J. Biochem. 128, 575–584 (2000)

Genetic Characterization of *rbt* Mutants That Enhance Basal Transcription from Core Promoters in *Saccharomyces cerevisiae*[1]

Tatsuki Kunoh, Takeshi Sakuno,[2] Takakazu Furukawa, Yoshinobu Kaneko, and Satoshi Harashima[3]

Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871

Received June 6, 2000; accepted July 18, 2000

While this *Saccharomyces cerevisiae SIN4* gene product is a component of a mediator complex associated with RNA polymerase II, various studies suggest the involvement of Sin4 in the alteration of higher-order chromatin structure. Our previous analysis of a *sin4* mutant suggested that the mechanisms of transcriptional repression by Sin4 (mediator) and the Tup1-Ssn6 complex (general repressor) are different. To elucidate the way in which these two repression systems are interrelated, we isolated mutants that exhibit enhanced transcription of a reporter gene harboring the upstream activation sequence (UAS), but still are subject to Tup1-Ssn6–mediated repression. Besides *sin4*, *rgr1*, *tup1*, and *ssn6* mutants, we also obtained new mutants that enhance basal transcription even from a core promoter without UAS. Such mutants, designated *rbt* for regulator of basal transcription, can be classified into at least six complementation groups, *i.e.*, four single (*rbt1* to *rbt4*) and two apparently double (*rbt5 rbt6* and *rbt7 rbt8*) mutations. The phenotype of *rbt* mutants is dependent on the TATA box and not specific to the integration site or kind of core promoter. No significant difference in micrococcal nuclease (MNase) accessibility to the core promoter of test genes was observed between *rbt* mutants and the wild-type strain, indicating that the higher-order chromatin structure of the core promoter region is not significantly altered in these mutants. The *rbt1* to *rbt4* mutations are suppressed by the Δ*gal11* mutation as in the case of the *sin4* mutation, but give rise to a different profile from the *sin4* mutation with regard to the activity of some of the promoters. From these observations, we suggest that *RBT* gene product(s) could be novel mediators that act with or in close association with Sin4 but have a function distinct from that of Sin4. Moreover, the fact that *rbt* mutations nullify Tup1-Ssn6 general repressor–mediated repression is consistent with the idea that the mechanisms of Rbt (mediator)- and Tup1-Ssn6 (general repressor)–mediated repression are interconnected but substantially different.

Key words: basal transcription, general repressor Tup1-Ssn6, mediator, *Saccharomyces cerevisiae*, Sin4.

Transcriptional regulation requires the interactions of specific regulatory proteins with components of the transcription machinery. The specific regulatory proteins that are implicated in transcriptional activation and/or repression bind, directly or indirectly, to an upstream element containing UAS or upstream repression sequence (URS). The transcription machinery binds to a core promoter region including a TATA box as an essential element. The basal transcription is dependent on the core-promoter and requires RNA polymerase (RNAP II), general transcription factors (GTFs) such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, and mediators that are associated with the C-terminal heptapeptide repeat domain (CTD) of RNAP II.

We have been interested in the role of mediators in the regulation of the basal transcription of various promoters. Genetic and biochemical analyses in *Saccharomyces cerevisiae* have revealed that mediators consist of a large protein complex including proteins such as Nut1, Nut2, Rox3, Gal11, Sin4, Rgr1, Pgd1, Srbs, and Meds, and that mutations in the components of the mediator complexes lead to disregulated expression of a wide variety of genes (*1–9*). We previously identified a mutation of *SIN4*, the product of which is one of the most poorly understood mediator components, as a suppressor mutation that suppresses a mutation of the gene encoding the transcriptional activator Gcn4, which activates the expression of the *HIS5p-PHO5* reporter gene [*HIS5* promoter fused to the open reading frame (ORF) of *PHO5* encoding repressible acid phosphatase; rAPase], and found that basal transcription is increased by the *sin4* mutation in all core promoters tested

[1] This study was partially supported by a Grant-in-Aid for Scientific Research on Priority Areas (No. 08250210 and No. 09277214) to S.H. from the Ministry of Education, Science, Sports and Culture of Japan and a grant to T.K. from Foundation of Osaka University. The first and second authors equally contributed to this work.

[2] Present address: Department of Cellular Biology, Institute of Molecular and Cellular Biosciences, The University of Tokyo.

[3] To whom correspondence should be addressed. Tel: +81-6-6879-7420, Fax: +81-6-6879-7421, E-mail: harashima@gen.bio.eng.osaka-u.ac.jp

to date (*10*). It has also been reported that the micrococcal nuclease (MNase)–sensitivity of bulk chromatin is increased in the *sin4* mutant relative to that in the wild-type strain, and that the *sin4* mutation alters the number of nucleosomes in closed circular DNAs (*11*), whereas no effect on nucleosome positioning (*12*) was observed. Therefore, it was assumed that the enhanced basal transcription in the *sin4* mutant is caused by the alteration of the higher-order chromatin structure and not by nucleosome positioning. We also reported that enhanced basal transcription in the *sin4* mutant is not repressed by the Tup1-Ssn6 complex–mediated repression system, which represses transcription through the control of nucleosome positioning (*10*, *13*). In addition, Tup1-Ssn6 complex–mediated repression has been shown to be diminished in mutants of other components of the mediator complex, such as Srb8, Srb10, and Srb11 (*14*, *15*), suggesting that the two repression systems operate through different pathways and therefore the mechanisms of the repression of basal transcription by the mediator complex and the Tup1-Ssn6 complex are different.

To learn more about the interrelationships between the two repression systems, we isolated mutants that exhibit enhanced transcription of a reporter gene harboring UAS and which is subject to Tup1-Ssn6–mediated repression. Some of mutations identified occurr in *sin4*, *rgr1*, *tup1*, and *ssn6* while others apparently occur in unidentified genes. Mutants, designated as *rbt* for regulator of basal transcription, were classified into six complementation groups, *i.e.*, four single (*rbt1* to *rbt4*) and two apparently double (*rbt5 rbt6* and *rbt7 rbt8*) mutations. *rbt1* to *rbt4* mutants display several phenotypes that are quite similar to but distinct from those of the *sin4* mutant. We suggest that *RBT* gene product(s) could be novel mediators that act with or in close association with Sin4 but harbor a function distinct from that of Sin4. We also suggest that mutations such as *rbt* that lead to a defect in the repression of basal transcription nullify Tup1-Ssn6 general repressor–mediated repression, consistent with the idea that the mechanisms of Rbt (mediator)- and Tup1-Ssn6 (general repressor)–mediated repression are interconnected but substantially different.

MATERIALS AND METHODS

Media and Genetic Methods—The nutrient high-P_i (YPDA) and minimal (SD) media for *S. cerevisiae* (*16*) and the LB medium for *Escherichia coli* (*17*) were prepared as described previously. The minimal medium was supplemented with appropriate nutrients when necessary (*16*). Solid media contained 20 g of agar per liter. The genetic methods for *S. cerevisiae* have been as described previously (*18*). *S. cerevisiae* was transformed by the lithium-acetate method (*19*) and *E. coli* was transformed by the method of Morrison (*20*).

Strains—The yeast strains used in this work are listed in Table I. To construct the *rbt1-1* Δ*gal11*-double mutant (SH5512), a Δ*gal11::LEU2* disruptant (SH5488) showing an rAPase$^-$ phenotype was crossed with an *rbt1-1* mutant (SH5494) that displayed an rAPase$^+$ phenotype and the resultant diploids were subjected to tetrad analysis. Since the Δ*gal11::LEU2* disruption mutation can be monitored by the Leu$^+$ phenotype, the presence of an *rbt1-1* mutation in some meiotic segregants showing an rAPase$^-$ Leu$^+$ phenotype, and, therefore, having the supposed genotype of either Δ*gal11::LEU2* or *rbt1-1* Δ*gal11::LEU2*, was tested by crossing the segregants with the *rbt1-1* mutant (SH5511) and determining the rAPase activity of the colonies of the resultant diploids. One among several segregants, designated SH5512 and exhibiting the rAPase$^-$ Leu$^+$ phenotype, was verified to have the *rbt1-1* Δ*gal11::LEU2* double mutation since the resultant diploid exhibited the rAPase$^+$ phenotype. The *rbt2-1* Δ*gal11*-double (SH5517), *rbt3-1* Δ*gal11*-double (SH5711), and *rbt4-1* Δ*gal11*-double (SH5682) mutants were constructed and verified as described above using *rbt2-1* mutants (SH5497 and SH5520), *rbt3-1* mutants (SH5709 and SH5710), and *rbt4-1* mutants (SH5681 and SH5680), respectively, and the Δ*gal11::LEU2* disruptant (SH5487 and SH5513) (data not shown). *E. coli* TG1 (*17*) was used to manipulate the plasmid DNAs.

Plasmids—Construction of YIp-type plasmids, pAAV, pAAV-A, and pAAV-B, harboring the ΔUAS*PHO84p-PHO5*, $IME1_A$-ΔUAS*PHO84p-PHO5*, and $IME1_B$-ΔUAS*PHO84p-PHO5* reporter genes, respectively, was described previously (*21*). Construction of YCp-type plasmids harboring the *SUC2p-PHO5* fusion reporter gene (p1039), the ΔUAS*PHO84p-PHO5* fusion reporter gene (p891) and the ΔTATA*PHO5p-PHO5* fusion reporter gene (p1319) was described previously (*10*). Construction of control reporter gene, containing only an ORF of the *PHO5* gene (pSH39) was described previously (*22*). To construct the ΔUAS*PHO84p-PHO5* fusion reporter gene (p1244), a 2.1-kbp *Hin*dIII–*Sph*I fragment containing the *PHO5p-PHO5* fusion reporter gene from p714 (*10*) was inserted into the *Hin*dIII–*Sph*I site of YIp33 (*23*), resulting in plasmid p813. Then, a 0.3-kbp *Hin*dIII–*Bam*HI fragment containing the ΔUAS*PHO84* promoter from pAAV was cloned into the *Hin*dIII–*Bam*HI gap of p813. To construct the ΔUAS*PHO5p-PHO5* fusion reporter gene (p890), a 3.3-kbp *Bam*HI fragment from pPHO5 (*24*) was inserted into the *Bam*HI site of YIp5 (*23*). Then, the core promoter region of the *PHO5* gene was amplified by PCR using the resultant plasmid as the template and oligonucleotides designated OLI184 and OLI142 (Table II) as the forward and reverse primers, respectively. The amplified product was doubly digested with *Hin*dIII and *Bam*HI, and cloned into the *Hin*dIII–*Bam*HI gap of pSH39, resulting in plasmid p890. To construct the YCp-type plasmid harboring the ΔUAS*CYC1p-PHO5* fusion reporter gene (p1762), a 0.3-kbp *Hin*dIII–*Bam*HI fragment carrying the core promoter region of the *CYC1* gene from p1108 (*10*) was inserted into the *Hin*dIII–*Bam*HI gap of p891. YCp-type plasmids carrying the *TUP1* (p1161) or *SSN6* (p1162) gene were derived from pRS315 (*25*). YEp-type plasmid carrying the *RGR1* gene (pSAKO34) (a generous gift from A. Sakai) was described previously (*26*). To construct YCp-type plasmid carrying the *NCB1* (p1854) gene, an approximately 1.8-kbp fragment was amplified by PCR using chromosomal DNAs of strain MCY3605 (*27*) as templates and oligonucleotides designated as OLIG27 and OLIG28 (Table II) as the forward and reverse primers, respectively. The amplified product was cloned into the pT7blue(R) Vector (Novagen). An approximately 1.8-kbp *Sal*I–*Sma*I fragment was inserted into the *Sal*I–*Sma*I gap of pRS313 (*25*). To construct YCp-type plasmid carrying the *NCB2* (p1855) gene, an approximately 1.6-kbp fragment was amplified by PCR using chromosomal DNAs of strain MCY3605 as templates and

TABLE I. *S. cerevisiae* strains used in this study.

Strain	Genotype	Source
SH682	*MATa ura1 ura2 trp1 lys2 pho3-1 pho5-1*	Our laboratory
SH2921	*MATa ura3-52 his1-29 trp1 pho3-1 pho5-1 gcn4-103 sin4-206*	Our laboratory
SH3700	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1*	Our laboratory
SH4761	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	Our laboratory
SH4358	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1*	Our laboratory
SH4433	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 ura3-52::[IMEIA-ΔUAS.PHO84p-PHO5+URA3]*	Our laboratory
SH5397	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt1-1 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5400	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt2-1 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5398	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt4-1 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5399	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 sin4-162 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5401	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt1-1*	This study
SH5402	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt2-1*	This study
SH5404	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt4-1*	This study
SH5403	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 sin4-162*	This study
SH5219	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt1-2 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5220	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt2-1 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5221	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt2-2 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5509	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt5 rbt6 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5222	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt7 rbt8 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5223	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt1-3*	This study
SH5224	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt3-1*	This study
SH5225	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt2-2*	This study
SH5510	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt5 rbt6*	This study
SH5226	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt7 rbt8*	This study
HSY5-3A	*MATa ura3 leu2 trp1 ade1 Δgal11::LEU2*	M. Nishizawa
SH5486	*MATa ura3 leu2 trp1 ade1 Δgal11::LEU2 ura3..[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5487	*MATa ura3 leu2 trp1 pho3-1 pho5-1 Δgal11::LEU2 ura3::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5488	*MATα ura3 leu2 trp1 pho3-1 pho5-1 Δgal11::LEU2 ura3::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5489	*MATa ura3-52 leu2-3,112 trp1 his4 lys2 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5491	*MATα ura3-52 leu2-3,112 trp1 lys2 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5494	*MATa ura3-52 leu2-3,112 trp1 lys2 pho3-1 pho5-1 rbt1-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5497	*MATα ura3-52 leu2-3,112 trp1 his4 lys2 pho3-1 pho5-1 rbt2-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5498	*MATα ura3-52 leu2-3,112 his4 trp1 lys2 pho3-1 pho5-1 rbt2-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5511	*MATa ura3-52 leu2-3,112 trp1 pho3-1 pho5-1 rbt1-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5512	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt1-1 Δgal11::LEU2 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5515	*MATa ura3-52 leu2-3,112 Δhis3 a/o his4 trp1 lys2 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5517	*MATα ura3-52 leu2-3,112 his4 pho3-1 pho5-1 rbt2-1 Δgal11::LEU2 ura3::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5518	*MATa ura3-52 leu2-3,112 trp1 pho3-1 pho5-1 ura3::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5520	*MATa ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt2-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5504	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 Δpho84::CgHIS3 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5506	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt2-1 Δpho84::CgHIS3 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5508	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 rbt4-1 Δpho84::CgHIS3 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5507	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2 pho3-1 pho5-1 sin4-162 Δpho84::CgHIS3 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5503	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 Δpho84::CgHIS3 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5505	*MATα ura3-52 leu2-3,112 Δhis3 trp1 ade2 pho3-1 pho5-1 rbt1-2 Δpho84::CgHIS3 ura3-52::[IME1A-ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5680	*MATa ura3-52 leu2-3,112 trp1 lys2-801 rbt4-1 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5681	*MATα ura3-52 leu2-3,112 trp1 lys2-801 rbt4-1 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5513	*MATa ura3-52 leu2-3,112 Δhis3 trp1 pho3-1 pho5-1 Δgal11::LEU2 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5682	*MATα ura3-52 leu2-3,112 Δhis3 trp1 rbt4-1 Δgal11::LEU2 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5687	*MATα ura3-52 his1-29 trp1 lys2 Δsin4::TRP1 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5686	*MATa ura3-52 trp1 lys2 Δsin4::TRP1 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5685	*MATα ura3-52 leu2 his1-29 trp1 Δsin4::TRP1 Δgal11::LEU2 pho3-1 pho5-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5709	*MATα ura3-52 leu2-3,112 trp1 pho3-1 pho5-1 rbt3-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5710	*MATa ura3-52 leu2-3,112 trp1 lys2-801 pho3-1 pho5-1 rbt3-1 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study
SH5711	*MATα ura3-52 leu2-3,112 Δhis3 trp1 lys2-801 pho3-1 pho5-1 rbt3-1 Δgal11:: LEU2 ura3-52::[ΔUAS.PHO84p-PHO5+URA3]*	This study

TABLE II. Oligonucleotides used.

Oligonucleotides	Sequence
OLIG21	5'- AAATCAGTGAGATCGGTGCAGTTATGCACCAAATGTCGTGCACAGGAAACAGCTATGACC 3'
OLIG22	5'- CAATATGAGCAAAATCATTCAAATGGTTGTGGAAGGCCATGTTGTAAAACGACGGCCAGT -3'
OLIG27	5'- TGTGTCGACGATCTATTACATTATGGGTGG -3'
OLIG28	5'- TGTCCCGGGAGAATCCTCGAGCTAGCCCTT -3'
OLIG29	5'- TGTCTCGAGTGTCTTTGATTTCGTAAGAAT -3'
OLIG30	5'- TGTCCCGGGAAATAGTGGAATGTGATAGTT -3'
OLIG31	5'- TGTCTCGAGAGGTGTACCAGTAAACAAATA -3'
OLIG32	5'- TGTCCCGGGGAAAAAGCATGCCTGTCTCAA -3'
PGD1c	5'- CTCGGATCCGTCGAGTACGAGAACCTTTC -3'
OLIG33	5'- TGTCTCGAGTATGTCCCTTTTTGGAGCAAT -3'
OLIG38	5'- CGCGGATCCTTTTAAAACAAAGAGTATAA -3'
OLIG39	5'- CGCGGATCCTTGAAAGGATTTTTTGAACA -3'
OLI184	5'- CTCAAGCTTTATCAAATTGGTCACCTTAC -3'
OLI142	5'- CTCGGATCCTTGCTCTATTTGTTGTTGTT -3'

oligonucleotides designated OLIG29 and OLIG30 (Table II) as the forward and reverse primers, respectively. The amplified product was cloned into the pT7blue(R) Vector. A *Xho*I–*Sma*I fragment of about 1.6-kbp was inserted into the *Sal*I-*Sma*I gap of pRS313. YCp-type plasmids carrying the *ROX3* (p1856) and *PGD1* (p1857) genes were con-

structed in the same way as p1855 using OLIG31 and OLIG32 for the *ROX3* gene, and PGD1c and OLIG33 for the *PGD1* gene. To construct a YCp-type plasmid carrying the *SRB11* (p2032) gene, an approximately 2.5-kbp fragment was amplified by PCR using chromosomal DNAs of strain MCY3605 as templates and oligonucleotides designated OLIG38 and OLIG39 (Table II) as the forward and reverse primers, respectively. The amplified product was cloned into the pT7blue(R) Vector. A *Bam*HI fragment of about 2.5-kbp was inserted into the *Bam*HI site of pRS313.

Disruption of the PHO84 Gene—Disruption of the *PHO84* gene was conducted by the method of PCR-mediated gene disruption described by Sakumoto *et al.* (*28*) using the plasmid pCgHIS3 carrying the *Candida glabrata HIS3* gene as the template and oligonucleotides designated OLIG21 and OLIG22 (Table II) as the forward and reverse primers, respectively. The disruption was verified by Southern blot analysis. A 0.9-kbp *Hin*dIII–*Bam*HI fragment from p602 (*10*) was used as a probe (data not shown).

Biochemical Methods—Methods to detect rAPase activity in colonies and to assay rAPase activity using intact *S. cerevisiae* cells were described previously (*18*). The preparation of RNAs from *S. cerevisiae* cells for Northern blot analysis was described previously (*18*). DNA manipulation was according to procedures described previously (*17*). The method for Southern blot analysis was described previously (*17*).

Isolation and Analysis of Chromatin—Yeast nuclei were isolated from exponentially growing (OD_{660} = 1.0) cells of each strain cultured in YPDA medium as previously described (*29*). Digestion with micrococcal nuclease (MNase) and DNA purification were performed as previously described (*30*) with the following modifications: (i) The nuclear pellet (1.0 g) was gently suspended in 7.0 ml of digestion buffer. (ii) The suspension was divided into 600 μl aliquots and the nuclei digested at 37°C for 5 min using a series of MNase dilutions (0, 1.25, 2.5, 5, 10, and 40 units/ml) (Boehringer Mannheim). The DNA samples were resolved by electrophoresis in a 1.2% agarose gel.

RESULTS

Isolation of Mutants That Enhance Transcription from a Reporter Gene Subjected to Tup1-Ssn6 Mediated Repression—To isolate mutants that exhibit enhanced transcription from a reporter gene that is subject to repression mediated by the Tup1-Ssn6 complex, we used the *IME1*$_A$-Δ*UASPHO84p-PHO5* (pAAV-A) and *IME1*$_B$-Δ*UASPHO84p-PHO5* (pAAV-B) reporter genes since we previously revealed that both *IME1*$_A$ and *IME1*$_B$ harbor a Tup1-Ssn6 complex–dependent URS (*21*). Wild-type α-haploid strains harboring the *IME1*$_A$-Δ*UASPHO84p-PHO5* (in SH4761) or *IME1*$_B$-Δ*UASPHO84p-PHO5* (in SH4433) reporter gene integrated at the *ura3-52* locus were mutagenized by EMS, and mutants that enhanced the activity of the reporter gene (rAPase$^+$ phenotype) were screened by staining (see "MATERIALS AND METHODS"). Of 140,000 colonies screened, we found 175 showing the rAPase$^+$ phenotype (Table III). To distinguish mutants exhibiting a dominant and nonmater phenotype, all mutants were crossed with wild-type **a**-cells (SH682). Of 175 mutants 158 were mating-competent, and colonies of the 158 resultant diploids were stained for rAPase activity to monitor the expression of the reporter gene. The number of dominant mutants was 52 because diploids crossed with these mutants showed the rAPase$^+$ phenotype. Analyses of these dominant mutants will be conducted in a separate study. Sterile mutants also were not analyzed in this study. However, seven of 17 sterile mutants were shown to harbor the *tup1* mutation as verified by suppression of the rAPase$^+$ phenotype of transformants of these mutants with a YCp-type plasmid carrying the *TUP1* gene (p1161). Interestingly, 75 of 106 recessive and mating-competent mutants were revealed to carry a *sin4* mutation because diploids constructed by crossing these mutants with the *MAT*a-*sin4* mutant (SH2921) showed the rAPase$^+$ phenotype. Since we knew from this result that these mutations include those of the mediators as well as *tup1*, we introduced a YCp-type plasmid carrying the *SSN6* gene (p1162) and a YEp-type plasmid carrying the *RGR1* gene (pSAKO34) into the 31 remaining mutants, and colonies of the resultant transformants were stained to determine rAPase activity. The rAPase$^+$ phenotypes of four and ten mutants were suppressed by introduction of the plasmid borne-*SSN6* gene and *RGR1* gene, respectively, indicating that these mutants harbored the *ssn6* and *rgr1* mutations, respectively.

These results led to us to examine whether mutations responsible for the rAPase$^+$ phenotype of the remaining mutants are effective in enhancing basal transcription from a core promoter. The Δ*UASPHO84p-PHO5* (pAAV) reporter gene was introduced into the 17 remaining mutants and colonies of the resultant transformants were stained for rAPase activity. All the transformants still showed the rAPase$^+$ phenotype, indicating that activation can occur even for reporter genes with only a core promoter. It should be noted that the rAPase$^+$ phenotype produced by these mutations cannot be repressed by Tup1-Ssn6 complex–mediated repression because the mutants were originally screened to isolate those that enhance the transcription of the *IME1*$_A$ and *IME1*$_B$ promoters harboring URS subject to repression by the Tup1-Ssn6 complex. All these observations suggest that mutations that destroy repression of basal transcription nullify Tup1-Ssn6–mediated repression.

TABLE III. **Summay of sceening for mutants that exhibited enhanced transcription from Δ*UASPHO84* promoter.**

Reporter gene	Mutation	Number of mutants
IME1p-Δ*UASPHO84p-PHO5*	Nonmater	17
(Region A or Region B)	Dominant	52
	tup1	7
	ssn6	4
	sin4	75
	rgr1	10
Δ*UASPHO84p-PHO5*	*rbt*	17
Total		175

TABLE IV. **Classification of *rbt* mutants.**

Complementation group	Number of mutants
rbt1	7
rbt2	2
rbt3	1
rbt4	5
rbt5 rbt6[a]	1
rbt7 rbt8[a]	1

[a]In these mutants, two or more genes are responsible for the *rbt* phenotype.

Seventeen recessive mutants that showed increased basal transcription from a core promoter were further analyzed in this study.

Genetic Complementation of rbt Mutants—To classify the 17 mutants, we determined by tetrad analysis whether the rAPase$^+$ phenotype segregates through meiosis as a single-gene trait. In 15 of 17 mutants, the rAPase$^+$ phenotype segregated as a single-gene trait in more than eight asci examined. However, two or more genes were suggested to be responsible for the rAPase$^+$ phenotype in the remaining two mutants (data not shown). We next performed complementation tests. From these analyses, we defined at least six complementation groups, two of which represent apparently double mutations as described above (data not shown). We designated these mutants *rbt1* to *rbt4* (regulator of basal transcription) for a single mutation and *rbt5 rbt6* and *rbt7 rbt8* for double mutations (Table IV). The staining of colonies and measurements of rAPase activity in representative mutants from each complementation group are shown in Fig. 1. We also conducted Northern blot analysis to determine whether enhanced rAPase activity is caused at the level of transcription. As shown in Fig. 2, the level of transcription of a reporter gene in the *rbt* mutants was higher than that in the wild-type strain, indicating that the rAPase$^+$ phenotype in *rbt* mutants is caused at the level of transcription.

rbt Mutations Do Not Occur in the NCB1, NCB2, PGD1, ROX3, and SRB11 Genes—The *rox3* mutant has been shown to enhance the basal transcription of a diverse set of promoters (*3*, *7*). It has been reported that basal transcription from a UAS less-*SUC2* promoter is activated in an *ncb1* mutant and that the Ncb1 protein binds to TBP as a heterodimer with Ncb2 (*31*, *32*). The *pgd1* mutant shows quite similar phenotypes to *sin4* and *rgr1* mutants (*2*, *33*). The *srb11* mutation was identified as a suppressor of the mutation of *SWI4*, which is a transcriptional activator for several genes (*7*). Therefore, we determined whether *RBT* genes are identical to the *NCB1, NCB2, PGD1, ROX3*, or *SRB11* gene; single *rbt1-1, rbt2-1, rbt3-1, rbt4-1*, and double *rbt5 rbt6* and *rbt7 rbt8* mutants were transformed with YCp-type plasmids carrying the *NCB1* (p1854), *NCB2* (p1855), *PGD1* (p1856), *ROX3* (p1857), or *SRB11*(p2032) gene and colonies of the resultant transformants were stained for rAPase activity. The levels of rAPase activity of colonies of these transformants were higher than those of transformants harboring the control plasmid pRS313, indicating that enhanced transcription in the *rbt* mutants is not suppressed by plasmid-borne *NCB1, NCB2, PGD1, ROX3*, or *SRB11* gene (data not shown), indicating that the *RBT* genes are not identical to the *NCB1, NCB2, PGD1, ROX3*, and *SRB11* genes.

rbt Mutations Are Effective Irrespective of Chromosomal Position and Kind of Core Promoter—We examined whether the enhanced basal transcription in the *rbt* mutants occurs only in reporter genes with a core promoter integrated at the *ura3* locus. The Δ*UASPHO84p-PHO5* reporter gene (p1244) is integrated at the *leu2-3,112* locus of the *rbt* mutants. Separately, the YCp-plasmid borne Δ*UASPHO84p-PHO5* reporter gene was also introduced into the *rbt* mutants. All transformants showed the rAPase$^+$ phenotype, indicating that enhanced basal transcription in the *rbt* mutants occurrs in the reporter gene

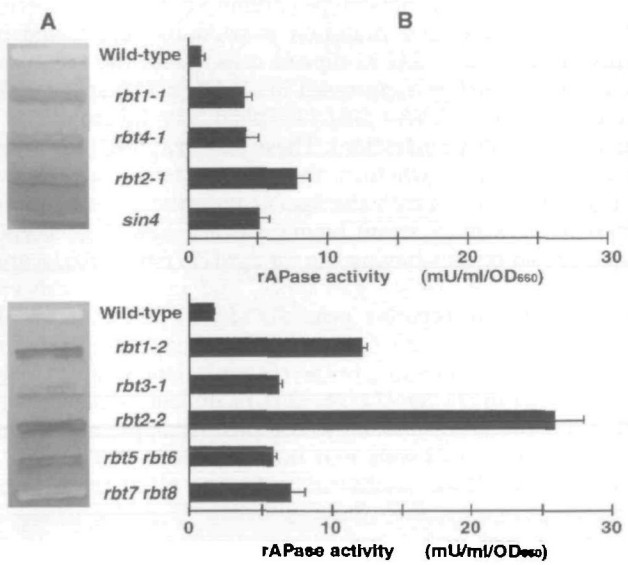

Fig. 1. **Staining of colonies and measurement of rAPase activity in representative *rbt* mutants from each complementation group.** Upper panel: wild-type strain (SH4761) and *rbt1-1* (SH5397), *rbt2-1* (SH5400), and *rbt4-1* (SH5398) mutants harboring the *IME1*$_A$*-PHO84p-PHO5* fusion reporter gene (pAAV-A) integrated at the *ura3-52* locus. Lower panel: the wild-type strain (SH4433) and *rbt1-2* (SH5219), *rbt3-1* (SH5220), *rbt2-2* (SH5221), *rbt5 rbt6* (SH5509), and *rbt7 rbt8* (SH5222) mutants harboring the *IME1*$_B$-Δ*UASPHO84p-PHO5* fusion reporter gene (pAAV-B) integrated at the *ura3* locus. The copy numbers of the fusion reporter genes in these strains were not determined. However, the wild-type and mutant strains listed in each panel must have the same copy number because each mutant strain was directly derived from the respective wild-type strains by *in vivo* mutagenesis. (A) These strains were grown on YPDA plates for one day and the colonies were stained using a staining method specific for rAPase activity (*18*). (B) For measurement of rAPase activity, cells of these strains were grown at 30°C in YPDA medium to an OD_{600} of 1.0, washed once with water and resuspended in an equal volume of water. The measurement of rAPase activity in cell suspensions has been described previously (see "MATERIALS AND METHODS"). The results are averages of at least three independent experiments and standard deviations are indicated.

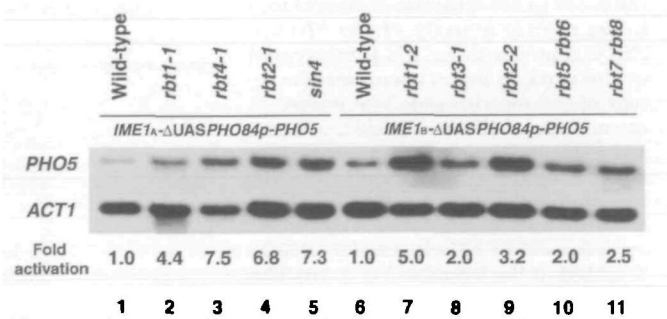

Fig. 2. **Enhanced rAPase activity in *rbt* mutants is caused at the transcriptional level.** RNA samples were prepared from cells of the strains described in the legend to Fig. 1, which were grown at 30°C in 10 ml of YPDA medium to an OD_{600} of 1.0. The Northern blot was hybridized with the *PHO5* probe. A 1.8-kbp *Sal*I-fragment from pAAV (*21*) was used as a probe to detect the *PHO5* transcript and an approximately 1.0-kbp *Bam*HI–*Hin*dIII fragment from plasmid p448 was used as a probe to detect the *ACT1* transcript. Fold activation compared with the wild-type strains was calculated using NIH Image 1.62.

integrated at the *leu2* locus and on the plasmid (Table V and data not shown).

We also examined whether *rbt* mutations result in enhanced transcription from the core promoter of genes other than *PHO84*. Plasmid-borne Δ*UASCYC1p-PHO5p* (p1762) and Δ*UASPHO5p-PHO5* (p890) reporter genes were introduced into the *rbt* mutants (Table V). The rAPase activity of the *rbt* mutants carrying the reporter genes was higher than that of the wild-type strain, indicating that the effects of *rbt* mutations are not specific to the Δ*UASPHO84* promoter. From these results, we suggest that the Rbt phenotype, *i.e.*, enhanced transcription from core promoters, is not specific to a particular chromosomal position of the reporter gene or to the kind of core promoter.

The Effect of rbt Mutations Requires a TATA-Element—Transcription from some promoters, including, promoters of *PHO5, IME1*, and *HIS5* genes integrated at the *ura3-52* locus, is increased by the *sin4* mutation even under repressive conditions, and enhanced basal transcription due to the *sin4* mutation is dependent on the TATA-element (*10*). Therefore, we investigated whether *rbt* mutations display similar characteristics to the *sin4* mutation. For this purpose, we used the *PHO5p-PHO5* reporter gene (p714). This gene was integrated at the *ura3-52* locus in the *rbt* mutants and rAPase staining of transformant colonies was conducted on YPDA medium in which conditions are repressive to the *PHO5* gene (Fig. 3A). In all but the *rbt4-1* mutant that was less affected by the *rbt4-1* mutation, basal transcription from the *PHO5* promoter was enhanced (Fig. 3). Then, to examine whether the effect of the *rbt* mutations is dependent on TATA-elements, the Δ*TATAPHO5p-PHO5* reporter gene (p1319) was integrated at the *ura3-52* locus in the *rbt* mutants (Fig. 3B). No stimulatory effect of the *rbt* mutations was observed for the *PHO5* promoter containing a mutation in the TATA element (TATA to TGCA) (Fig. 3B and Ref. *10*). These observations indicate that enhanced basal transcription in the *rbt* mutations requires TATA elements.

rbt Mutations Display Genetic Interaction with the Δgal11 Mutation—We have isolated a dominant *ABE1-1* (Activator of Basal Expression) mutant that suppresses the *sin4* mutation, and found that the *ABE1* gene is identical to the *GAL11* gene (Mizuno *et al.*, manuscript in preparation). Gal11 is known to be a component of the mediator complex and is suggested to activate transcription by stimulating CTD phosphorylation by TFIIH (*34*). We also noticed that the *sin4* mutant and the *Δgal11* disruptant exhibited the haplo-insufficiency phenotype: enhanced basal transcription due to the *sin4* mutation is partially suppressed in *sin4 Δgal11/sin4 GAL11*-diploid cells, while the enhancement is completely suppressed in *sin4 Δgal11*-haploid cells and *sin4 Δgal11/SIN4 GAL11*-diploid cells (Mizuno *et al.*, manuscript in preparation). These observations motivated us to determine whether the *rbt* mutations also show genetic interaction with the *Δgal11* mutation. We have constructed a haploid strain having the *rbt Δgal11* genotype, and diploid strains having the *rbt Δgal11/RBT GAL11* and *rbt Δgal11/rbt GAL11* genotypes, and examined the expression of the reporter gene. As shown in Fig. 4, in all cases of the *rbt1* to *rbt4* mutants, the enhanced expression of the reporter gene due to the *rbt* mutations was partially suppressed in *rbt Δgal11/rbt GAL11* diploid cells, whereas that due to *rbt* mutations was completely suppressed in the *rbt Δgal11* haploid cells and the *rbt Δgal11/RBT GAL11* diploid cells. These results indicate that all of the *rbt1* to

TABLE V. **rAPase activity of plasmid-borne reporter genes harboring several core promoters in *rbt* mutants.**

Strains	rAPase activity (mU/ml/OD_{660})		
	Δ*UASPHO84p-PHO5*	Δ*UASCYC1p-PHO5*	Δ*UASPHO5p-PHO5*
Wild-type	4.3 ± 1.7 (—)[a]	3.1 ± 0.1 (—)[a]	2.5 ± 0.1 (—)[a]
rbt1-1	10.2 ± 4.3 (2.4)	8.1 ± 0.3 (2.7)	4.5 ± 0.3 (1.8)
rbt2-1	21.1 ± 2.0 (4.9)	5.9 ± 0.1 (1.9)	3.4 ± 0.3 (1.4)
rbt4-1	5.7 ± 1.4 (1.3)	33.6 ± 1.5 (11.0)	14.8 ± 3.5 (6.0)
sin4	11.8 ± 1.0 (2.8)	14.4 ± 0.3 (4.7)	6.9 ± 2.6 (2.8)
Wild-type	1.8 ± 0.2 (—)	3.1 ± 0.1 (—)	1.6 ± 0.4 (—)
rbt1-2	2.4 ± 0.4 (1.4)	5.0 ± 0.1 (1.6)	2.4 ± 0.2 (1.5)
rbt2-2	3.0 ± 0.2 (1.7)	6.3 ± 0.6 (2.0)	5.6 ± 0.7 (3.5)
rbt3-1	3.3 ± 0.9 (1.8)	7.1 ± 0.3 (2.3)	3.0 ± 0.6 (1.8)
rbt5 rbt6	10.0 ± 0.5 (5.8)	8.4 ± 0.2 (2.7)	3.5 ± 0.1 (2.1)
rbt7 rbt8	4.3 ± 0.8 (2.5)	12.3 ± 0.1 (4.0)	4.5 ± 1.1 (2.8)

[a]Fold activation compared with the wild-type strain.

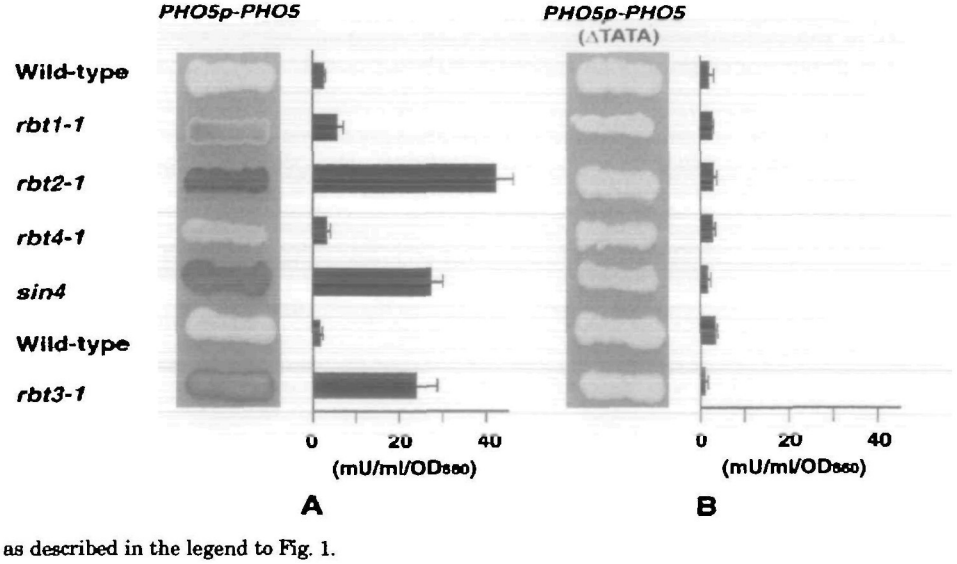

Fig. 3. **(A) In *rbt* mutants as in *sin4* mutants, rAPase activity of the *PHO5p-PHO5* reporter gene (p714) integrated at the *ura3-52* locus is enhanced.** One copy of the reporter gene was present in *rbt* mutants (SH5401, SH5402, SH5404, and SH5224), the *sin4* mutant (SH5403), and the isogenic wild-type strains (SH3700 and SH4358) as determined by Southern-blot analysis. Methods for staining and the measurement of rAPase activity were as described in the legend to Fig. 1. **(B) The enhanced transcription in the *rbt* mutants is dependent on the TATA-element.** *rbt* mutants, *sin4* mutant and the isogenic wild-type strains described in (A) were transformed with the Δ*TATAPHO5p-PHO5* reporter gene (p1319) integrated at the *ura3-52* locus. One copy of the reporter gene was present in these transformants, as determined by Southern-blot analysis. These transformants were stained on YPDA plates. rAPase activity was measured as described in the legend to Fig. 1.

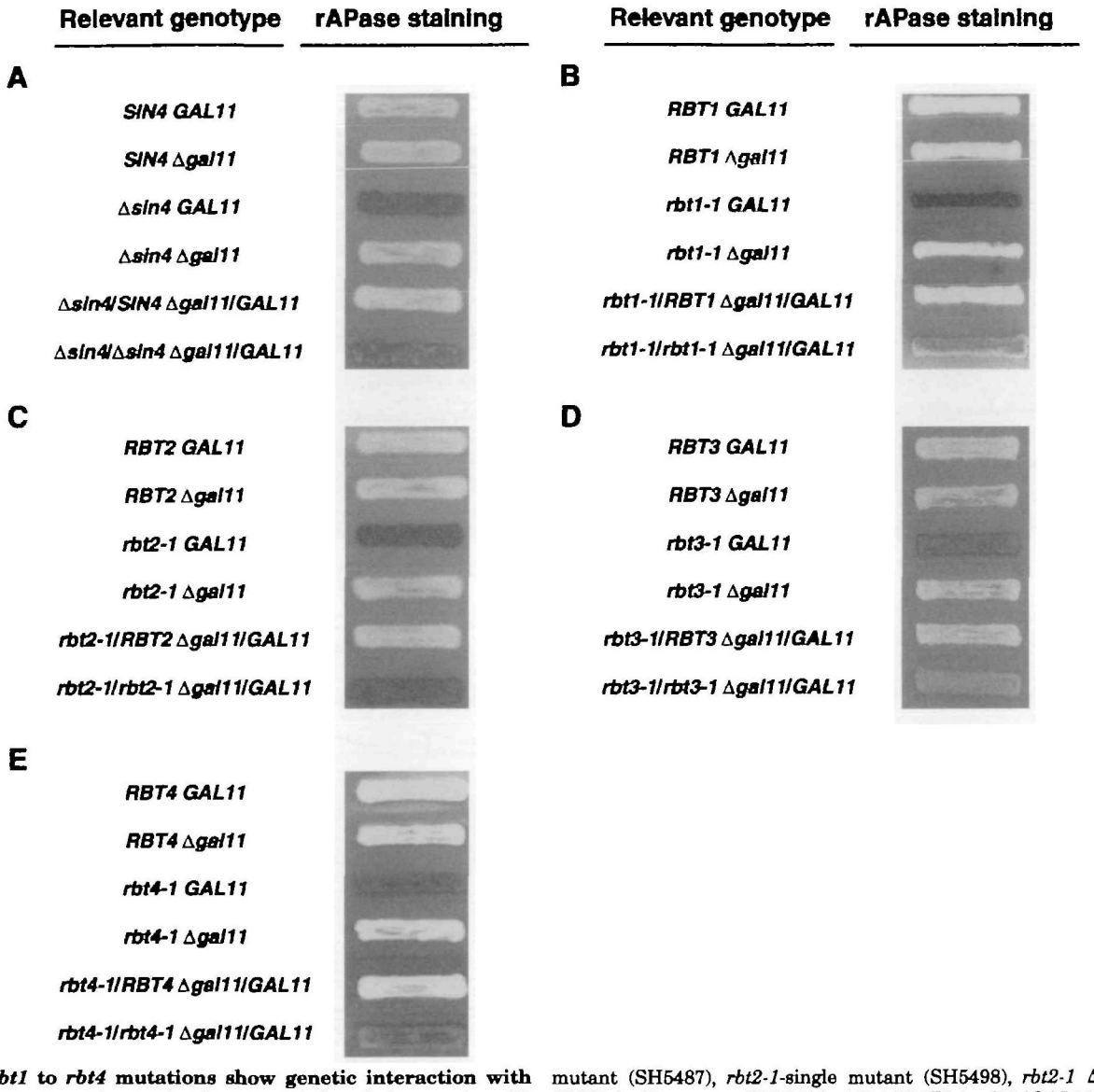

Fig. 4. ***rbt1* to *rbt4* mutations show genetic interaction with the Δ*gal11* mutation as in the case of the *sin4* mutation.** All strains harbored the ΔUAS*PHO84p-PHO5* reporter gene (pAAV) integrated at the *ura3-52* locus. (A) Colonies of the wild-type strain (SH5489), Δ*gal11::LEU2* mutant (SH5513), Δ*sin4::TRP1* mutant (SH5696), Δ*gal11::LEU2* Δ*sin4::TRP1*-double mutant (SH5695), Δ*sin4::TRP1/SIN4* Δ*gal11/GAL11*-diploid (SH5695 X SH5489), and Δ*sin4::TRP1/*Δ*sin4::TRP1* Δ*gal11/GAL11*-diploid (SH5695 X SH5696) were stained as described in the legend to Fig. 1. (B) The wild-type strain (SH5491), Δ*gal11::LEU2* mutant (SH5487), *rbt1-1*-single mutant (SH494), *rbt1-1* Δ*gal11::LEU2*-double mutant (SH5512), *rbt1-1/RBT1* Δ*gal11/GAL11*-diploid (SH5512 X SH5515) and *rbt1-1/rbt1-1* Δ*gal11/GAL11*-diploid (SH5512 X SH5511) were stained on YPDA plates. (C) Colonies of the wild-type strain (SH5491), Δ*gal11::LEU2* mutant (SH5487), *rbt2-1*-single mutant (SH5498), *rbt2-1* Δ*gal11::LEU2*-double mutant (SH5517), *rbt2-1/RBT2* Δ*gal11/GAL11*-diploid (SH5517 X SH5518), and *rbt2-1/rbt2-1* Δ*gal11/GAL11*-diploid (SH5517 X SH5520) were stained as described in the legend to Fig. 1. (D) The wild type-strain (SH5489), Δ*gal11::LEU2* mutant (SH5513), *rbt3-1*-single mutant (SH5709), *rbt3-1* Δ*gal11::LEU2*-double mutant (SH5711), *rbt3-1/RBT3* Δ*gal11/GAL11*-diploid (SH5711 X SH5489), and *rbt3-1/rbt3-1* Δ*gal11/GAL11*-diploid (SH5711 X SH5710) were stained on YPDA plates. (E) The wild type-strain (SH5489), Δ*gal11::LEU2* mutant (SH5513), *rbt4-1*-single mutant (SH5680), *rbt4-1* Δ*gal11::LEU2*-double mutant (SH5682), *rbt4-1/RBT4* Δ*gal11/GAL11*-diploid (SH5682 X SH5489), and *rbt4-1/rbt4-1* Δ*gal11/GAL11*-diploid (SH5682 X SH5680) were stained as described in the legend to Fig. 1.

rbt4 mutations interact genetically with the Δ*gal11* mutation as *sin4* mutation does. Since this behavior of the *rbt* mutations is very similar to that of the *sin4* mutation, we conclude that Rbt1 to Rbt4 function in close association with Sin4 and Gal11 in the regulation of basal transcription.

Chromatin Structure of the Core Promoter Region in rbt Mutants—MNase that cleaves linker DNA located between nucleosomes is commonly used to monitor chromatin structure. Macatee *et al.* (*12*) have reported that MNase accessibility is altered at histone loci (H3 and H4) and at the *HMLα* locus as a result of the loss of the *SIN4* gene. Therefore, we examined whether the chromatin structure in the core promoter region of the ΔUAS*PHO84p-PHO5* reporter gene integrated at the *ura3-52* locus is altered in the *rbt* mutants. The MNase digestion pattern, determined by staining with EtBr, of bulk chromatin isolated from the wild-type strain, and *rbt1-2*, *rbt2-1*, *rbt4-1*, and *sin4* mu-

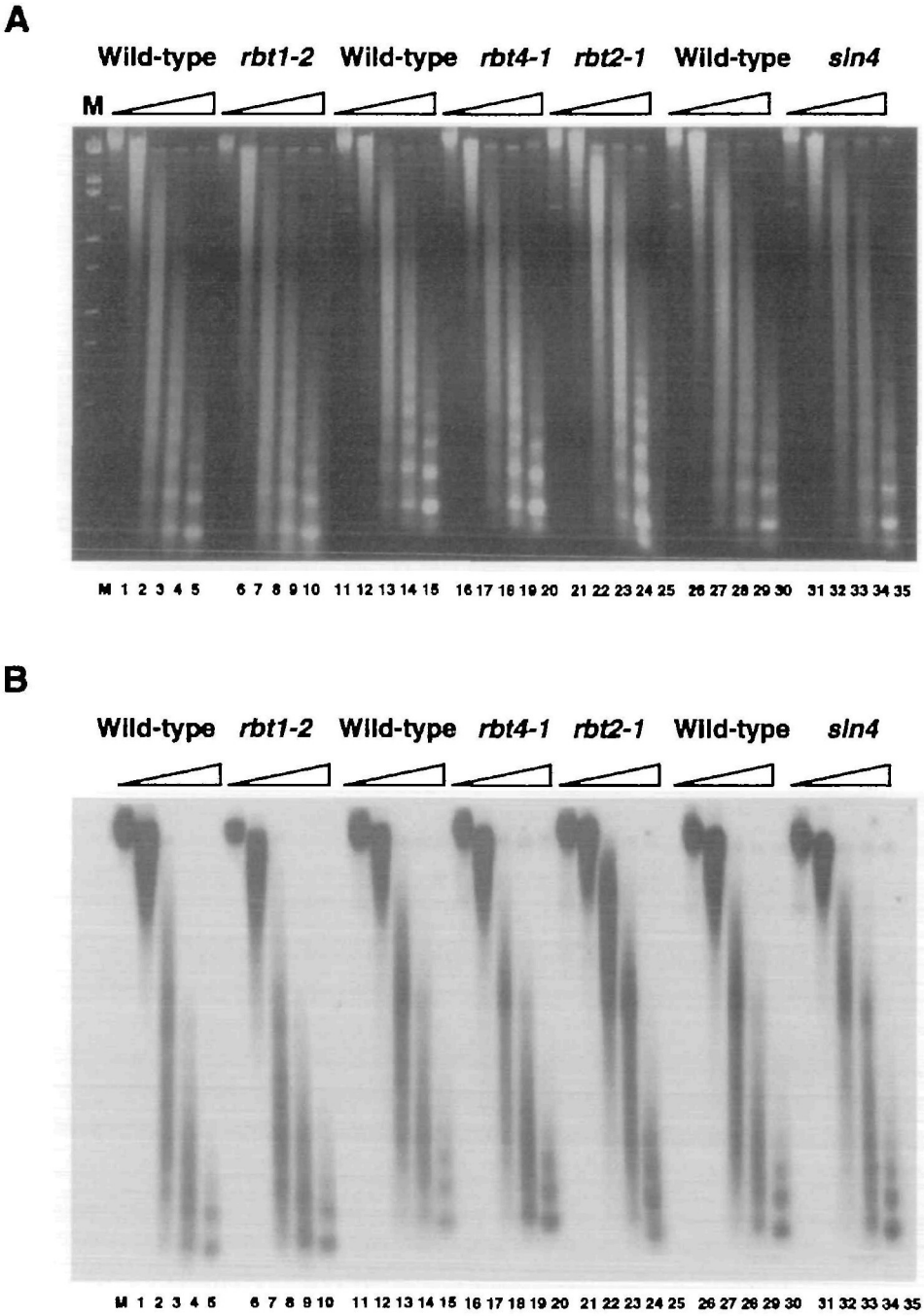

Fig. 5. **(A) MNase sensitivity of bulk chromatins is not altered in the *rbt1-2*, *rbt2-1*, *rbt4-1*, and *sin4* mutants.** Methods for isolation and digestion with MNase of bulk chromatins are described in "MATERIALS AND METHODS." The bulk chromatin from the *rbt1-2* mutant (SH5505) (lane 6 to 10), the isogenic wild-type strain for the *rbt1-2* mutant (SH5503) (lane 1 to 5), the *rbt2-1* (SH5506) (lane 21 to 25), *rbt4-1* (SH5508) (lane 16 to 20), and *sin4* (SH5507) (lane 31 to 35) mutants, and the isogenic wild-type strain for these mutants (SH5504) (lane 11 to 15 and 26 to 30) was digested with MNase. The concentrations of MNase used to digest the chromatins were as follows: no treatment (lanes 1, 6, 11, 16, 21, 26, and 31); 2.5 units/ml (lanes 2, 7, 12, 17, 22, 27, and 32); 5 units/ml (lanes 3, 8, 13, 18, 23, 28, and 33); 10 units/ml (lanes 4, 9, 14, 19, 24, 29, and 34); 40 units/ml (lanes 5, 10, 15, 20, 25, 30, and 35). After resolution by electrophoresis, agarose gels were stained with ethidium bromide. **(B) The digestion patterns using MNase around the core-promoter region of the reporter gene are not altered in the *rbt1-2*, *rbt2-1*, and *rbt4-1* mutants.** After taking photographs of the agarose gels described in (A), the DNA was transferred to nitrocellulose membranes and Southern blot analysis was conducted. A 0.2-kbp *Bam*HI-*Hin*dIII fragment containing the core promoter region of the *PHO84* gene from pAAV was used as a probe.

tants was almost identical (Fig. 5A). Southern blot analysis was subsequently performed using the core promoter region of the *PHO84* gene as a probe. Since the chromosomal *PHO84* gene was deleted in all these strains, the probe was expected to detect the core promoter region of the *PHO84* gene integrated at the *ura3-52* locus. As shown in Fig. 5B, the MNase digestion patterns of these mutants were also the same as that of the wild-type strain. These observations indicate that the global aspect of chromatin accessibility and the chromatin structure of the core promoter region of the Δ*UASPHO84p-PHO5* reporter gene integrated at the *ura3-52* locus are not altered by the *rbt* mutations. Therefore, we conclude that the enhancement of basal transcription in the *rbt* mutants is not caused by alterations in higher-order chromatin structure.

DISCUSSION

In this study, we isolated mutants that abolished Tup1-Ssn6–mediated repression and found them to contain mutations of mediators such as *sin4* and *rgr1* that enhance the basal transcription of core promoters. Such mutations also include seventeen recessive mutations that appear to be unidentified novel mediator mutations, and these were analyzed further. Genetic complementation analysis revealed that the seventeen mutants can be classified into at

least six complementation groups (Tables III and IV). It has been reported that *sin4*-mediated activation is repressed by the Rme1- but not by the Tup1-Ssn6 complex–mediated repression system (*10*). Since enhanced transcription from core promoters was observed even when $IME1_A$ or $IME1_B$, both of which harbor URS that are subject to repression by the Tup1-Ssn6 complex (*21*), was inserted upstream of the core promoters, we conclude that the *rbt* mutations somehow overcome or weaken the Tup1-Ssn6–complex mediated repression as seen in the *sin4* mutant (Table III, Fig. 1, and data not shown). Similarities between the *rbt* mutation and the *sin4* mutation were also noted in that the enhancement of core-promoter activity by *rbt* mutations require TATA elements and is not specific to any particular chromosomal locus or kind of core-promoter (Fig. 3 and Table V). In addition, *rbt* mutations, as well as the *sin4* mutation, display genetic interaction with the Δ*gal11* mutation (Fig. 4). Based upon these observations, we propose that Rbt proteins are factors, possibly mediators, that act in conjugation or in close association with Sin4 and Gal11.

Interestingly, the effect of the *rbt* and *sin4* mutations on the enhancement of transcription from individual core-promoters differs (Table V). For example, the level of enhancement is higher in the case of the ΔUAS*CYC1p* reporter (4.7-fold compared with that of the wild-type strain) than in the case of the ΔUAS*PHO84p* (2.8-fold) and the ΔUAS*PHO5p* (2.8-fold) reporters in *sin4* mutants. Conversely, a higher level of enhancement was observed in the case of the ΔUAS*PHO84p* reporter (4.9-fold) compared with the ΔUAS*CYC1p* (1.9 fold) and ΔUAS*PHO5p* (1.4 fold) reporters in the *rbt2-1* mutants (Table V). This differential effect was also seen between the *rbt4* and *sin4* mutations for the *PHO5p-PHO5* and ΔUAS*PHO5p-PHO5* promoters, as the *rbt4* mutation results in a higher level of transcription from the ΔUAS*PHO5p-PHO5* promoter than the *PHO5p-PHO5* promoter, while the *sin4* mutation has a stronger effect on the *PHO5p-PHO5* promoter than the ΔUAS*PHO5p-PHO5* promoter (Fig. 3 and Table V). These observations suggest that Rbt2 and Rbt4 harbor different functions from Sin4 even though all of the *rbt2*, *rbt4*, and *sin4* mutations cause enhanced basal transcription from all tested core-promoters.

There are several lines of evidence showing that Sin4 plays a role in the maintenance of higher-order chromatin structure (*11*, *12*). Therefore, we determined whether the enhancement in basal transcription in *rbt* mutants is also caused by alterations of higher-order chromatin structure. However, the chromatin structure of the core-promoter region of the *PHO84* gene integrated at the *ura3-52* locus is not altered in the *rbt* mutants (Fig. 5, A and B). In addition, contradictory to a previous report (*12*), no clear alteration of MNase accessibility was observed between the *sin4* mutant and the wild-type strain (Fig. 5, A and B). However, since we have successfully shown, using the same protocol as employed in this study, that MNase accessibility to the telomere region and promoter regions of the *HSP12* and *HXK1* genes is increased as a result of the loss of the *SIR3* gene (Iida *et al.*, manuscript submitted), we assume that the enhancement of transcription from core promoters in the *rbt* mutants occurs through mechanisms other than the alteration of higher-order chromatin structure.

Mutations in many genes have been reported to enhance basal transcription (*2*, *15*, *35–41*). Some of these mutants show pleiotropic phenotypes. For example, mutations of the *BUR6/NCB1* and *BUR3/MOT1* genes result in the Gal$^-$ phenotype in addition to increased basal transcription from core promoters of the *SUC2* and *CYC1* genes (*32*). Mutations of *SUD1/SPT10* gene cause temperature-sensitive (TS) growth, reduced sporulation efficiency, and sensitivity to heat shock and nitrogen starvation, in addition to increased basal transcription from core promoters of the *STA1, CYC1, CUP1, HIS3, PUT1*, and *PUT2* genes (*41*). In contrast, the *rbt* mutants isolated in this study display no such pleiotropic phenotypes. Therefore, we suggest that the *RBT* genes are not identical to the above-mentioned genes. Since some of the above-mentioned genes, *i.e.*, the *BUR* genes and several *SPT* genes, have been shown to suppress defects in UAS or supposed activator(s) of the *SUC2* gene, we examined the transcription of the *SUC2p-PHO5* reporter gene integrated at the *ura3-52* locus in the *rbt* mutants. rAPase activity in the *rbt1-1, rbt2-1, rbt3-1*, and *rbt4-1* mutants harboring this reporter gene was not significantly increased in comparison with the wild-type strain (data not shown), suggesting that the *rbt1* to *rbt4* mutations are not present in those genes. From these observations, we suggest that Rbt proteins are novel factors that act in conjunction with or in close association with Sin4 and have similar but distinct functions from that of Sin4. We also suggest that mutations, such as *rbt*, that lead to the defect in the repression of basal transcription nullify Tup1-Ssn6 general repressor-mediated repression, consistent with the idea that the mechanisms of Rbt (mediator)- and Tup1-Ssn6 (general repressor)–mediated repression are interconnected but substantially different.

We thank A. Sakai and M. Nishizawa for providing the plasmid and the cell strain. We also thank Y. Mukai, N. Ogawa, and T. Mizuno for helpful discussions.

REFERENCES

1. Gustafsson, C.M., Myers, L.C., Beve, J., Spahr, H., Lui, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R.D. (1998) Identification of new mediator subunits in the RNA polymerase II holoenzyme from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273**, 30851–30854
2. Piruat, J.I., Chavez, S., and Aguilera, A. (1997) The yeast *HRS1* gene is involved in positive and negative regulation of transcription and shows genetic characteristics similar to *SIN4* and *GAL11*. *Genetics* **147**, 1585–1594
3. Rosenblum-Vos, L.S., Rhodes, L., Evangelista, C.C., Boayke, K.A.Jr., and Zitomer, R.S. (1991) The *ROX3* gene encodes an essential nuclear protein involved in *CYC7* gene expression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **11**, 5639–5647
4. Lee, Y.C., Min, S., Gim, B.S., and Kim, Y.J. (1997) A transcriptional mediator protein that is required for activation of many RNA polymerase II promoters and is conserved from yeast to humans. *Mol. Cell. Biol.* **17**, 4622–4632
5. Papamichos-Chronakis, M., Conlan, R.S., Gounalaki, N., Copf, T., and Tzamarias, D. (2000) Hrs1/Med3 is a Cyc8-Tup1 corepressor target in the RNA polymerase II holoenzyme. *J. Biol. Chem.* **275**, 8397–8403
6. Stillman, D.J., Dorland, S., and Yu, Y. (1994) Epistasis analysis of suppressor mutations that allow *HO* expression in the absence of the yeast *SWI5* transcriptional activator. *Genetics* **136**, 781–788
7. Tabtiang, R.K. and Herskowitz, I. (1998) Nuclear proteins Nut1p and Nut2p cooperate to negatively regulate a Swi4p-dependent *lacZ* reporter gene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **18**, 4707–4718

8. Carlson, M. (1997) Genetics of transcriptional regulation in yeast: connections to the RNA polymerase II CTD. *Annu. Rev. Cell. Dev. Biol.* **13**, 1–23
9. Perez-Martin, J. (1999) Chromatin and transcription in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* **23**, 503–523
10. Mizuno, T. and Harashima, S. (2000) Activation of basal transcription by a mutation in *SIN4*, a yeast global repressor, occurs through a mechanism different from activator-mediated transcriptional enhancement. *Mol. Gen. Genet.* **263**, 48–59
11. Jiang, Y.W. and Stillman, D.J. (1992) Involvement of the *SIN4* global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 4503–4514
12. Macatee, T., Jiang, Y.W., Stillman, D.J., and Roth, S.Y. (1997) Global alterations in chromatin accessibility associated with loss of *SIN4* function. *Nucleic Acids Res.* **25**, 1240–1247
13. Edmondson, D.G., Zhang, W., Watson, A., Xu, W., Bone, J.R.,Yu, Y., Stillman, D., and Roth, S.Y. (1998) In vivo functions of histone acetylation/deacetylation in Tup1p repression and Gcn5p activation. *Cold Spring Harb. Symp. Quant. Biol.* **63**, 459–468
14. Kuchin, S., Yeghiayan, P., and Carlson, M. (1995) Cyclin-dependent protein kinase and cyclin homologs *SSN3* and *SSN8* contribute to transcriptional control in yeast. *Proc. Natl. Acad. Sci. USA* **92**, 4006–4010
15. Wahi, M. and Johnson, A.D. (1995) Identification of genes required for alpha 2 repression in *Saccharomyces cerevisiae*. *Genetics* **140**, 79–90
16. Adams, A., Gottschling, D.E., Kaiser, C.A., and Stearns, T. (1997) *Methods in Yeast Genetics. A Cold Spring Harbor Laboratory Course Manual*, pp. 145–157, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
17. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, pp. 9.31–9.57 and A9-A13, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Harashima, S., Mizuno, T., Mabuchi, H., Yoshimitsu, S., Ramesh, R., Hasebe, M., Tanaka, A., and Oshima, Y. (1995) Mutations causing high basal level transcription that is independent of transcriptional activators but dependent on chromosomal position in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **247**, 716–725
19. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**, 163–168
20. Morrison, D.A. (1977) Transformation in *Escherichia coli*: cryogenic preservation of competent cells. *J. Bacteriol.* **132**, 349–351
21. Mizuno, T., Nakazawa, N., Remgsamrarn, P., Kunoh, T., Oshima, Y., and Harashima, S. (1998) The Tup1-Ssn6 general repressor is involved in repression of *IME1* encoding a transcriptional activator of meiosis in *Saccharomyces cerevisiae*. *Curr. Genet.* **33**, 239–247
22. Mukai, Y., Harashima, S., and Oshima, Y. (1993) Function of the Ste signal transduction pathway for mating pheromones sustains *MATα1* transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 2050–2060
23. Parent, S.A., Fenimore, C.M., and Bostian, K.A. (1985) Vector systems for the expression, analysis and cloning of DNA sequences in *S. cerevisiae*. *Yeast* **1**, 83–138
24. Arima, K., Oshima, T., Kubota, I., Nakamura, N., Mizunaga, T., and Toh-e, A. (1983) The nucleotide sequence of the yeast *PHO5* gene: a putative precursor of repressible acid phosphatase contains a signal peptide. *Nucleic Acids Res.* **11**, 1657–1672
25. Sikorski, R.S. and Hieter, P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19–27
26. Sakai, A., Shimizu, Y., Kondou, S., Chibazakura, T., and Hishinuma F. (1990) Structure and molecular analysis of *RGR1*, a gene required for glucose repression of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10**, 4130–4138
27. Kunoh, T., Kaneko, Y., and Harashima, S. (2000) *YHP1*, encodes a new homeoprotein that binds to *IME1* promoter in *Saccharomyces cerevisiae*. *Yeast* **16**, 439–449
28. Sakumoto, N., Mukai, Y., Uchida, K, Kouchi, T., Kuwajima, J., Nakagawa, Y., Sugioka, S., Yamamoto, E., Furuyama, T., Mizubuchi, H., Ohsugi, N., Sakuno, T., Kikuchi, K., Matsuoka, I., Ogawa, N., Kaneko, Y., and Harashima, S. (1999) A series of protein phosphatase gene disruptants in *Saccharomyces cerevisiae*. *Yeast* **15**, 1669–1679
29. Roth, S. and Simpson, R. (1991) Yeast minichromosomes. *Methods Cell. Biol.* **35**, 289–314
30. Szent-Gyorgyi, C., Finkelstein, D.B., and Garrard, W.T. (1987) Sharp boundaries demarcate the chromatin structure of a yeast heat-shock gene. *J. Mol. Biol.* **193**, 71–80
31. Kim, S., Na, J.G., Hampsey, M., and Reinberg, D. (1997) The Dr1/DRAP1 heterodimer is a global repressor of transcription *in vivo*. *Proc. Natl. Acad. Sci. USA* **94**, 820–825
32. Prelich, G. (1997) *Saccharomyces cerevisiae BUR6* encodes a DRAP1/NC2alpha homolog that has both positive and negative roles in transcription *in vivo*. *Mol. Cell. Biol.* **17**, 2057–2065
33. Li, Y., Bjorklund, S., Jiang, Y.W., Kim, Y.J., Lane, W.S., Stillman, D.J., and Kornberg, R.D. (1995) Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. *Proc. Natl. Acad. Sci. USA* **92**, 10864–10868
34. Sakurai, H. and Fukasawa, T. (1998) Functional correlation among Gal11, transcription factor (TF) IIE, and TFIIH in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273**, 9534–9538
35. Clark-Adams, C.D., Norris, D., Osley, M.A., Fassler, J.S., and Winston, F. (1988) Changes in histone gene dosage alter transcription in yeast. *Genes Dev.* **2**, 150–159
36. Fassler, J.S., and Winston, F. (1988) Isolation and analysis of a novel class of suppressor of Ty insertion mutations in *Saccharomyces cerevisiae*. *Genetics* **118**, 203–212
37. Katcoff, D.J., Yona, E., Hershkovits, G., Friedman, H., Cohen, Y., and Dgany, O. (1993) *SIN1* interacts with a protein that binds the URS1 region of the yeast *HO* gene. *Nucleic Acids Res.* **21**, 5101–5109
38. Malone, E.A., Clark, C.D., Chiang, A., and Winston, F. (1991) Mutations in *SPT16/CDC68* suppress *cis-* and *trans*-acting mutations that affect promoter function in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **11**, 5710–5717
39. Neigeborn, L., Celenza, J.L., and Carlson, M. (1987) *SSN20* is an essential gene with mutant alleles that suppress defects in *SUC2* transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7**, 672–678
40. Wechser, M.A., Kladde, M.P., Alfieri, J.A., and Peterson, C.L. (1997) Effects of Sin$^-$ versions of histone H4 on yeast chromatin structure and function. *EMBO J.* **16**, 2086–2095
41. Yamashita, I. (1993) Isolation and characterization of the *SUD1* gene, which encodes a global repressor of core promoter activity in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **241**, 616–626