B) A Northern blot was probed with a CTSl probe and an Actin probe as an internal control. RNA was prepared from the following strains: lane 1, A488 (wild type); lane 2, A479 (rgr1). Cells were grown in YEPD media. Total RNA was prepared from cells growing logarithmically in YEPD media.

RGR1 regulates CTSl expression. Isogenic wild-type, sin4, and rgr1 mutants containing an integrated CTSl-lacZ reporter were constructed and β-galactosidase activity was measured (Figure 1). CTSl-lacZ expression is reduced more than fivefold by an rgr1 mutation. Northern blot analysis demonstrates that expression of the chromosomal CTSl gene is similarly reduced. We have shown previously that expression of HIS4-lacZ, Ty1-lacZ, and MATAalpha2-lacZ reporters is reduced 5–20-fold in a sin4 mutant (JIANG and STILLMAN 1995), and an rgr1 mutation causes a similar reduction in expression of these genes (data not shown). We conclude that genes such as CTSl that require SIN4 for full expression also require RGR1.

Expression of yeast genes normally requires both a UAS and a TATA element. It has been shown that a sin4 mutation causes inappropriate expression of promoters
It has been suggested that this inappropriate activation is seen when histone H4 levels are experimentally decreased (CLARK-ADAMS et al. 1988; FASSLER and WINSTON 1988). Thus, these genes can confer an Spt− phenotype either through loss of function or when overproduced. Neither SIN4 nor RGR1 are capable of conferring an Spt− phenotype when overproduced.

**RGR1 is a multicopy suppressor of sin4.** Although SIN4 and RGR1 appear to play similar roles in transcriptional activation, several observations suggest that RGR1 has a more important role. A strain with a sin4 gene deletion is viable and relatively healthy (at least at 30°), whereas deletion of RGR1 is lethal at all temperatures. A strain with the rgr1-A2 truncation allele is viable but temperature sensitive (SAKAI et al. 1988). However, this temperature sensitivity can be suppressed by overexpression of RGR1 from a multicopy plasmid. This result is consistent with the idea that SIN4 and RGR1 play similar roles but that RGR1 is more important. Interestingly, Yep-RGR1 cannot suppress the sin4 defect at the HO locus. We have identified another multicopy suppressor of the sin4 growth defect, and characterization of this gene is in progress (Y. Yu, Y. W. JIANG and D. J. STILLMAN, unpublished results). An rgr1 mutation also confers temperature sensitive growth (SAKAI et al. 1988, 1990). However, this temperature sensitivity can be suppressed by overexpression of RGR1 from a multicopy plasmid. This result is consistent with the idea that SIN4 and RGR1 play similar roles but that RGR1 is more important. Interestingly, Yep-RGR1 cannot suppress the sin4 defect at the HO locus. We have identified another multicopy suppressor of the sin4 growth defect, and characterization of this gene is in progress (Y. Yu, Y. W. JIANG and D. J. STILLMAN, unpublished results).

### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>CYC1 (ΔUAS)-lacZ</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY2273</td>
<td>wild type</td>
<td>5.1 ± 0.2</td>
<td>1X</td>
</tr>
<tr>
<td>DY2273</td>
<td>sin4</td>
<td>84 ± 7</td>
<td>16X</td>
</tr>
<tr>
<td>DY22010</td>
<td>rgr1</td>
<td>290 ± 20</td>
<td>57X</td>
</tr>
</tbody>
</table>

Plasmid pLG4-178, which has a UAS-less CYC1 promoter driving lacZ expression, was used to measure UAS-independent expression.

### Table 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>his4-9126-lacZ</th>
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<tr>
<td>A. Expression of a his4-9126-lacZ reporter*</td>
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<tr>
<td>DY2273</td>
<td>Wild type</td>
<td>0.3 ± 0.1</td>
<td>1X</td>
</tr>
<tr>
<td>DY2277</td>
<td>sin4</td>
<td>13 ± 0.4</td>
<td>22X</td>
</tr>
<tr>
<td>DY2010</td>
<td>rgr1</td>
<td>56 ± 3</td>
<td>98X</td>
</tr>
<tr>
<td>B. Suppression of his4-9126 and lys2-1286b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FY56</td>
<td>Wild type</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DY2081</td>
<td>sin4</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DY2578</td>
<td>rgr1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Strains DY2273 (wild type), DY2278 (sin4), and DY2010 (rgr1) were transformed with plasmid pL525, which contains a his4-9126-lacZ reporter, and grown under uracil selection. Quantitative measurements of β-galactosidase activity were made.

**Strains**

Strain Description

- FY56: Wild type
- DY2278: sin4
- DY2010: rgr1

**Expression**

- Strain FY56: Wild type
- Strain DY2081: sin4
- Strain DY2578: rgr1

**Suppression**

- FY56: Wild type
- DY2081: sin4
- DY2578: rgr1

**Additional Information**

- Table 2: CYC1 (ΔUAS)-lacZ
- Table 3: his4-9126-lacZ

**References**

- CLARK-ADAMS et al. 1988
- FASSLER and WINSTON 1988
- SAKAI et al. 1988, 1990
- Y. Yu, Y. W. JIANG and D. J. STILLMAN, unpublished results
- Y. Y. Yu, Y. W. JIANG and D. J. STILLMAN, unpublished results
The *sin4* and *rgrl* mutations affect DNA linking number: Each nucleosome induces a single superhelical turn in closed circular DNA, and thus determination of superhelical density, or linking number, gives a measurement of nucleosome content. We demonstrated previously that a *sin4* mutation leads to a change in the linking number of plasmid DNA using two-dimensional electrophoresis on agarose gels containing chloroquine (Jiang and Stillman 1992), and we therefore decided to determine if an *rgrl* mutation has the same effect. As shown in Figure 2 the *sin4* and *rgrl* mutations each cause a substantial increase in the linking number of the YCp50 plasmid. In this figure the two-dimensional gel resolves topoisomers of DNA in a broad arc, from the lower left, up to the center, and then down to the right, with linking number increasing as one proceeds clockwise. In *sin4* and *rgrl* mutants the distribution of YCp50 topoisomers shows a clockwise shift on the two-dimensional gel. The linking number of other plasmids is also altered in a *sin4* mutant (Jiang and Stillman 1992), and thus the effect is not specific to YCp50. We suggest that the *sin4* and *rgrl* mutations cause a decrease in nucleosome density or stability, and that these changes in chromatin structure lead to the observed increase in linking number.

**Sin4p and Rgrlp physically interact:** The fact that the *sin4* and *rgrl* mutations cause similar phenotypes suggested that the Rgrlp1 and Sin4p proteins might physically interact. We decided to use a copurification assay to test this hypothesis. The first experiment used a yeast strain that expresses a Glutathione-STransferase-Rgrlp fusion protein. These cells also express Sin4p from the *ADH1* promoter, since we have been unable to immunologically detect Sin4p expressed from its native promoter. Protein extracts were prepared and the GST-Rgrlp1 protein was purified by glutathione chromatography. The results shown in Figure 3 (lane 3) demonstrate that Sin4p copurified with GST-Rgrlp1. Control experiments demonstrate the specificity of the Sin4p-Rgrlp1 copurification: Sin4p was not copurified from a yeast strain expressing GST instead of the GST-Sin4p fusion (Figure 3, lane 6).

The reciprocal experiment was performed, using a GST-Sin4p fusion protein, to further demonstrate the interaction. In this experiment, the yeast expressed Rgrlp fused to LexA, and antibody to lexA was used to detect the LexA-Rgrlp1 fusion protein. The results show that LexA-Rgrlp copurifies with GST-Sin4p (Figure 3, lane 9). LexA-Rgrlp1 does not bind to GST alone (Figure 3, lane 12), and thus GST-Sin4p is required for copurification of LexA-Rgrlp1.

Although unlikely, it is possible that many proteins bind nonspecifically to the GST-Rgrlp1 and GST-Sin4p columns. To demonstrate specificity of the Rgrlp1-Sin4p interaction, a Western blot containing the protein fractions from yeast expressing GST-Rgrlp1 or GST-Sin4p was probed with antiserum to Spt2p (= Sin1p). The Spt2p protein does not bind to GST-Rgrlp1 or GST-Sin4p (Figure 3, lanes 13–18). Additionally, silver staining of protein gels demonstrates that the flow through and bound fractions from the GST-Rgrlp1 and GST-Sin4p columns contain very different protein species (data not shown).

We conclude that Sin4p and Rgrlp1 physically interact, although we cannot determine whether these two proteins interact directly or via intermediate proteins. It is worth noting that the fusion proteins used in these assays are functional in vivo, since they complement *sin4* or *rgrl* mutations.

**DISCUSSION**

We have shown that *sin4* and *rgrl* mutants have many properties in common. Both mutants have a defect in
cell separation, an abnormal morphology, and temperature sensitive lethality (Sakai et al. 1990; Jiang and Stillman 1992). SIN4 and RGR1 both function as negative regulators of HO and IME1 expression, yet they are also required for the full transcriptional activation of other genes, such as HIS4 and CTS1. We have suggested that the effects of a sin4 mutation may be through alterations in chromatin structure, based on the expression of promoters containing 6 element insertions, the inappropriate activation of UAS-less promoters, and the altered superhelical density of circular DNA molecules (Jiang and Stillman 1992). In this report we demonstrate that an rgrl mutation also causes all of these phenotypes. Moreover, RGR1 overexpression from a multicopy plasmid suppresses the temperature sensitive growth defect of a sin4 mutant, although SIN4 overexpression does not suppress the rgrl temperature sensitive lethality. Finally, we have demonstrated that the Sin4p and Rgrlp proteins are associated in vivo. Thus, each mutation leads to a similar spectrum of phenotypes, and we conclude that SIN4 and RGR1 function together in vivo.

Sin4p and Rgrlp apparently function in a multi-subunit protein complex in vivo. However, our data does not allow us to determine whether any other proteins are present in the complex. Sin4p and Rgrlp are both large proteins, at 111 and 123 kD, respectively. The predicted amino acid sequences derived from SIN4 and RGR1 do not provide clues as to function. Although there are no genes with significant homology to SIN4 in the database, the Caenorhabditis elegans C38C10.5 and the Aspergillus niger A. nidulans CreA genes show some homology to RGR1 (Sulston et al. 1992; Drysdale et al. 1993).

SIN4 and RGR1 have been described as global transcriptional regulators because they affect many diverse yeast genes. There are several other examples of yeast global transcriptional regulators that are proposed to form multiprotein complexes. The SSN6 (CYCL8) and TUP1 gene products, which repress the transcription of many genes, are present in a complex of apparent size of 1200 kD (Williams et al. 1991). Genetic criteria suggest that the SPT4, SPT5, and SPT6 genes function together in transcriptional regulation, possibly by affecting chromatin structure, and immune precipitation experiments demonstrate that at least the Spt5p and Spt6p proteins physically interact (Swanson and Winston 1992). The Ccr4p transcriptional regulator is in a complex with several other proteins (Draper et al. 1994).

The SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 genes are required for the activation of a large number of yeast genes, and it has been suggested that they act by altering the local chromatin structure at promoters (Winston and Carlson 1992). It has been recently demonstrated that these SWI/SNF gene products, along with four additional proteins, are associated in a large
complex that has DNA-dependent ATPase activity (CAIRNS et al. 1994; PETERSON et al. 1994). Finally, COLLART and STRUHL (1994) recently demonstrated that the NOT1/CDC39, NOT2/CDC36, NOT3 and NOT4 genes, which function as global negative regulators of transcription, form a multiprotein complex. Thus, large multiprotein complexes play important roles in transcriptional regulation. Interestingly, it appears that none of these proteins present in these complexes can bind to DNA directly.

**SIN4 and RGR1** both function as negative regulators of some genes and positive regulators of other genes. We suggest that sin4 and rgr1 mutations affect the structure of chromatin. We note that HO and IME1 are under strong negative regulation, which may be mediated in part by chromatin structure, and that sin4 and rgr1 mutations disrupt this negative regulation (COVITZ et al. 1994; STILLMAN et al. 1994). In contrast, the chromatin structure at genes such as HIS4 makes it ready for rapid transcriptional activation, and sin4 or rgr1 mutations reduce the level of HIS4 expression (DEVLIN et al. 1991; JIANG and STILLMAN 1995). Although there are other explanations, we suggest that these different genes are affected differently by the sin4 and rgr1 mutations because their chromatin is organized in different ways.

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**LITERATURE CITED**


**SARKA, A., Y. SHIMIZU, S. KONDO, T. CHIBAZUKA and F. HISHINUMA, 1990** Structure and molecular analysis of RGR1, a gene re-


Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122: 19–27.


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