MOLECULAR AND CELLULAR BIOLOGY, Dec. 1991, p. 6317–6327
0270-7306/91/126317-11$02.00/0

Vol. 11, No. 12

RPD3 Encodes a Second Factor Required To Achieve Maximum Positive and Negative Transcriptional States in *Saccharomyces cerevisiae*

MARC VIDAL AND RICHARD F. GABER*

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208-3500

Received 30 April 1991/Accepted 24 September 1991

In *Saccharomyces cerevisiae*, *TRK1* and *TRK2* encode the high- and low-affinity K^+ transporters, respectively. In cells containing a deletion of *TRK1*, transcription levels of *TRK2* are extremely low and are limiting for growth in media containing low levels of K^+ (Trk^- phenotype). Recessive mutations in *RPD1* and *RPD3* suppress the Trk^- phenotype of *trk1*Δ cells. We show here that *rpd3* mutations derepress *TRK2*, conferring an approximately fourfold increase in transcription. *rpd3* mutations confer pleiotropic phenotypes, including (i) mating defects, (ii) hypersensitivity to cycloheximide, (iii) inability to sporulate as homozygous diploids, and (iv) constitutive derepression of acid phosphatase. *RPD3* was cloned and is predicted to encode a 48-kDa protein with no extensive similarity to proteins contained in current data bases. Deletion of *RPD3* is not lethal but confers phenotypes identical to those caused by spontaneous mutations. *RPD3* is required for both full repression and full activation of transcription of target genes including *PHO5*, *STE6*, and *TY2*. *RPD3* is the second gene required for this function, since *RPD1* is also required. The effects of mutations in *RPD1* and *RPD3* are not additive, suggesting that these genes are involved in the same transcriptional regulatory function or pathway.

Genetic selections and screenings for yeast mutants that exhibit increased expression of a structural gene have proven successful in the identification of transcription factors. The selection schemes described thus far have used as model systems structural genes required for growth in defined media. In the starting wild-type strain, transcription of the structural gene is limiting for growth and therefore mutations that increase its transcription can be selected.

Some of these mutational studies have identified genes that encode proteins whose role in transcription is known or readily determined. Genetic mapping of the *sit1* and *sit2* mutations demonstrated that they reside in the genes that encode the two largest subunits of RNA polymerase II (1). *SPT15* (8, 47) was shown to encode TATA-binding factor TFIID (7, 13). *SPT11* and *SPT12* (8) are allelic to structural genes *HTA1* and *HTB1*, which encode histone proteins H2A and H2B (6, 15, 29). It was recently shown that *SPT2* (31) is allelic to *SIN1* (30, 36), whose sequence reveals a protein with significant similarity to nonhistone chromatin component HMG1 (2, 12). Finally, *GAL11* (*SPT13*) (9, 28, 39) has recently been shown to function by establishing or maintaining phosphorylation of transactivator GAL4 (24). The roles of a large number of other genes, identified through similar genetic selections, remain to be determined and are likely to define new functions required for transcriptional regulation.

We have used as a model system transcription of *TRK2*, a yeast gene that encodes the low-affinity K^+ transporter. In cells deleted for *TRK1*, the high-affinity K^+ transporter gene, expression of *TRK2* is limiting for growth on media containing low levels of K^+ (Trk^- phenotype; 11). We have isolated recessive mutations in two genes, *RPD1* and *RPD3* (reduced potassium dependency), that confer a Trk^+ phenotype to *trk1*Δ *TRK2* cells but not to *trk1*Δ *trk2*Δ cells (42). We have now shown that *rpd1* mutations increase *TRK2* transcription and that *RPD1* is a global transcriptional regulator required for both full repression and full activation of many yeast genes (43). In *rpd1* mutants, the regulated genes exhibit increased levels of transcription under repression conditions and decreased levels of transcription under activation conditions. *RPD1* regulates a wide spectrum of genes, including cell type-specific and cell differentiation-specific genes, as well as genes regulated by external signals. Not surprisingly, given their global effect on gene expression in this organism, mutations in this gene have also been identified by other laboratories; hence, *RPD1* is also known as *SIN3* (27, 36) and *UME4* (37). *RPD1* is required for accurate binding of specific activators and repressors to the promoters of *RPD1*-regulated genes (45, 46), although the mechanism involved remains unknown.

In this report, we describe molecular and genetic analyses of *RPD3*. Our results show that *RPD3*, like *RPD1*, is a global regulator required for target genes to achieve maximal transcriptional states. *RPD3* is not essential and is capable of encoding a 48-kDa protein that shows little, if any, sequence similarity to other proteins. Mutations in *RPD3* confer pleiotropic phenotypes indistinguishable from those exhibited by *rpd1* mutants. Phenotypic analysis and gene expression assays performed on *rpd1 rpd3* double mutants suggest that these genes are involved in the same transcriptional regulatory function or pathway.

MATERIALS AND METHODS

Media. The genetic techniques and standard media used were previously described (34). Synthetic low-salt (LS) and low-phosphate media were prepared essentially as previously described (11, 43). LS medium contained less than 2 μM Na^+ and virtually no potassium prior to addition. Desired potassium levels were added as KCl and are indicated in millimolar concentrations; for example, LS(0.2K) is LS plus 0.2 mM KCl. Sporulation medium was prepared as

* Corresponding author.

TABLE 1. Strains used in this study

Strain	Relevant characteristic	Source or reference
JY383	*MAT***a** *ura3-52 trp1Δ1 his6 lys2Δ201 sst1-3*	J. Trueheart
M186	*MAT***a** *ura3-52 trp1Δ1 trk1Δ rpd1-41*	42
M209	*MAT***a** *ura3-52 trp1Δ1 his4-15 trk1Δ rpd3-4*	42
M211	*MAT*α *ura3-52 lys9 trk1Δ rpd1-41 rpd3-4*	42
M398	*MAT*α *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ*	42
M445	*MAT*α *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd3-72*	This work
M517	Diploid strain obtained by mating strains M537 and M398	42
M537	*MAT***a** *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ*	42
M613	*MAT***a** *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd1Δ::TRP1*	42
M771	*MAT*α *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd3Δ::HIS3*	This work
M774	*MAT*α *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd3Δ::URA3*	This work
M778	*MAT***a** *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd3Δ::HIS3*	This work
M798	Diploid strain obtained by mating strains M771 and M778	This work
M833	*MAT***a** *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd3Δ::HIS3*	This work
M834	*MAT*α *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd1Δ::TRP1*	This work
M835	*MAT***a** *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ*	This work
M836	*MAT*α *ura3-52 trp1Δ1 his3-200 leu2-1 trk1Δ rpd3Δ::HIS3 rpd1Δ::TRP1*	This work
M850	Diploid strain obtained by mating strains M771 and M613	This work
Mx134	Diploid strain obtained by mating strains R1624 and R1155	42
R757	*MAT*α *ura3-52 his4-15 lys9*	11
R1155	MATα *ura3-52 his4-15 lys9 trk1Δ*	11
R1174	*MAT***a** *ura3-52 trp1Δ1 trk1Δ*	11
R1624	*MAT***a** *ura3-52 trp1Δ lys9 trk1Δ rpd3-1*	42
R1680	*MAT*α *ura3-52 his4-15 lys9 trk1Δ rpd3-4*	42
R1689	*MAT*α *ura3-52 his4-15 lys9 trk1Δ rpd1-41*	42

previously described (20). Cycloheximide medium was prepared as previously described (10). Ethidium bromide was added to YEP medium (34) containing 4% glycerol to a final concentration of 1 μg/ml.

Plasmids. pCB3 and pCB4 are primary clones from a yeast genomic library constructed in YCp50 (32) and were isolated on the basis of the ability to confer normal levels of cycloheximide resistance on *rpd3* mutants. Different *RPD3* subclones (see Fig. 3) were used to delimit the borders of the gene. To construct pMV29-11, pCB3 was digested with *Bam*HI and the largest fragment was recircularized by ligation. To construct pMV29-21, pCB3 was digested with *Sph*I and the largest fragment was recircularized by ligation. To construct pMV34, pMV29-21 was digested with *Cla*I and the largest fragment was recircularized. To construct pMV28-33, the 2-kb *Eco*RI-*Eco*RI fragment from pCB3 was inserted into pRG415 (11) linearized with *Eco*RI. To construct pMV59, the 1.3-kb *Hin*dIII-*Sph*I fragment from pMV29-21 was inserted into YCp50 (32) linearized with *Hin*dIII and *Sph*I.

A complete deletion of *RPD3* (*rpd3Δ*) was constructed in vitro by the gamma deletion method (35). To facilitate further genetic analysis, the same deletion allele was constructed in two pRS integrative plasmids containing different selectable markers (*HIS3* and *URA3*) (35). (i) The 3.4-kb *Cla*I-*Sph*I fragment from pMV34 was inserted into pGEM4Z (Promega) linearized with *Cla*I and *Sph*I to generate pMV107. The 550-bp *Eco*RV-*Hin*dIII fragment from pMV34 was inserted into pGEM7Z (Promega) linearized with *Hin*dIII and *Sma*I to generate pMV42-51. (ii) The 450-bp *Eco*RI-downstream *Bgl*II fragment from pMV107 was inserted into plasmids pRS303 and pRS306 (35) such that the *Bgl*II site located in *RPD3* (see Fig. 3 and 4) was located near the T3 promoter of pRS303 and pRS306 to generate pMV127 (*HIS3*-containing plasmid) and pMV128 (*URA3*-containing plasmid), respectively. (iii) The 500-bp *Eco*RV-*Hin*dIII fragment was obtained by *Sac*I-*Xba*I digestion of plasmid pMV42-51 and subsequently inserted into pMV127 and pMV128 linearized with *Sac*I-*Xba*I with the *Xba*I site near the T7 promoter. The plasmids containing *rpd3Δ* deletion alleles are pMV129 (*rpd3Δ::HIS3*) and pMV130 (*rpd3Δ::URA3*).

The promoter-*lacZ* fusion plasmids used as reporters in transcription assays were the following: *TRK2::lacZ* integrative plasmid pAB138 (contains the entire promoter and the first 1.4 kb of the *TRK2* open reading frame [22]); *TRK2::lacZ* multicopy plasmid pAB137 (the *TRK2::lacZ* construct is identical to pAB138); *SPO13::URA3* multicopy plasmid pBW2 (38); *STE6::lacZ* multicopy plasmid pΔHA (for construction details, see reference 43); *HO::lacZ* multicopy plasmid YEpHO::lacZ (gift of P. Dorhmann and D. Stillman); *TY2::lacZ* centromeric plasmid p1033 (for construction details, see reference 43); and *PHO5::lacZ* centromeric plasmid pMH313 (14).

Sequencing of *RPD3*. A series of small (300- to 400-bp) overlapping subclones were constructed encompassing the region between the *Eco*RV and the downstream *Bgl*II sites of *RPD3* (see Fig. 3). Dideoxy sequencing of double-stranded plasmid DNA (33) was carried out by using the Sequenase Sequencing Kit from United States Biochemical Co. Gradient gel electrophoresis was performed as previously described (33). All DNA sequences were read from both strands. DNA sequence data were stored and analyzed by using the DNA Inspector IIe program from Textco, Inc.

Strains. The genotypes of the *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Isogenic *rpd3Δ* strains were constructed in the M398 strain background (43) by transformation of plasmids pMV129 and pMV130 into M398 (*MAT*α) to generate strains M771 and M774, respectively, and into M537 (*MAT***a**) to generate strains M778 and M777, respectively. The M445 strain is a spontaneous revertant of M398 isolated on LS(0.2K) plates and contains the *rpd3-72* allele. The homozygous *rpd3Δ/rpd3Δ* M798 diploid was obtained by crossing M771 with M778. Heterozygous *RPD3/rpd3Δ rpd1Δ/RPD1* strain M850 was obtained by

crossing M613 with M778, and strains M833, M834, M835, and M836 are spores of a tetratype of that cross.

Phenotypic characterization. The colony-staining overlay assay used to observe acid phosphatase (APase) activity qualitatively was previously reported (40). APase activity was quantified by the method of Torriani as described by Toh-e et al. (40). The values obtained are averages of six assays. One enzymatic unit is defined as the amount of enzyme necessary to liberate 1 μmol of *p*-nitrophenylphosphate per h; one specific unit is the number of enzymatic units per unit of optical density of cells at 660 nm (OD_{660}).

Mating assays were performed essentially as described by Trueheart et al. (41). Mid-log-phase *MAT***a** and *MAT*α cells were mixed onto a nitrocellulose filter at a density of 3×10^6 cells per parent. The filter was then transferred to a plate containing YEPD medium (34) and incubated for 3 h at 30°C to allow mating. The cells were then removed from the filters and diluted, and for each cross, the same cell concentration was plated on medium selective for diploids. Some dilutions were used to count the number of diploids over the number of cells present in the assay.

The halo assay was performed as described by Herskowitz (16 and references therein). About 10^4 cells of α-factor-hypersensitive tester strain RC634 (*sst1*) were spread onto a YEPD (34) plate, and subsequently, 10^5 exponentially growing cells to be tested were spotted onto this lawn. Halos were zones of inhibition visible after 2 days of incubation at 30°C.

β-Galactosidase assays. For each promoter tested, the *lacZ* fusion-containing plasmids were introduced into the appropriate wild-type and *rpd1*Δ isogenic recipients by the method of Ito et al. (18). A minimum of four independent transformants were purified and subsequently grown to the stationary phase in synthetic medium lacking the appropriate amino acid or purine. The cultures were diluted in the same medium and, after overnight incubation, harvested in the late exponential phase. For assay of *PHO5* expression, cells were grown in LS(100K) medium (42) containing low levels of phosphate (0.015 g of KH_2PO_4 per ml) for derepression conditions or high levels of phosphate (0.9 g of KH_2PO_4 per ml) for repression conditions. For *CYC1*, cells were grown in glucose- or glycerol-containing medium for conditions of repression or activation, respectively. The cells were washed twice with Z buffer and resuspended at various cell densities, depending on the basal activities of the different promoters tested. After resuspension, the final OD_{600} was measured in a Beckman 25 spectrophotometer. At least two different dilutions were assayed for β-galactosidase activity in permeabilized cells as described previously (26, 48). Specific activities were defined as $(1{,}000 \times OD_{420})$/(time [minutes] × volume [milliliters] × cell density [OD_{600}]). No differences in cell size, clumpiness, or correlation between OD_{600} and actual cell concentrations were detected between *RPD3* and *rpd3*Δ cells. Therefore, since the global effect of *rpd3* mutations on transcription could alter total cellular protein concentrations, we normalized the expression assays to OD_{600}.

RESULTS

Transcriptional repression of *TRK2* by *RPD3*. Yeast cells deleted for *TRK1*, the gene that encodes the high-affinity K^+ transporter, exhibit a Trk^- phenotype; i.e., they are unable to grow on media containing low concentrations of potassium (11). We previously showed that recessive *rpd3* mutations restore the ability of *trk1* cells to grow on low-potassium medium (Trk^+) (42). The Trk^+ phenotype of *trk1 rpd3* cells requires the presence of low-affinity K^+ transporter gene *TRK2*, suggesting that *RPD3* is a negative regulator of *TRK2* (21, 22, 42). To determine what effect *rpd3* mutations have on the expression of *TRK2*, we measured β-galactosidase activity expressed from constructs containing *TRK2*::*lacZ* fusions. The results showed that *rpd3* mutations confer a two- to fivefold increase in *TRK2* expression (Table 2). Similar results were obtained whether the *TRK2*::*lacZ* fusion was present as a single copy within the yeast chromosome or on a multicopy plasmid. The *rpd3*-dependent increase in *TRK2* expression was not allele specific, since similar effects were observed in cells that contained either the spontaneous *rpd3-75* mutation or a null allele (*rpd3*Δ; see below). Both the basal and derepressed levels of β-galactosidase activity driven by the *TRK2* promoter indicated that *TRK2* is expressed at extremely low levels. This was consistent with previous observations (43), and consequently, detection of *TRK2* RNA was not performed.

TABLE 2. Repression of *TRK2* by *RPD3*

Strain	Mean β-galactosidase units[a] ± SD	
	Single[b]	Multiple[c]
M398 (*RPD3*)	0.6 ± 0.1	4.3 ± 1.3
M445 (*rpd3-75*)	1.2 ± 0.2	22.2 ± 4.3
M778 (*rpd3*Δ::*HIS3*)	1.2 ± 0.3	19.6 ± 3.5

[a] Enzyme activity units are defined in Materials and Methods.
[b] Chromosomal integration of *TRK2*::*lacZ*.
[c] *TRK2*::*lacZ* carried on multicopy plasmid.

Mutations at *RPD3* confer pleiotropic phenotypes. The derepression of *TRK2* caused by mutations in *RPD3* mimicked the effect we observed for mutations in *RPD1* (43). To determine the extent to which *RPD3* and *RPD1* might play similar roles in the regulation of transcription, we compared wild-type, *rpd1*, *rpd3*, and *rpd1 rpd3* cells for phenotypes known to be altered in *rpd1* mutants, including sensitivity to cycloheximide, acriflavin, and ethidium; production of APase; mating efficiency; and the ability to sporulate. Compared with isogenic wild-type cells, *rpd3* cells were found to be hypersensitive to acriflavin (data not shown), ethidium, and cycloheximide (Fig. 1). Acid phosphatase overlay assays suggested that under conditions of repression (high phosphate concentration) *rpd3* mutants produce more APase (Pho^+ phenotype) than do wild-type cells (Fig. 1). Quantitation of the Pho phenotype revealed that *rpd3* mutants exhibited a threefold increase in total APase activity compared with the wild type (Table 3).

The ability of *rpd3* cells to mate with wild-type cells was also diminished (Fig. 1). Quantitation of this phenotype showed that although mating ability was defective in *rpd3* × *RPD* crosses, the phenotype was more obvious in *rpd3* × *rpd1* crosses and most severe in homozygous *rpd3* × *rpd3* (and *rpd1* × *rpd1*) crosses, where a greater than 10-fold decrease in the frequency of diploid formation was observed (Table 4). The mating defect in *rpd3* mutants is due, at least in part, to decreased α-factor production in *MAT*α *rpd3* cells compared with that of *MAT*α *RPD* cells (Fig. 2). In the accompanying report (43), we show that decreased α-factor production in *rpd1 MAT*α cells is due to aberrant expression of **a**-specific gene *BAR1*, which encodes an α-factor protease (17, 25).

Finally, homozygous *rpd3*/*rpd3* diploid cells failed to

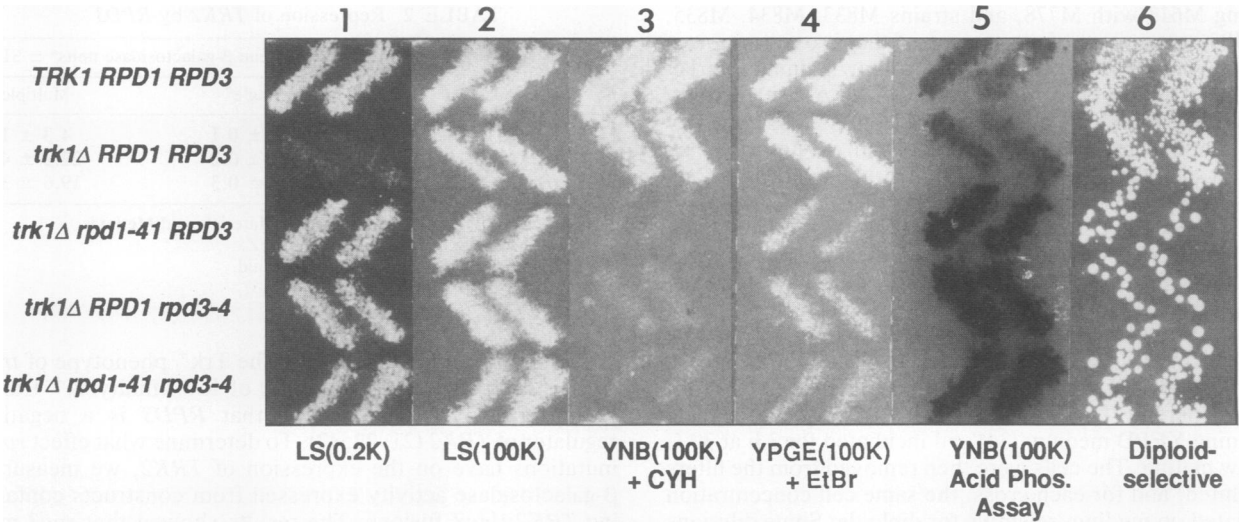


FIG. 1. Pleiotropic phenotypes of *rpd1* and *rpd3* strains. *rpd* mutants were patched on a master plate containing YPD(100K) and then replica plated on the next day onto the different media. Lanes: 1, LS(0.2K) plate (see Materials and Methods); 2, LS(100K) plate; 3, standard YNB plates containing a high concentration of potassium (100 mM KCl) and a low (normally sublethal) concentration of cycloheximide (0.04 μg/ml); 4, standard YPDGE containing a high concentration of potassium (100 mM KCl) and a low (normally sublethal) concentration of ethidium bromide (EtBr; 1 μg/ml); 5, YNB(100K) plate incubated overnight and then overlaid with a low-pH buffered α-naphthylphosphate substrate (see Materials and Methods) for 10 min (the color intensity indicates the level of APase activity); 6, master plate with the five *MAT*α *rpd lys9* mutants first replica plated onto a YPD(100K) plate covered by a lawn of a *MAT***a** *rpd3-4 trp1Δ1* tester strain (M209 [Table 1]) incubated for 4 h at 30°C and then replica plated onto diploid-selective medium (YNB containing all amino acids except Lys and Trp) to select for diploids. The strains used were R757 (*TRK1 RPD1 RPD3*), R1155 (*trk1Δ RPD1 RPD3*), R1689 (*trk1Δ rpd1-41 RPD3*), R1680 (*trk1Δ RPD1 rpd3-4*), and M211 (*trk1Δ rpd1-41 rpd3-4*). The complete genotypes of the strains are indicated in Table 1.

sporulate. Microscopic examination revealed no four-spored asci among over 50,000 *rpd3/rpd3* diploid cells examined microscopically, whereas *rpd3/RPD* and *rpd3/rpd1* cells yielded more than 50% four-spored asci under the same conditions (see Materials and Methods).

In each of the cases described above, the phenotypes of the *rpd3* mutants were indistinguishable from those of *rpd1* mutants (Fig. 1 and 2; Tables 3 and 4). We recently showed that the pleiotropic phenotypes of *rpd1* cells are due to aberrant transcriptional regulation of specific yeast genes (43). *rpd1* mutations abolish the ability of many genes to respond fully to repression and derepression signals. Our observations that *rpd3* mutations confer the same phenotypes as *rpd1* mutations suggested that *RPD3* encodes a transcription factor required for similar transcriptional regulation in *S. cerevisiae*.

Isolation of the *RPD3* gene. DNA fragments containing the *RPD3* gene were cloned from a yeast library (32) by the ability to suppress the cycloheximide hypersensitivity (Cyh^{hs}) of an *rpd3* recipient strain (R1680). Among approximately 4,000 Ura^+ transformants screened, 4 exhibited wild-type levels of cycloheximide resistance (Cyh^+). DNA was prepared from each of the Ura^+ Cyh^+ transformants, and the corresponding plasmids were retrieved by transformation of *Escherichia coli* HB101 to ampicillin resistance. Restriction maps of the recovered plasmids indicated that they represented two different inserts, contained in pCB3 and pCB4, that overlapped the same region of DNA (Fig. 3). Plasmids pCB3 and pCB4 were chosen and used to retransform *ura3-52 trk1Δ rpd3-4* recipient strain R1680. All Ura^+ transformants exhibited the phosphate-dependent repression of APase activity observed in wild-type *RPD3* cells. Furthermore, they had lost the ability to grow on media containing minimal concentrations of potassium, suggesting that *TRK2* repression was comparable to that in wild-type *RPD3* cells containing a *trk1Δ* allele.

TABLE 3. APase assays

Mutations	Mean sp act[a] ± SD	
	High phosphate concn (repressed)	Low phosphate concn (derepressed)
RPD1 RPD3	0.69 ± 0.09	6.16 ± 0.35
rpd1-41 RPD3	2.98 ± 0.15	10.64 ± 0.4
RPD1 rpd3-4	2.27 ± 0.08	11.80 ± 0.4

[a] The values reflect averages of six determinations of each culture in a single experiment. Independent experiments were performed three times without significant deviation from the reported values. The relatively high APase activity measured in the case of the *RPD1 RPD3* strain under repression conditions is due to the presence of the wild-type *PHO3* gene in its genetic background (40). APase specific activity units are defined in Materials and Methods.

TABLE 4. Efficiency of diploid formation in crosses with *rpd1* and *rpd3* mutants

Mutation[a] (mating type)	Mean % diploid cells ± SD[b]		
	RPD3 (α)	*rpd1-41* (α)	*rpd3-4* (α)
RPD3 (**a**)	50.6 ± 1.5	32.8 ± 1.0	37.4 ± 1.4
rpd1-41 (**a**)	34.2 ± 2.0	4.0 ± 0.5	13.1 ± 1.9
rpd3-4 (**a**)	33.4 ± 1.1	4.6 ± 0.5	4.3 ± 0.7

[a] The strains used were R1174 and R1155 for *RPD3*, M186 and R1689 for *rpd1-41*, and M209 and R1680 for *rpd3-4*. Details of the diploid formation assay are presented in Materials and Methods.

[b] The values shown were obtained after 3 h of mating and are averages of three independent experiments.

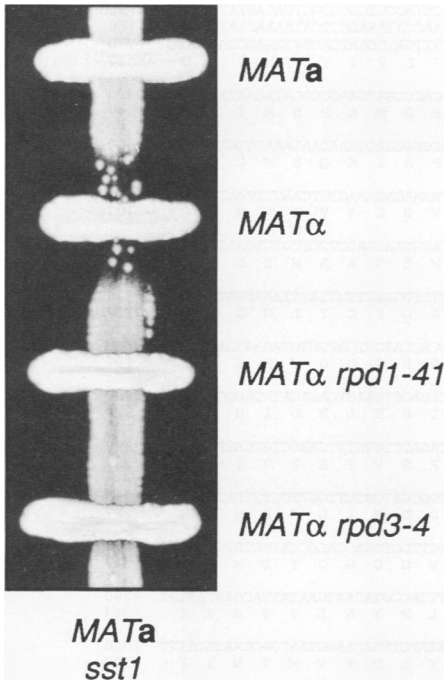


FIG. 2. α-Factor production defect in *rpd1* and *rpd3* mutants. The three *MAT*α strains tested are isogenic, except for the respective *rpd* loci; the *MAT***a** strain is congenic. The tests were performed on plates containing YPD(100K) at pH 5.5. α-Factor production was estimated on the basis of the sizes of the inhibition zones around the tested strains in a lawn of α-factor-hypersensitive *MAT***a** *sst1* tester strain BC161 (gift of J. Trueheart). The strains used were R1174 (*MAT***a**), R1155 (*MAT*α), R1689 (*MAT*α *rpd1-41*), and R1680 (*MAT*α *rpd3-4*). The complete genotypes of the strains are indicated in Table 1.

We performed a directed-integration experiment to determine whether the cloned DNA fragments encode the *RPD3* gene. The 3.3-kb *Bam*HI-*Sal*I fragment shared by pCB3 and pCB4 was subcloned into integrative vector YIp5, resulting in recombinant plasmid pMV19-1 (Fig. 3). pMV19-1 was linearized by digestion with *Bgl*II to enhance the frequency of integration and used to transform a heterozygous *rpd3-1*/*RPD3* diploid to Ura^+. After sporulation of three independent transformants, tetrad analysis was performed on the resulting meiotic progeny. In two diploids analyzed (13 tetrads), the Ura^+ phenotype cosegregated with the Cyh^{hs}, Pho^+, and Trk^+ phenotypes. In the third diploid (eight tetrads), the Ura^+ phenotype cosegregated with the Cyh^+, Pho^-, and Trk^- phenotypes. The complete genetic linkage between the integrated Ura^+ plasmid and the Rpd phenotypes confirmed that the cloned DNA fragments carried the authentic *RPD3* gene.

Subcloning experiments localized the *RPD3* gene to a

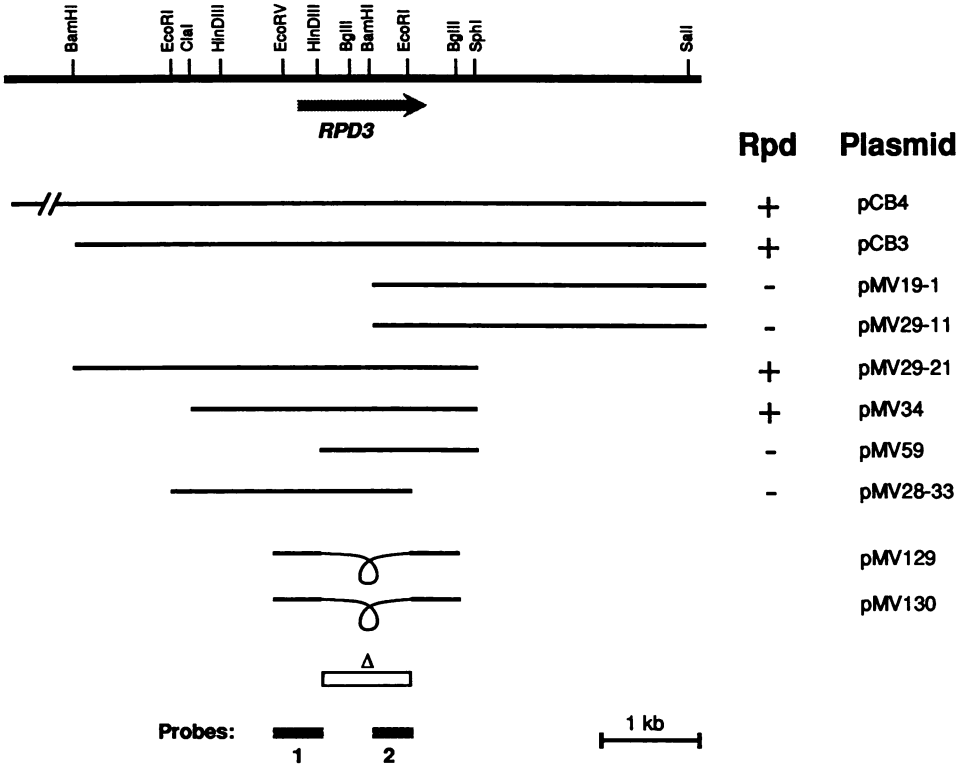


FIG. 3. Restriction map of the *RPD3* locus. The top line represents an abbreviated restriction map of the *RPD3* region. The arrow represents the open reading frame and direction of transcription of *RPD3* (see Fig. 5). The lines represent *RPD3* subclone fragments that delimit the borders of the gene. Each clone was tested for the Rpd phenotypes in a *trk1*Δ *rpd3-4* mutant strain (a plus sign indicates growth on cycloheximide and no growth on low-K^+ media). The open box represents the genomic sequence, between the *Hin*dIII and *Eco*RI sites, deleted in the *rpd3*Δ allele. Plasmid constructions are described in Materials and Methods. The probes refer to the radiolabeled DNA fragments used for Fig. 6.

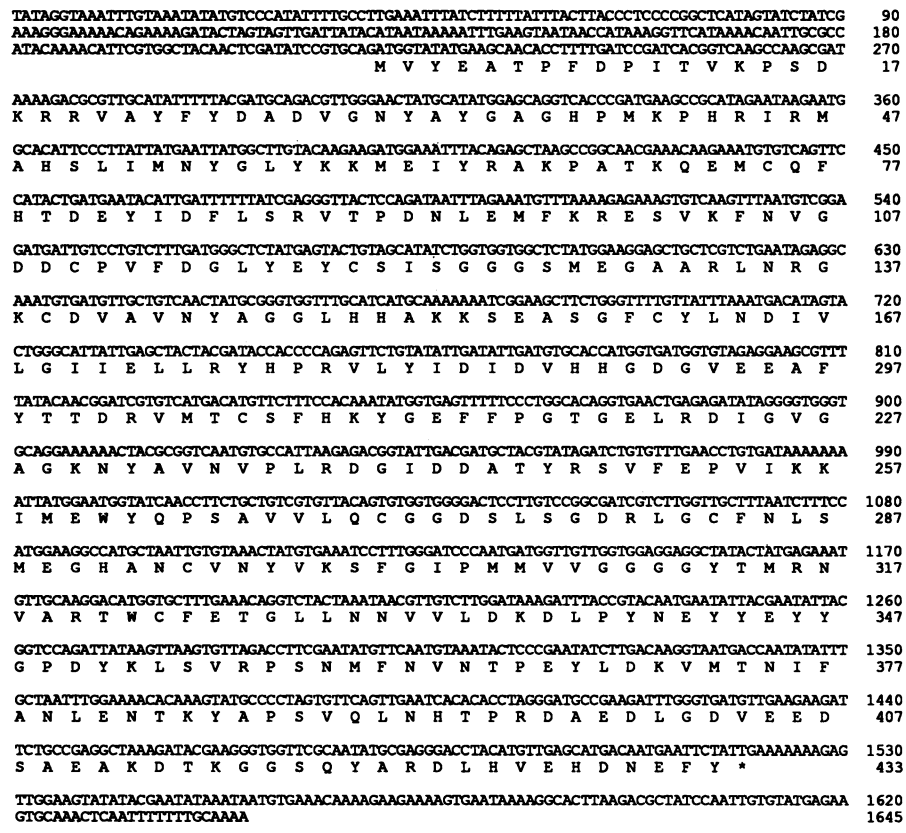


FIG. 4. Nucleotide and predicted amino acid sequences of *RPD3*. Nucleotides are numbered from the first position of the putative initiating methionine. The initiator methionine was assigned on the basis of the upstream in-frame termination codon at −180. The termination codon is indicated by an asterisk.

2.4-kb *Cla*I-*Sph*I fragment (Fig. 3) sufficient for complementation of the Rpd$^-$ phenotypes of *rpd3-4* cells and cells containing a null allele of *RPD3* (*rpd3*Δ::*HIS3*; described below).

Sequence of *RPD3*. DNA sequence analysis of the region spanned by the *Eco*RV and second *Bgl*II sites (Fig. 3) revealed a single large open reading frame capable of encoding a protein of 433 amino acids with a molecular mass of 48 kDa (Fig. 4). Deletion of approximately 70% of this open reading frame from the chromosome resulted in the Rpd$^-$ phenotypes (see below), indicating that this region encodes the *RPD3* gene product. Comparison of the inferred RPD3 amino acid sequence with the available data bases failed to identify other proteins with extensive similarity. Thus, although RPD3 has global effects on transcription (see below), it contains none of the known DNA-binding motifs.

Deletion of the *RPD3* gene. The pleiotropic phenotypes of *rpd3* cells implied a general function for the *RPD3* gene product. To determine the effects of a null allele, a deletion mutation that replaced most of the open reading frame of *RPD3* with integrative plasmid sequences was generated by the method of Sikorski and Hieter (35; Materials and Methods). To facilitate further genetic analysis, the same deletion was constructed with two different markers (*URA3* in pMV129 and *HIS3* in pMV130; Fig. 3).

In separate experiments, pMV129 and pMV130 were linearized by digestion with *Xba*I and used to transform homozygous *ura3-52*/*ura3-52 his3*Δ*200*/*his3*Δ*200 trk1*Δ/*trk1*Δ diploids (strain M517) to Ura$^+$ and His$^+$, respectively. After sporulation, the transformants gave rise to tetrads in which cosegregation of the Ura$^+$ (or His$^+$) phenotypes with the Rpd$^-$ phenotypes (Cyh^{hs} and Trk$^+$) was observed, indicating that one copy of the *RPD3* gene in each diploid had been disrupted. The viability of all four spores indicated that the *rpd3* deletion mutation is not lethal. To generate a series of isogenic strains differing only at the *RPD3* and *MAT* loci, haploid cells of strains M398 and M537 were transformed with pMV129 or pMV130. As anticipated, these transformants acquired the pleiotropic Rpd$^-$ phenotypes.

Two criteria were used to demonstrate that the Rpd$^-$ phenotypes of cells transformed with pMV129 or pMV130 were due to a deletion in *RPD3*. (i) Matings with *rpd3-4* strains produced diploids in which the *rpd3*Δ mutations failed to complement the *rpd3-4* mutation. (ii) Physical evidence was obtained showing that plasmids pMV129 and pMV130 had integrated at the *RPD3* locus, replacing the wild-type allele with the deletion construct (Fig. 5). DNA hybridization experiments were performed with genomic DNAs prepared from transformants M774 (*rpd3*Δ::*URA3*) and M771 (*rpd3*Δ::*HIS3*) and from nontransformed strain M398. Two radiolabelled probes were used to determine the accuracy of the deletion mutation. One probe (no. 2) was internal to the gene and demonstrated that the 0.8-kb *Hin*dIII-*Eco*RI fragment within the *RPD3* reading frame was missing in the transformants; the alternate probe (no. 1) hybridized to sequences external to the gene and confirmed the replacement of the *RPD3* coding region by the integrative plasmids (Fig. 5).

***RPD3* is required for maximum transcriptional responses.** To determine whether *RPD3*, like *RPD1*, is required for

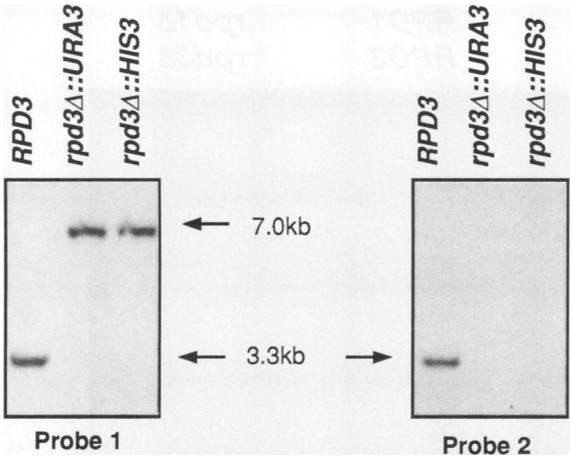


FIG. 5. Southern analysis of the *rpd3*Δ strains. The radiolabelled probes used are represented in Fig. 4. The strains used were M398 (*RPD3*), M774 (*rpd3*Δ::*URA3*), and M771 (*rpd3*Δ::*HIS3*). Probe 1 hybridized to sequences located outside the deleted region and gave rise to shifted signals because of the plasmid insertion. Probe 2 hybridized to sequences within the region to be deleted and, as expected, failed to detect any homologous sequence in *rpd3*Δ::*URA3* and *rpd3*Δ::*HIS3* strains. The blots were washed under conditions of high stringency (65°C, 0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% sodium dodecyl sulfate).

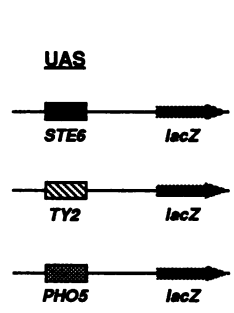
RPD-regulated genes to achieve their maximum transcriptional responses, we measured the expression, in *RPD3* and *rpd3*Δ cells, of a variety of these genes under activation and repression conditions. Promoter-*lacZ* fusion constructs were used to determine the extent of regulation of cell type-specific genes and genes regulated by extracellular signals. β-Galactosidase activity measured in *MAT***a** *RPD3* (M537) and *MAT***a** *rpd3*Δ (M778) cells transformed with a *cyc1*::*lacZ* fusion in which the endogenous *CYC1* upstream activating sequences (UAS) have been replaced with the *STE6* UAS showed that activation of the **a**-specific UAS element was fivefold lower in *MAT***a** *rpd3*Δ cells than in wild-type *MAT***a** *RPD3* cells (Fig. 6). In contrast, β-galactosidase activity measured in *MAT*α *RPD3* (M398) and *MAT*α *rpd3*Δ (M771) cells transformed with the same reporter plasmid showed that the level of expression of the **a**-specific UAS element was increased by at least fivefold in *MAT*α *rpd3*Δ cells compared with that in *MAT*α *RPD3* cells (Fig. 6). *lacZ* transcription driven by the *TY2* and *PHO5* promoters was also altered in *rpd3*Δ mutants. The role of *RPD3* in the activation of some genes, e.g., *PHO5*, and the repression of others, *e.g.*, *TY2*, can be very small. However, the ratio of activation levels to repression levels (A/R in Fig. 6) revealed that the overall effect of *RPD3* on transcriptional regulation is significant. These results revealed that the decrease of transcriptional responses in cells containing a null allele of *RPD3* is similar both qualitatively and quantitatively to that observed in cells deleted for *RPD1*.

Tests for additivity between *rpd1* and *rpd3* mutations. The results described above indicate that *RPD3* is required for many genes to achieve their maximum state of transcriptional regulation. The role of *RPD3* thus appears to be identical to that of *RPD1*. Two possibilities could explain the similarity of *rpd1*- and *rpd3*-determined phenotypes. RPD1 and RPD3 proteins may mediate the effects on transcriptional regulation independently or function in the same molecular pathway. The former scenario seemed unlikely, since the amino acid sequences of RPD3 and RPD1 are unrelated and RPD3 contains none of the putative protein-protein interaction domains found in RPD1 (43, 44). To help determine the functional relationship between *RPD1* and *RPD3*, we performed experiments to detect additivity between the effects of *rpd1* and *rpd3* mutations.

Wild-type, *rpd1*, *rpd3*, and *rpd1 rpd3* cells were obtained from a tetratype tetrad generated from heterozygous *rpd1*/*RPD1 rpd3*/*RPD3* congenic diploid strain M798 after sporulation. The Trk, Cyh, and Pho phenotypes of these isolates were tested under conditions that would allow detection of additivity (Fig. 7a). For example, cells were tested for growth on medium containing 0.08 mM KCl to test the Trk phenotype and on medium containing 0.04 μg of cycloheximide per ml (incubated at 30°C) to test the Cyh phenotype. In addition, expression of *RPD*-regulated genes *TRK2*, *SPO13*, and *HO* was measured in each of the isolates by using the appropriate *lacZ* fusion plasmids (Fig. 7b). Each of these tests demonstrated absence of additivity between the effects *rpd1* and *rpd3* mutations. On the basis of these results, we conclude that the *RPD1* and *RPD3* gene products function in the same molecular pathway (Fig. 8).

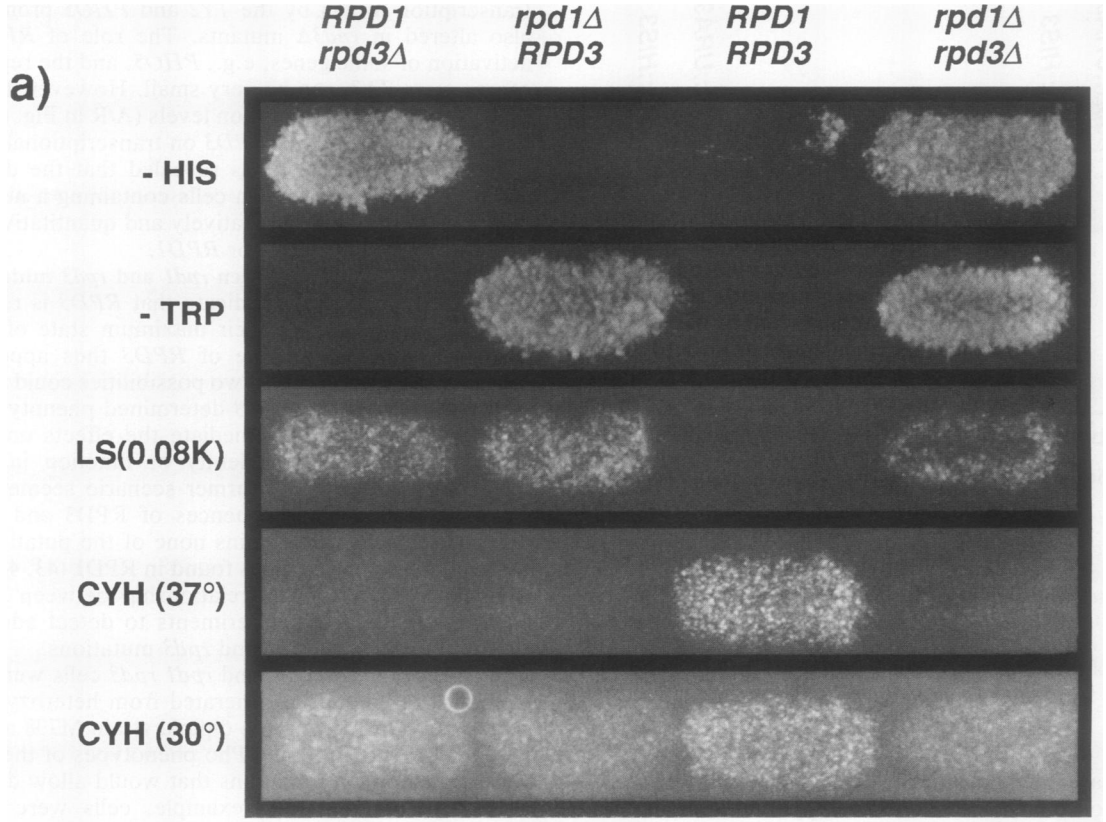
DISCUSSION

A simplified view of the universe of transcriptional regulators is one in which these factors constitute two mutually exclusive groups: DNA-binding proteins and proteins that



	Repression (R)		Activation (A)		A/R	
UAS	*RPD3*	*rpd3*Δ	*RPD3*	*rpd3*Δ	*RPD3*	*rpd3*Δ
STE6–*lacZ*	< 0.2	1.0 (±0.2)	486 (±14)	99 (±9)	> 2400	99
TY2–*lacZ*	0.7 (±0.15)	1.2 (±0.3)	3.0 (±0.3)	1.3 (±0.3)	4.3	1.1
PHO5–*lacZ*	11 (±1.0)	26 (±4.2)	151 (±15)	121 (±20)	13.7	4.6

FIG. 6. Activation and repression in *rpd3* mutants. Mean β-galactosidase specific activities (units are defined in Materials and Methods) exhibited by *RPD3* and *rpd3* cells (standard deviations shown in the parentheses). *PHO5*, *PHO5*:*lacZ* fusion on a centromeric plasmid under repression (high phosphate concentration) and activation (low phosphate concentration) conditions; *STE6*, *cyc1*:UAS(*STE6*):*lacZ* on a multicopy plasmid under repression (*MAT*α cells) and activation (*MAT***a** cells) conditions; *TY2*, *TY2*:*lacZ* fusion on a centromeric plasmid under repression (diploid cells) and activation (haploid cells) conditions.



b)

	RPD1 rpd3Δ	rpd1Δ RPD3	RPD1 RPD3	rpd1Δ rpd3Δ
TRK2	47	49	11	39
SPO13	1.23	0.73	< 0.4	1.18
HO	45	100	7	39

FIG. 7. Tests for additivity between *rpd1* and *rpd3*. The experiment was performed on a tetratype tetrad from heterozygous *rpd1*/*RPD1* *RPD3*/*rpd3* congenic diploid strain M850. (a) Growth tests. −HIS, medium containing YNBAA lacking histidine; −TRP, medium containing YNBAA lacking tryptophan; CYH(37°), medium containing YNBAA supplemented with cycloheximide (0.04 μg/ml) and incubation at 37°C; CYH(30°), medium containing YNBAA supplemented with cycloheximide (0.04 μg/ml) and incubation at 30°C. (b) β-Galactosidase specific activities (units are defined in Materials and Methods) exhibited by wild-type, *rpd1*, *rpd3*, and *rpd1* *rpd3* cells under repression conditions. *TRK2*, *TRK2*:*lacZ* fusion in a multicopy plasmid; *SPO13*, *SPO13*:*lacZ* fusion in a centromeric plasmid during vegetative growth; *HO*, *HO*:*lacZ* fusion in a multicopy plasmid.

regulate the activity of DNA-binding proteins. Recent studies that have focused on the latter group have revealed previously unknown mechanisms of transcriptional regulation. Some of these include (i) negative regulation by interactions between transcriptional activators and non-DNA-binding proteins (e.g., MyoD-Id, [3]), (ii) modification of transcriptional activity by phosphatases (*SIT4* [38]), (iii) positive regulation by proteins that establish or maintain the phosphorylated level of transactivators (GAL11/SPT13 [24]), and (iv) molecular communication between transactivator proteins and the transcriptional machinery via adaptor proteins (4, 5, 19).

In the accompanying report, we describe a novel mechanism of gene regulation that is dependent on *RPD1* (43). Under the appropriate regulatory conditions, *RPD1* ensures both full activation and full repression of transcription.

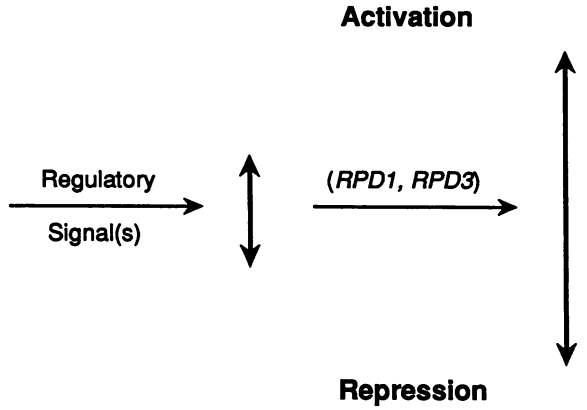


FIG. 8. RPD1 and RPD3 are required for maximum transcriptional responses. The vertical arrows represent the differences in levels of transcriptional activity between repression and activation conditions of a given gene. Horizontal arrows represent pathways leading to specific regulation (left) or enhancement of regulation. *RPD1* and *RPD3* act in the same pathway that leads to enhanced transcriptional regulation; the parentheses indicate that the order of function is unknown. For details, see the text.

Unlike the products of the *RAP1* and *MCM1* genes, which act as positive or negative regulators in a gene-specific manner (23), *RPD1* acts as both a positive and a negative regulator of the same genes. Furthermore, the role of *RPD1* in transcriptional regulation is global; many diversely regulated yeast genes are also subject to regulation by *RPD1* (43). Not surprisingly, several investigators have genetically identified this locus by selecting for inappropriate expression of different genes, including *HO* (27, 36), *SPO13* (37), and *TRK2* (42).

RPD1 maximizes the transcriptional responsiveness of many yeast genes, including (i) cell type-specific genes, (ii) cell differentiation-specific genes, (iii) genes that respond to external signals, and (iv) *TRK2*, which encodes the low-affinity K^+ transporter. *RPD1* (also known as *SIN3* [36], *SDI1* [27], and *UME4* [37]) does not encode a DNA-binding protein (43, 45, 46). Instead, *RPD1* appears to confer its effects on transcription by altering the activities of transcriptional activators and/or repressors in an unknown manner.

Given the diversity of genes under *RPD* regulation, it is likely that additional factors are required for its implementation. Our search for other genes involved in this regulation included analysis of *rpd3* mutants since they were identified in the same genetic selection scheme that gave rise to *rpd1* mutants (42). In this report, we describe genetic and molecular experiments that show that *RPD3* encodes a second factor required for full transcriptional response of many genes to their regulatory stimuli.

***RPD3* maximizes transcriptional states (positive and negative) of yeast genes.** Mutations in *RPD3* confer pleiotropic phenotypes, including suppression of the Trk^- phenotype of *trk1*Δ cells; hypersensitivity to cycloheximide, acriflavin, and ethidium bromide; increased production of APase; inability to sporulate when homozygous; and, in *MAT*α cells, decreased production of α-factor. These phenotypes are identical to those exhibited by cells containing mutations in *RPD1*, suggesting that *RPD3* is also involved in a mechanism that allows diverse genes to achieve maximal transcriptional states. To assess the role of *RPD3* further, we cloned the wild-type allele by its ability to suppress the cycloheximide hypersensitivity phenotype of *rpd3* recipient cells. The *RPD3* DNA sequence encodes a 433-amino-acid protein with a molecular mass of 48 kDa. The RPD3 protein shows little similarity to the sequences of other proteins contained in the available data bases (11a). Gene disruption experiments demonstrated that *RPD3* is not essential.

The identical phenotypes of *rpd1*Δ and *rpd3*Δ cells supported the notion that *RPD3* encodes a second factor required for maximal transcriptional regulation. This was confirmed by experiments in which expression of *lacZ*, driven by different yeast promoters, was measured in *RPD3* and *rpd3* cells. The results of these experiments demonstrated that *RPD3* is required for proper regulation of the low-affinity K^+ transporter (*TRK2*), cell type-specific genes (*STE6*, *TY2*, and *HO*), cell differentiation-specific genes (*SPO13*), and genes that are regulated by extracellular signals (*PHO5*). Surprisingly, although we showed that *rpd3* mutations lead to aberrant derepression of both *HO* and *SPO13*, there is no evidence to suggest allelism between *rpd3* mutations and *sin* and *ume* mutants (36a).

We tested whether *RPD1* and *RPD3* function in the same or different molecular pathways by testing for additivity of the *rpd1* and *rpd3* mutations. If RPD1 and RPD3 function in independent pathways, the pleiotropic phenotypes would be expected to be more severe in *rpd1 rpd3* double mutants than in either single mutant. Conversely, if RPD1 and RPD3 function in the same molecular pathway, the effects of double mutations would not be additive. We compared the effects on transcription and the phenotypes of cells containing the *rpd1*Δ or *rpd3*Δ single mutation with those of cells containing the *rpd1*Δ *rpd3*Δ double mutation. Phenotypic analysis included tests for cycloheximide hypersensitivity and the ability to grow on potassium-limiting media (dependent on derepression of *TRK2*). Gene expression assays included the measurement of β-galactosidase activity driven by the promoters of several *RPD*-regulated genes (*TRK2*, *SPO13*, and *HO*). In each of these tests, the effects of the double mutation were essentially indistinguishable from those of either one of the single mutations, suggesting that RPD1 and RPD3 function either at different steps in a single pathway or as a heterologous complex. Support for an RPD1-RPD3 complex is inferred from our observations that mutations in either *RPD1* or *RPD3* confer allele-specific subsets of the multiple phenotypes observed in *rpd1*Δ or *rpd3*Δ cells (unpublished data). These observations suggest that each of the two proteins takes part in the downstream functions that lead to the effects on transcriptional regulation.

The isolation of extragenic suppressors of *rpd1* and *rpd3* missense mutations should reveal the identity of proteins with which RPD1 and RPD3 interact. Results from these experiments will help to determine how RPD1 and RPD3 convey their global effects on transcriptional regulation.

ACKNOWLEDGMENTS

We thank C. Burd, D. Stillman, P. Farabaugh, M. Grunstein, C. Ko, P. Hieter, I. Herskowitz, G. Sprague, A. Mitchell, K. Tatchell, V. McKay, and F. Hilger for generous gifts of strains, plasmids, genomic libraries, promoter-*lacZ* fusions, and S1 probes.

This work was supported by grants from the National Science Foundation (DCB-8711346 and DCB-8657150) and the National Institutes of Health (GM45739) to R.F.G.

ADDENDUM IN PROOF

Two additional genes, distinct from *RPD1* and *RPD3*, have recently been shown to be involved in the transcrip-

tional regulation of diverse yeast genes, including *PHO5* and *ST*. Like the *RPD* genes, *SPT10* and *SPT21* can play both positive and negative roles in the transcriptional regulation of target genes (G. Natsoulis, C. Dollard, F. Winston, and J. D. Boeke, New Biol. 3:1–9, 1991). It will be of interest to determine whether these regulators constitute elements of the same system.

REFERENCES

1. **Arndt, K. T., C. A. Styles, and G. R. Fink.** 1989. A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell **56**:527–537.
2. **Basson, M. E., R. L. Moore, J. O'Rear, and J. Rine.** 1987. Identifying mutations in duplicated functions in Saccharomyces cerevisiae: recessive mutations in HMG-CoA reductase genes. Genetics **117**:645–655.
3. **Benezra, R., R. L. Davis, D. Lockson, D. L. Turner, and H. Weintraub.** 1990. The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. Cell **61**:49–59.
4. **Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61**:1199–1208.
5. **Chasman, D. I., N. F. Lue, A. R. Buchman, J. W. Lapointe, Y. Lorch, and R. D. Kornberg.** 1990. A yeast protein that influences the chromatin structure of UAS_G and functions as a powerful auxiliary gene activator. Genes Dev. **4**:503–514.
6. **Clark-Adams, C. D., D. Norris, M. A. Osley, J. S. Fassler, and F. Winston.** 1988. Changes in histone gene dosage after transcription in yeast. Genes Dev. **2**:150–159.
7. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell **58**:1183–1191.
8. **Fassler, J. S., and F. Winston.** 1988. Isolation and analysis of a novel class of suppressor of Ty insertion mutations in *Saccharomyces cerevisiae*. Genetics **118**:203–212.
9. **Fassler, J. S., and F. Winston.** 1989. The *Saccharomyces cerevisiae SPT13/GAL11* gene has both positive and negative regulatory roles in transcription. Mol. Cell. Biol. **9**:5602–5609.
10. **Gaber, R. F., D. M. Copple, B. K. Kennedy, M. Vidal, and M. Bard.** 1989. The yeast gene *ERG6* is required for normal membrane function but is not essential for biosynthesis of the cell-cycle-sparking sterol. Mol. Cell. Biol. **9**:3447–3456.
11. **Gaber, R. F., C. A. Styles, and G. R. Fink.** 1988. *TRK1* encodes a plasma membrane protein required for high-affinity potassium transport in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:2848–2859.

11a. **Goebl, M.** Personal communication.

12. **Haggren, W., and D. Kolodrubetz.** 1988. The *Saccharomyces cerevisiae ACP2* gene encodes an essential HMG1-like protein. Mol. Cell. Biol. **8**:1282–1289.
13. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the *SPT15* suppressor of Ty element insertions. Cell **58**:1173–1181.
14. **Han, M., and M. Grunstein.** 1988. Nucleosome loss activates yeast downstream promoters in vivo. Cell **55**:1137–1145.
15. **Hereford, L., K. Fahrner, J. Woolford, M. Rosbash, and D. B. Kaback.** 1979. Isolation of yeast histone genes H2A and H2B. Cell **18**:191–199.
16. **Herskowitz, I.** 1988. Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:536–553.
17. **Hicks, J. B., and I. Herskowitz.** 1976. Evidence for a new diffusible element of mating pheromones in yeast. Nature (London) **260**:246–248.
18. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
19. **Kelleher, R. J. I., P. M. Flanagan, and R. D. Kornberg.** 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61**:1209–1215.
20. **Klapholz, S., and R. E. Esposito.** 1980. Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics **96**:589–611.
21. **Ko, C. H., A. M. Buckley, and R. F. Gaber.** 1990. *TRK2* is required for low-affinity K^+ transport in *Saccharomyces cerevisiae*. Genetics **125**:305–312.
22. **Ko, C. H., and R. F. Gaber.** 1991. *TRK1* and *TRK2* encode structurally related K^+ transporters in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:4266–4273.
23. **Kurtz, S., and D. Shore.** 1991. RAP1 protein activates and silences transcription of mating-type genes in yeast. Genes Dev. **5**:616–628.
24. **Long, R. M., L. M. Mylin, and J. E. Hopper.** 1991. GAL11 (SPT13), a transcriptional regulator of diverse yeast genes, affects the phosphorylation state of GAL4, a highly specific transcriptional activator. Mol. Cell. Biol. **11**:2311–2314.
25. **McKay, V. L., S. K. Welch, M. Y. Insley, T. R. Manney, J. Holly, G. C. Saari, and M. L. Parker.** 1988. The *Saccharomyces cerevisiae BAR1* gene encodes an exported protein with homology to pepsin. Proc. Natl. Acad. Sci. USA **85**:55–59.
26. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Nasmyth, K., D. Stillman, and D. Kipling.** 1987. Both positive and negative regulators of *HO* transcription are required for mother-cell-specific mating-type switching in yeast. Cell **48**:579–587.
28. **Nogi, Y., and T. Fukusawa.** 1980. A novel mutation that affects utilization of galactose in *Saccharomyces cerevisiae*. Curr. Genet. **2**:115–120.
29. **Norris, D., and M. A. Osley.** 1985. Two gene pairs encoding H2A and H2B play different roles in the *Saccharomyces cerevisiae* life cycle. Mol. Cell. Biol. **7**:3473–3481.
30. **Peterson, C. L., W. Kruger, and I. Herskowitz.** 1991. A functional interaction between the C-terminal domain of RNA polymerase II and the negative regulator SIN1. Cell **64**:1135–1143.
31. **Roeder, G. S., C. Beard, M. Smith, and S. Keranen.** 1985. Isolation and characterization of the *SPT2* gene, a negative regulator of Ty-controlled yeast gene expression. Mol. Cell. Biol. **5**:1543–1553.
32. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1988. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60**:237–243.
33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
34. **Sherman, F., G. R. Fink, and J. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **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.
36. **Sternberg, P. W., M. J. Stern, I. Clark, and I. Herskowitz.** 1987. Activation of the yeast *HO* gene by release from multiple negative controls. Cell **48**:567–577.

36a. **Stillman, D., and R. E. Esposito.** Personal communication.

37. **Strich, R., M. R. Slater, and R. E. Esposito.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86**:10018–10022.
38. **Sutton, A., D. Immanuel, and K. T. Arndt.** 1991. The SIT4 protein phosphatase functions in late G_1 for progression into S phase. Mol. Cell. Biol. **11**:2133–2148.
39. **Suzuki, Y., Y. Nogi, A. Abe, and T. Fukasawa.** 1988. *GAL11* protein, an auxiliary transcription activator for genes encoding galactose-metabolizing enzymes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:4991–4999.
40. **Toh-e, A., Y. Ueda, S.-I. Kakimoto, and Y. Oshima.** 1973. Isolation and characterization of acid phosphatase mutants in *Saccharomyces cerevisiae*. J. Bacteriol. **113**:727–738.

41. **Trueheart, J., J. D. Boeke, and G. R. Fink.** 1987. Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. Mol. Cell. Biol. **7**:2316–2328.
42. **Vidal, M., A. M. Buckley, F. Hilger, and R. F. Gaber.** 1990. Direct selection for mutants with increased K^+ transport in *Saccharomyces cerevisiae*. Genetics **125**:313–320.
43. **Vidal, M., R. Strich, R. E. Esposito, and R. F. Gaber.** 1991. *RPD1* (*SIN3*) is required for maximal activation and repression of diverse yeast genes. Mol. Cell. Biol. **11**:6306–6316.
44. **Wang, H., I. Clark, P. R. Nicholson, I. Herskowitz, and D. Stillman.** 1990. The *Saccharomyces cerevisiae SIN3* gene, a negative regulator of *HO*, contains four paired amphipathic helix motifs. Mol. Cell. Biol. **10**:5927–5936.
45. **Wang, H., P. R. Nicholson, and D. J. Stillman.** 1990. Identification of a *Saccharomyces cerevisiae* DNA-binding protein involved in transcriptional regulation. Mol. Cell. Biol. **10**:1743–1753.
46. **Wang, H., and D. J. Stillman.** 1990. In vitro regulation of a *SIN3*-dependent DNA-binding activity by stimulatory and inhibitory factors. Proc. Natl. Acad. Sci. USA **87**:9761–9765.
47. **Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink.** 1984. Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. Genetics **107**:179–197.
48. **Yocum, R. R., S. Hanley, R. West, Jr., and M. Ptashne.** 1984. Use of *lacZ* fusions to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1985–1988.