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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 Rgr1p proteins are physically associated *in vivo*. These results indicate that Sin4p and Rgr1p 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; NASMYTH 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 *TSF3* 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 *MAT*α*2* (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 α-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 *rgr1-*Δ*2::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 *sin4* 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 Rgr1p proteins are physically associated *in vivo*.

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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

Strain	Genotype
DY150	*MATa ade2 can1 his3 leu2 trp1 ura3*
DY881	*MATa ade2 his3 leu2 lys2 trp1 ura3*
DY1717	*MATa sin4::TRP1 ade2 his3 leu2 lys2 trp1 ura3*
DY2010	*MATa rgr1-Δ2::TRP1 can1 leu2 trp1 ura3*
DY2081	*MATα his4-912δ lys2-128δ sin4::URA3 ura3*
DY2273	*MATa ade2 leu2 lys2 trp1 ura3*
DY2278	*MATa sin4::TRP1 ade2 leu2 lys2 trp1 ura3*
DY2578	*MATα rgr1-Δ2::URA3 his4-912δ lys2-128δ ura3*
DY2610	*MATa URA3::CTS1-lacZ ade2 his3 leu2 lys2 trp1 ura3*
DY2643	*MATa URA3::CTS1-lacZ sin4::LEU2 ade2 leu2 lys2 trp1 ura3*
DY2645	*MATa URA3::CTS1-lacZ rgr1-Δ2::TRP1 ade2 his3 leu2 lys2 trp1 ura3*
DY2699	*MATa/MATα HO::lacZ HMRa HMLa +/swi5::URA3 ade2 ade6 can1 his3 leu2 trp1 ura3*
DY2736	*MATa/MATαHO::lacZ HMRa HMLa +/swi5::URA3 +/rgr1-Δ2::TRP1 ade2 ade6 can1 his3 leu2 trp1 ura3*
FY56	*MATα his4-912δ lys2-128δ ura3-52*
A488	*MATα aro7 can1 gal2 trp1 ura3*
A479	*MATa rgr1-Δ2::URA3 can1 leu2 trp1 ura3*

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 *StuI* 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 YIplac204 and YIplac211 (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 YIp-*CTS1::lacZ*-integrating reporter plasmid M1593 is derived from the YIp353 (MYERS *et al.* 1986) and contains 1.3 kb of the *CTS1* promoter-driving expression of the *lacZ* gene. Plasmid pLL53, containing a *HIS4:lacZ* gene fusion under control of the his4-912d promoter (with a *Ty δ* insert), and plasmid pLGΔ-178, which has a UAS-less *CYC1* promoter driving *lacZ* expression, have been described (JIANG and STILLMAN 1992).

Plasmid pRD56, which is plasmid pRS316 (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-Rgr1p 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 ExoIII (for DNA sequencing), and the *EcoRI* and *XhoI* 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 *SalI-SacI* 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-Rgr1p 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-Rgr1p and LexA-Rgr1p fusion proteins lack the first five amino acids of Rgr1p, 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° with continual rotation. The slurry was poured into a column, the flow-through was collected by washing with 100 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 50 μg of the total protein were electrophoresed on SDS-polyacrylamide gels and electroblotted. The Western blots were incubated either with the anti-GST-Sin4p sera, anti-LexA sera (obtained from ROGER BRENT), or anti-Spt2p (SPT2 = SIN1) sera (obtained from WARREN KRUGER 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 (DOHR-

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-Δ2::URA3* truncation allele were not isogenic with the parent strain and the *sin4* single mutant. The *rgr1-Δ2* truncation allele was originally constructed in an S288C strain (SAKAI *et al.* 1990). We therefore introduced a *rgr1-Δ2* truncation mutation into the K1107 strain background that contains a chromosomal *HO::lacZ* reporter. When this diploid DY2699 containing the *rgr1-Δ2::TRP1* truncation mutation was sporulated, no viable Trp+ progeny were recovered, suggesting that K1107 strains bearing the *rgr1-Δ2* truncation allele are not viable. Alternatively, the *rgr1-Δ2* mutation may cause a germination defect in K1107 strains.

We investigated the phenotype of the *rgr1-Δ2* truncation mutation in two other commonly used strain backgrounds, W303 and YPH499/YPH500 (S288C). The *rgr1-Δ2* truncation mutation was introduced into diploids, and we were able to isolate haploid *rgr1-Δ2* 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-Δ2* allele used is a truncation allele expressing the first two-thirds of the Rgr1p protein.

Similar phenotypes in *rgr1* and *sin4* mutants: It has been shown that expression of the *CTS1* gene is reduced in *sin4* mutants (JIANG and STILLMAN 1992, 1995), and we therefore decided to determine whether

A.

Strain	Genotype	*CTS1-lacZ*	Percentage
DY2610	wildtype	2900 ± 290	100 %
DY2643	*sin4*	1000 ± 20	34 %
DY2645	*rgr1*	520 ± 30	18 %

B.

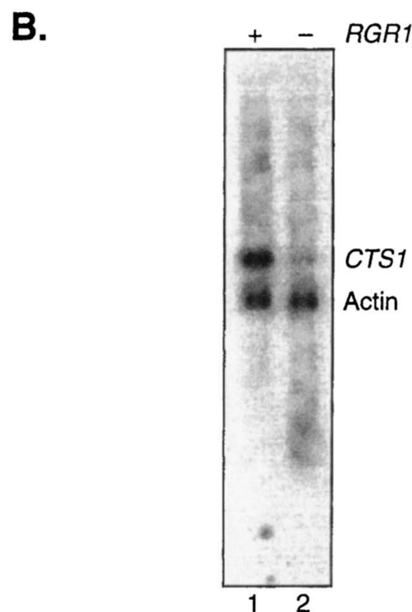

FIGURE 1.—*CTS1* expression is decreased in an *rgr1* mutant. (A) Quantitative measurements of β-galactosidase activity were made from extracts prepared from strains DY2610 (wild type), DY2643 (*sin4*), and DY2645 (*rgr1*), which contain an integrated *CTS1-lacZ* reporter. Extracts for β-galactosidase assays were prepared from cells growing logarithmetically in YEPD media. (B) A Northern blot was probed with a *CTS1* 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 logarithmetically in YEPD media.

RGR1 regulates *CTS1* expression. Isogenic wild-type, *sin4*, and *rgr1* mutants containing an integrated *CTS1-lacZ* reporter were constructed and β-galactosidase activity was measured (Figure 1). *CTS-lacZ* expression is reduced more than fivefold by an *rgr1* mutation. Northern blot analysis demonstrates that expression of the chromosomal *CTS1* 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 *CTS1* 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

TABLE 2

sin4 and rgr1 mutations allow expression from promoters lacking a UAS element

Strain	Genotype	*CYC1* (ΔUAS)-*lacZ*	Increase
DY2273	wild type	5.1 ± 0.2	1×
DY2278	*sin4*	84 ± 7	16×
DY2010	*rgr1*	290 ± 20	57×

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

lacking a UAS element (JIANG and STILLMAN 1992; CHEN *et al.* 1993). As shown in Table 2, an *rgr1* mutation also permits activation of a UAS-less promoter. A variety of other mutations have been shown to cause UAS-less activation, including *spt6/ssn20/cre2* (NEIGEBORN *et al.* 1987; PRELICH and WINSTON 1993), *cre1/spt10* (DENIS 1990; PRELICH and WINSTON 1993), *cdc68/spt16* (MALONE *et al.* 1991), *mot1* (DAVIS *et al.* 1992), *gal11/spt13* (CHEN *et al.* 1993), *sud1* (YAMASHITA 1993), *spt21, bur1/sgv1, bur2, bur3,* and *bur6* (PRELICH and WINSTON 1993). It has been suggested that this inappropriate activation of UAS-less promoters results from these mutations affecting chromatin structure, since mutations in *HHT1/BUR5* (encoding histone H3) activate UAS-less promoters (PRELICH and WINSTON 1993), and a similar effect is seen when histone H4 levels are experimentally depleted (HAN and GRUNSTEIN 1988).

***rgr1* is a weak *Spt*− mutation:** Promoter inactivation may result from insertion of *Ty* transposon (WINSTON *et al.* 1984). The *SPT* genes were identified by mutations that suppress these *Ty* promoter insertions (WINSTON *et al.* 1984). Because *sin4* has a weak Spt− phenotype (JIANG and STILLMAN 1992), we determined the effect of an *rgr1* mutation on promoters containing *Ty* insertions. In plasmid *his4*-912d-*lacZ*, expression of *lacZ* is driven by the *HIS4* promoter interrupted by a *Ty* δ insertion element. This reporter plasmid was transformed into wild-type, *sin4*, and *rgr1* mutants, and *lacZ* activity was measured. The *sin4* and *rgr1* mutations cause 20- and 90-fold increases, respectively, in expression of the *his4*-912δ-*lacZ* reporter (Table 3A). To determine the ability of *sin4* and *rgr1* to confer an Spt− phenotype, we constructed derivatives of strain FY56 (which contains the *his4*-912δ and *lys2*-128δ alleles) that contain the *sin4* null and *rgr1*-Δ2 truncation mutations. Both the *sin4* and *rgr1* mutations are able to suppress the *his4*-912d mutation, since these strains are phenotypically His+ (Table 3B). However, *sin4* and *rgr1* mutations are unable to suppress the *lys2*-128δ mutation. This result is not surprising for a weak *spt* mutation, since other *spt* mutations are known that suppress *his4*-912δ but not *lys2*-128δ (PRELICH and WINSTON 1993).

It has been shown that the *SPT6*, *SPT11* (*HTA1*), and *SPT12* (*HTB1*) genes, when present on a multicopy plasmid, can suppress the *his4*-912δ and *lys2*-128δ mutations (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.

TABLE 3

sin4 and rgr1 mutants have a weak Spt phenotype

Strain	Genotype	*his4*-912δ-*lacZ*	Increase
	A. Expression of a *his4*-912δ-*lacZ* reporter[a]		
DY2273	Wild type	0.3 ± 0.1	1×
DY2278	*sin4*	13 ± 0.4	22×
DY2010	*rgr1*	56 ± 3	93×
Strain	**Genotype**	**His phenotype**	**Lys phenotype**
	B. Suppression of *his4*-912δ and *lys2*-128δ[b]		
FY56	Wild type	−	−
DY2081	*sin4*	+	−
DY2578	*rgr1*	+	−

[a] Strains DY2273 (wild type), DY2278 (*sin4*), and DY2010 (*rgr1*) were transformed with plasmid pLL53, which contains a *his4*-912δ-*lacZ* reporter, and grown under uracil selection. Quantitative measurements of β-galactosidase activity were made.

[b] Suppression of the *his4*-912δ and *lys2*-128δ alleles in strains FY56 (wild type), DY2081 (*sin4*), and DY2578 (*rgr1*), was scored after replica plating to SC plates lacking histidine or lysine, respectively.

***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*-Δ2 truncation allele is viable but extremely unhealthy. The *sin4 rgr1*-Δ2 double mutant grows as well as the *rgr1*-Δ2 single mutant, and thus *rgr1* is epistatic to *sin4*.

A strain with a *sin4* mutation shows temperature sensitive growth at 37° (JIANG and STILLMAN 1992; CHEN *et al.* 1993). 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, *SIN4* overexpression from a multicopy plasmid is not able to suppress the *rgr1* temperature sensitivity. We have used a multicopy suppression screen to identify other genes capable of suppressing the 37° growth defect due to the *rgr1*-Δ2 mutation (L. LIAN, Y. W. JIANG and D. J. STILLMAN, unpublished results).

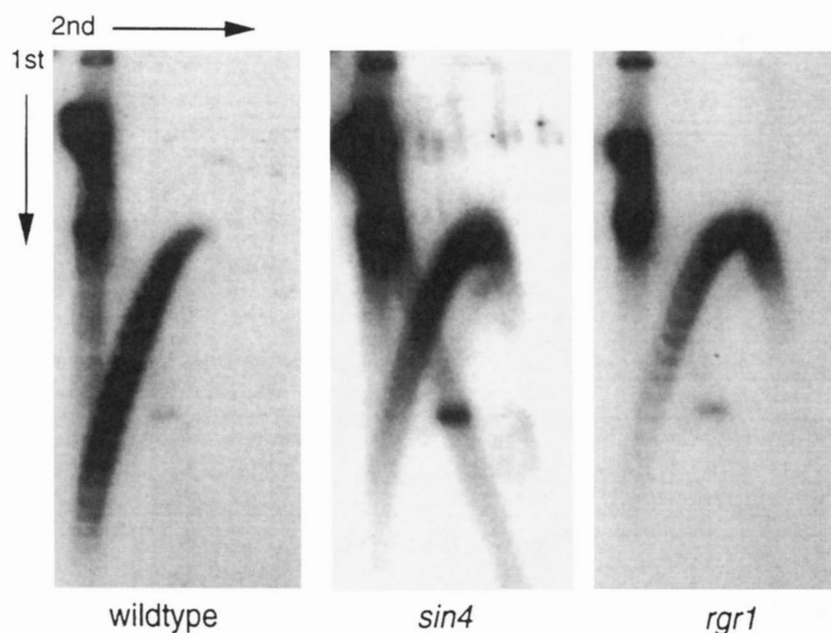

FIGURE 2.—Linking number of episomal DNA molecules is altered by *sin4* or *rgr1* mutations. DNA was isolated from logarithmically growing yeast cells, electrophoresed in two-dimensional chloroquine-agarose gels and subjected to Southern blot analysis with ^{32}P-labeled YCp50 DNA. Left, DY2273 (wild type); middle, DY2278 (*sin4*); right, DY2010 (*rgr1*). The directions of the first and second dimensions are indicated.

The *sin4* and *rgr1* 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 *rgr1* mutation has the same effect. As shown in Figure 2 the *sin4* and *rgr1* 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 *rgr1* 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 *rgr1* 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 Rgr1p physically interact: The fact that the *sin4* and *rgr1* mutations cause similar phenotypes suggested that the Rgr1p 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-S-Transferase-Rgr1p 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-Rgr1p protein was purified by glutathione chromatography. The results shown in Figure 3 (lane 3) demonstrate that Sin4p copurified with GST-Rgr1p. Control experiments demonstrate the specificity of the Sin4p-Rgr1p 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 Rgr1p fused to LexA, and antibody to lexA was used to detect the LexA-Rgr1p fusion protein. The results show that LexA-Rgr1p copurifies with GST-Sin4p (Figure 3, lane 9). LexA-Rgr1p does not bind to GST alone (Figure 3, lane 12), and thus GST-Sin4p is required for copurification of LexA-Rgr1p.

Although unlikely, it is possible that many proteins bind nonspecifically to the GST-Rgr1p and GST-Sin4p columns. To demonstrate specificity of the Rgr1p-Sin4p interaction, a Western blot containing the protein fractions from yeast expressing GST-Rgr1p or GST-Sin4p was probed with antisera to Spt2p (= Sin1p). The Spt2p protein does not bind to GST-Rgr1p 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-Rgr1p and GST-Sin4p columns contain very different protein species (data not shown).

We conclude that Sin4p and Rgr1p 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 *rgr1* mutations.

DISCUSSION

We have shown that *sin4* and *rgr1* mutants have many properties in common. Both mutants have a defect in

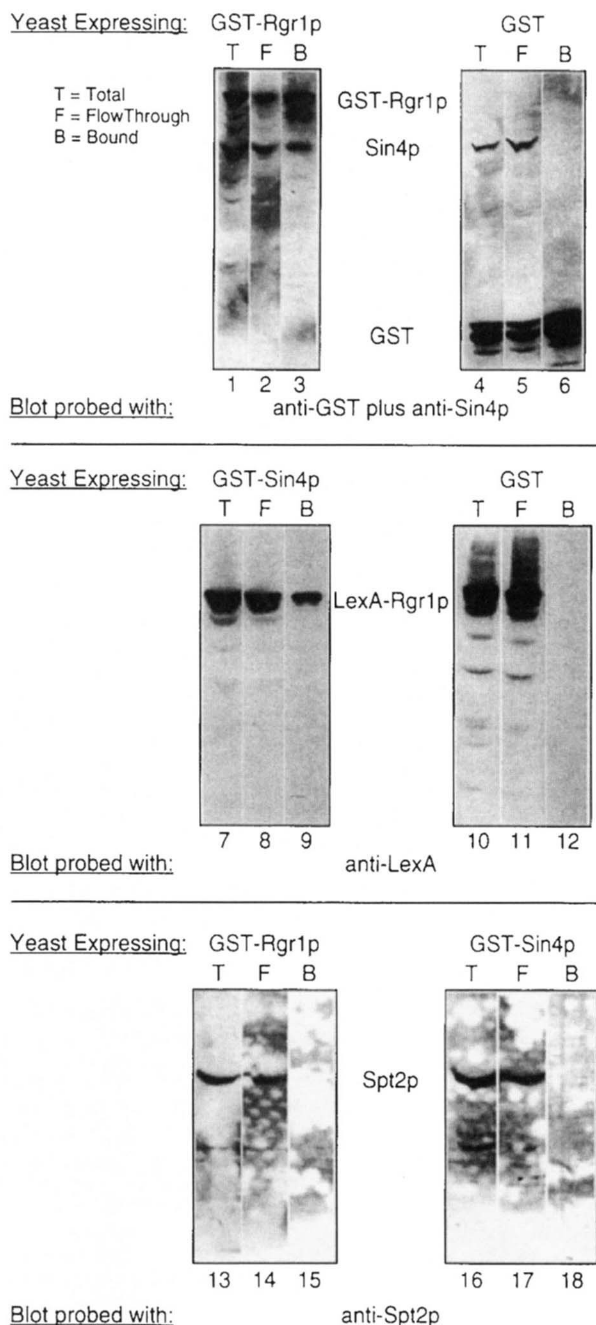

FIGURE 3.—Copurification of Sin4p and Rgr1p. Strain DY150 (wild type) contained the following plasmids: lanes 1–3, M1967 (GST-Rgr1p) and M1391 (*ADH*-Sin4p); lanes 4–6, pRD56 (GST vector) and M1391 (*ADH*-Sin4p); lanes 7–9, M1968 (GST-Sin4p) and M1981 (pLexA-Rgr1p); lanes 10–12, pRD56 (GST vector) and M1981 (pLexA-Rgr1p); lanes 13–15, M1967 (GST-Rgr1p) and M1391 (*ADH*-Sin4p); lanes 16–18, M1968 (GST-Sin4p) and M1981 (pLexA-Rgr1p). Protein extracts were prepared and fractionated by glutathione-agarose chromatography into flowthrough (F) and bound (B) fractions. The flowthrough and bound fractions (50 μl of each), along with 50 μg of the unfractionated total (T) protein mixture, were analyzed by immunoblotting with anti-GST-Sin4p antibody (lanes 1–6), anti-LexA antibody (lanes 10–12), or anti-Spt2p antibody (lanes 13–18). Some of the GST-Rgr1p (lane 2) and the GST (lane 5) protein remained in the supernatant, indicating that the GST proteins were not quantitatively absorbed by the glutathione-agarose beads.

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 δ 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 *rgr1* 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 *rgr1* temperature sensitive lethality. Finally, we have demonstrated that the Sin4p and Rgr1p 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 Rgr1p 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 Rgr1p 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* and *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* (*CYC8*) 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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