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RPD1 (*SIN3*/*UME4*) Is Required for Maximal Activation and Repression of Diverse Yeast Genes

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We show that the extent of transcriptional regulation of many, apparently unrelated, genes in *Saccharomyces cerevisiae* is dependent on *RPD1* (and *RPD3* [M. Vidal and R. F. Gaber, Mol. Cell. Biol. 11:6317–6327, 1991]). Genes regulated by stimuli as diverse as external signals (*PHO5*), cell differentiation processes (*SPO11* and *SPO13*), cell type (*RME1*, *FUS1*, *HO*, *TY2*, *STE6*, *STE3*, and *BAR1*), and genes whose regulatory signals remain unknown (*TRK2*) depend on *RPD1* to achieve maximal states of transcriptional regulation. *RPD1* enhances both positive and negative regulation of these genes: in *rpd1Δ* mutants, higher levels of expression are observed under repression conditions and lower levels are observed under activation conditions. We show that several independent genetic screens, designed to identify yeast transcriptional regulators, have detected the *RPD1* locus (also known as *SIN3*, *SDI1*, and *UME4*). The inferred RPD1 protein contains four regions predicted to take on helix-loop-helix-like secondary structures and three regions (acidic, glutamine rich, and proline rich) reminiscent of the activating domains of transcriptional activators.

Until recently, transcriptional regulation studies have focused primarily on components directly involved in activation of gene expression (20, 33, 42). Now, however, such studies have been extended to mechanisms that modulate the activity of the *trans* activators themselves (4). Although the molecular details vary from case to case, two classes of protein-protein interaction have been inferred to mediate a regulatory effect(s) on *trans* activators. (i) Interaction between a transcriptional activator and its regulator can occur through homologous domains. For example, MyoD is negatively regulated by Id via homologous helix-loop-helix (HLH) domains. (ii) Modulation of DNA-binding activity can occur through interactions of nonhomologous protein domains. For example, the glucocorticoid receptor and the transcription factor cJun-cFos (AP1) can exhibit negative effects on one another (21) through protein-protein interactions involving the zinc finger DNA-binding domain of the glucocorticoid receptor and the leucine zipper domain of cJun and/or cFos (6, 45, 64).

Transcriptional repressors, too, are subject to functional regulation through protein-protein interaction. Transcriptional regulator MCM1, which regulates cell type-specific genes in *Saccharomyces cerevisiae* (40), can play opposite roles in the transcriptional regulation of the genes under mating type control. MCM1 acts as either a repressor or an activator, depending on the factors with which it interacts (reviewed in reference 13): it participates in **a**-specific gene activation in *MAT***a** cells but also increases the binding ability of α2, a repressor of **a**-specific genes in *MAT*α cells (1, 22). In addition, MCM1 acts synergistically with α1 to enhance transcription of α-specific genes in *MAT*α cells (3, 18, 39).

We describe a new type of transcriptional regulation in *S. cerevisiae* that is mediated through products of the *RPD1* (this report) and *RPD3* (59) genes. We also show that several independent genetic screens, designed to identify new transcriptional regulators in *S. cerevisiae*, have detected the same gene: *RPD1* is allelic to *UME4* (52), *SIN3* (50), and *SDI1* (37). *RPD1* and *RPD3* were previously identified as negative regulators of *TRK2* (57), the gene that encodes the low-affinity K^+ transporter (24, 25). Through derepression of *TRK2*, *rpd* mutations confer reduced potassium dependency, allowing growth on low-potassium medium. *UME4* was identified as a mitotic repressor of a class of meiotic genes (*SPO11*, *SPO13*, and *SPO16*) coexpressed early in meiosis (52). Mutations in *UME4* allow unscheduled meiotic gene expression through increased transcription of these genes during vegetative growth. Mutations in *SIN3* (*SDI1*) were identified in a screen for variants that allow expression of *HO* in the absence of *SWI5* and are thus SWI5 independent. *SIN3* is proposed to be required for repression of *HO* in daughter cells (37, 50).

We show that for many, seemingly unrelated, yeast genes, both full repression and full activation of transcription are *RPD1* dependent; in the absence of *RPD1*, higher levels of expression under repression conditions and lower levels of expression under activation conditions are observed. *RPD1* does not appear to encode a DNA-binding protein (62). Nevertheless, *RPD1* regulates cell differentiation-specific and metabolically regulated genes in addition to cell type-specific genes. *RPD1* encodes a protein that contains different types of domains that may be involved in protein-protein interactions, including four HLH-like motifs and regions similar to the activating domains of transcriptional activators (acidic, Gln rich, and Pro rich). The diversity of these domains could account for the global effects of *rpd1* mutations on gene expression in *S. cerevisiae*.

MATERIALS AND METHODS

Media. The genetic crosses and standard media used were previously described (46). Synthetic low-salt and low-phosphate media were prepared essentially as previously de-

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TABLE 1. Strains used in this work

Strain	Genotype	Source or reference
A138	*MAT***a** *pho80*	B. Mortimer
KT43	*MATα ura3 leu2 trp1 his4*	K. Tatchell
KT53	*matα-182 ura3 leu2 trp1 his4*	K. Tatchell
KT146	*MAT***a** *ura3 leu2 trp1 his4*	K. Tatchell
M398	*MATα ura3-52 trp1Δ his3-200 leu2-1 trk1Δ*	
M444	*MATα ura3-52 trp1Δ his3-200 leu2-1 trk1Δ rpd1-73*	
M476	*MATα ura3-52 trp1Δ his3-200 leu2-1 trk1Δ rpd1Δ::TRP1*	
M482	*MATα ura3-52 trp1Δ his3-200 leu2-1 trk1Δ rpd1Δ::URA3*	
M517	M537/M398	
M537	*MAT***a** *ura3-52 trp1Δ his3-200 leu2-1 trk1Δ*	
M613	*MAT***a** *ura3-52 trp1Δ his3-200 leu2-1 trk1Δ rpd1Δ::TRP1*	
M617	M613/M476	
M624	*MATα ura3-52 trp1Δ his3-200 leu2-1 trk1Δ rpd1Δ::HIS3*	
M664	*MATα ura3-52 leu2-1 trp1Δ his4-15 rpd1Δ::TRP1*	
M708, M709	*MATα ura3-52 leu2-1 trp1Δ his4-15 rpd1Δ*::TRP1 *bar1Δ::LEU2*	
R1689	MATα *ura3-52 his4-15 lys9 trk1Δ rpd1-41*	57
RC634	*MAT***a** *sst1*	J. Thorner
RSY142	*MAT***a** *his3-11,15 leu2-3,112 ume4-1 ura3-1*	
RSY143	*MATα ade6 can1-100 his4-C leu2-3,112 trp1-1 rpd1-1 ura3-1*	

scribed (8, 57). Sporulation medium was prepared as previously described (23).

Cloning *RPD1*. The *RPD1* gene was cloned by transforming strain R1689 (*rpd1-41 trk1Δ ura3-52*) (Table 1) with a yeast YCp50 genomic library containing the *URA3* selectable marker (44). Approximately 10,000 Ura$^+$ transformants were screened for complementation of two *rpd1* phenotypes: cycloheximide hypersensitivity (Cyh^{hs}) and ability to grow on medium containing low concentrations of potassium (Trk$^+$). Plasmid DNAs from seven Ura$^+$ Cyh^r Trk$^-$ transformants were recovered in *Escherichia coli* and retransformed into R1689 to confirm their phenotypes. Overlapping DNA fragments were present in all of the plasmids; pRPD1, which contains the shortest genomic fragment, was retained for further analysis (see Fig. 1). The 3.6-kb *Eco*RI fragment from pRPD1 containing the 5′ end of *RPD1* was inserted into *URA3*-containing integrative vector pRS306 (50), linearized, and used to transform strain M444 (*ura3-52 trk1Δ rpd1-73*) (Table 1). Two Ura$^+$ transformants were crossed with strain M537 (*ura3-52 trk1Δ RPD1*) (Table 1), and the resulting diploids were sporulated. Among 22 tetrads analyzed, the Ura$^+$, Cyh^{hs}, and Trk$^+$ phenotypes cosegregated, demonstrating that the 3.6-kb *Eco*RI-*Eco*RI fragment of pRPD1 directed integration at the *rpd1* locus.

Cloning *UME4*. The strategy used to clone *UME4* relied on the ability to select against expression of the *URA3* gene by using the analog 5-fluoro-orotic acid (5-FOA) (5). *ume4-1 ura3-1 leu2-3,112* cells transformed by a *SPO13*:*URA3* promoter fusion express *URA3* because of the *ume4*-dependent aberrant mitotic expression and therefore are sensitive to 5-FOA. A library inserted in *LEU2*-containing plasmid p366 (P. Hieter) was transformed into strain RSY143 (*ume4-1 ura3-1 leu2-3,112*) (Table 1) by selection for Leu$^+$, and complementing plasmids were selected by resistance to 5-FOA. Nine independent isolates containing overlapping sequences were recovered in *E. coli* and retransformed into *ume4-1* strains. The absence of mitotic *SPO13* mRNA and the ability to complement the Spo$^-$ defect indicated that the observed phenotypes were plasmid borne. The complementing fragment was localized by deletion analysis (see Fig. 1) and was able to direct integration of the *LEU2* gene into the *UME4* locus as determined by meiotic cosegregation analysis (18 tetrads).

Sequencing *RPD1*. A region of pUME4 sufficient for complementation of both the *rpd1-41* and *ume4-1* mutations (Fig. 1) was subcloned into pRS316 (48): pU3 contains the 3.2-kb *Pst*I-*Sau*3A fragment (*Sau*3A is located 500 nucleotides upstream of the potential initiating ATG); pU11 and pU12 contain the 2.0-kb (*RPD1*-internal) and 2.2-kb (contains the *RPD1* 5′ end) *Bgl*II-*Bgl*II fragments, respectively. Two sets of nested deletions, one for each strand, were generated in each of the plasmids pU3, pU11, and pU12 by using the exonuclease III procedure of Henikoff (11), as recommended in the instructions supplied with the Pharmacia Nested Deletion Kit. Dideoxy sequencing of double-stranded plasmid DNA (44) was carried out by using the Sequenase Sequencing Kit from United States Biochemical Co. Gradient gel electrophoresis was performed as previously described (44). The DNA sequence encompassing the region that encodes *RPD1* was read on both strands. DNA sequence data were stored and analyzed by using the DNA Inspector IIe program from Textco, Inc.

Construction of isogenic *RPD1* and *rpd1Δ* strains. A complete deletion of *RPD1* (*rpd1Δ*) was constructed in vitro by the gamma deletion method (48). To facilitate further genetic analysis, the same deletion allele was constructed in three pRS integrative plasmids containing different selectable markers (*HIS3*, *TRP1*, and *URA3*) (48). The 3.6-kb *Eco*RI fragment of pRPD1 was inserted into the pRS plasmids such that the *Eco*RI site located in the *RPD1* open reading frame (ORF) (see Fig. 1) was located near the T3 promoter. The 2.0-kb *Bgl*II fragment of pRPD1 was subsequently inserted to position the *Xba*I site (see Fig. 1) near the T7 promoter. The plasmids containing the deletion were pMV117 (*rpd1Δ::URA3*), pMV120 (*rpd1Δ::HIS3*), and pMV121 (*rpd1Δ::TRP1*). Table 1 lists the yeast strains used in this study.

A strain containing the markers *MATα ura3-52 trp1Δ1 his3-200 leu2Δ1 trk1Δ* (M398) was obtained in a genetic cross between R1174 (8) and YPH252 (48). To generate an isogenic *MAT***a** strain, M398 was transformed with a centromeric plasmid containing the *HO* gene. Strain M537 (*MAT***a**, isogenic to M398) was recovered as a meiotic segregant of a Ura$^-$ M398/M398 diploid that had lost the YCp50-*HO* plasmid. M398 was crossed with M537 to generate diploid strain M517. Isogenic *rpd1Δ* strains were constructed by transformation of M398 with plasmids pMV117, pMV120, and pMV121, generating strains M482, M624, and M476, respectively. Southern blot analysis confirmed that integration of pMV117, pMV120, and pMV121 occurred at and deleted the *RPD1* gene (data not shown). Strain M613 (*MAT***a** *rpd1Δ*) was obtained as a meiotic segregant from a diploid generated by crossing isogenic strains M476 and M537. Homozygous *rpd1Δ/rpd1Δ* diploid strain M617 was obtained by crossing M613 with M476.

Strain M664 was obtained by transformation of strain KT43 with pMV121. M708 was obtained by transformation of M664 with pZV77 (30).

Phenotypic characterization. The colony-staining overlay assay used to observe acid phosphatase (APase) activity qualitatively was previously reported (55). APase activity was quantified by the method of Torriani as described by Toh-e et al. (55). The values obtained are averages of six assays. One enzymatic unit was defined as the amount of enzyme necessary to liberate 1 μmol of *p*-nitrophenylphosphate per h; one specific unit was the number of enzymatic units per optical density unit of cells at 660 nm (OD_{660}).

Mating assays were performed essentially as described by Trueheart et al. (56). 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 (46) 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. We typically observed 50% mating in wild-type crosses, 30% mating in heterozygous crosses, and 3 to 5% mating in *MAT***a** *rpd1* × *MAT*α *rpd1* crosses.

The halo assay was performed as described by Herskowitz (12 and references therein). About 10^4 cells of α-factor-hypersensitive tester strain RC634 (*sst1*) were spread onto a YEPD (46) 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.

Nuclease S1 analyses. Total and poly(A)$^+$ RNAs were isolated, and reactions were performed essentially as described previously (52). The 3′ *FUS1* (pSL589 (29), 3′ *STE2* (pSL628), and 5′ *STE3* (pSL774) probes were provided by G. Sprague. The 5′ *STE6* probe, pKAK5, was provided by J. Trueheart, courtesy of Karl Kuchler. The 5′ *TRK2* probe was constructed for this study. The *ACT1* and *LYS2* probes were constructed by R. Surosky. The *SPO11* and *SPO13* probes were as described in reference (52). The resulting S1-protected probe was quantitated either by excising the corresponding band from the gel and determining radioactivity in a liquid scintillation counter or by tracing the intensities of exposure on film by using an LKB Ultroscan XL. In one experiment, in which the amount of total Poly(A)$^+$ RNA was standardized by optical density, the amounts of *ACT1* (actin) message in *RPD1* and *rpd1*Δ extracts were found to be indistinguishable. In subsequent experiments, the poly(A)$^+$ loading was standardized to actin levels in each preparation because of the differences in rRNA contamination of such preparations.

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. (17). 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 a low-salt medium (57) containing 100 mM KCl and 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. The cells were washed twice with Z buffer (31) and resuspended at various densities, depending on the basal activities of the different promoters tested. After resuspension, the final OD_{600} was measured in a Beckman 25 spectrophotometer. A minimum of three different dilutions were assayed for β-galactosidase activity in permeabilized cells as described previously (31, 65). Specific activities were defined as (1,000 × OD_{420})/(time [minutes], × volume [milliliters × cell density [OD_{600}]). For the average values and standard deviations for a minimum of 12 measurements for each promoter tested, see Fig. 2 and 5. In our search for pleiotropic phenotypes, no difference in cell size or clumpiness or correlation between OD_{600} and actual cell concentrations was detected. Therefore, we normalized the expression assays to OD_{600}.

The following is a list of the *lacZ* fusions tested; in each case, the *lacZ* gene is fused in frame to the ORF corresponding to the promoter tested (Strich et al. [52] determined that *rpd1* [*ume4*] mutations do not affect plasmid copy number): *TRK2*::*lacZ*, *URA3* integrative plasmid pAB138 (contains the entire promoter and first 1.4 kb of the *TRK2* open reading frame [25]); *TRK2*::*lacZ*, *URA3* multicopy plasmid pAB137 (*TRK2*::*lacZ* construct identical to pAB138); *SPO11*::*lacZ* *TRP1* multicopy plasmid pMS4 (52); *SPO13*::*lacZ* *URA3* multicopy plasmid pBW2 (52); *STE6*::*lacZ*, *URA3* multicopy plasmid pΔHA (see the legend to Fig. 2 for details of construction; gift of I. Herskowitz); *HO*::*lacZ*, *URA3* multicopy plasmid YEpHO::lacZ (gift of P. Dorhmann and D. Stillman); *TY2*::*lacZ*, *URA3* centromeric plasmid p1033 (*lacZ* fused at nucleotide 1033 of *TY2*; gift of P. Farabaugh); *PHO5*::*lacZ*, *URA3* centromeric plasmid pMH313 (10).

RESULTS

Three genetic screenings identified *RPD1*. Yeast cells deleted for *TRK1*, the gene that encodes the high-affinity K^+ transporter, exhibit a Trk$^-$ phenotype, i.e., they fail to grow on media containing low concentrations of K^+ (8). Recessive *rpd1* mutations were identified as suppressors of the Trk$^-$ phenotype in *trk1*Δ cells (57) by the constitutive ability to derepress transcription of *TRK2* (see below), the gene that encodes the low-affinity K^+ transporter gene (24). *rpd1* mutations cause recessive pleiotropic phenotypes, including hypersensitivity to cycloheximide (Cyh^{hs}; data not shown). The wild-type *RPD1* gene was cloned by virtue of its ability to suppress the Cyh^{hs} phenotype of *rpd1* recipient cells (see Materials and Methods). Two overlapping clones were recovered that, upon reintroduction into *ura3-52 trk1*Δ *TRK2 rpd1-41* cells, restore repression of *TRK2*. A genomic fragment from plasmid pRPD1 (Fig. 1) directed integration of a vector to the *rpd1* locus, demonstrating that the clones contain the *RPD1* gene (see Materials and Methods).

A number of genes required for meiosis and spore formation have been shown to be transcriptionally regulated during development; they are repressed during mitotic cell division and induced at specific stages of sporulation (2, 41, 63). The wild-type *UME4* gene was cloned by its ability to suppress expression of *URA3* from a *SPO13* promoter fusion in *ume4-1 ura3-52* recipient cells. In contrast to Ura$^+$ cells, the Ura$^-$ transformants were resistant to the analog 5-FOA (see Materials and Methods). Two overlapping clones were obtained (Fig. 1) that, upon transformation into *ume4-1 ura3-52 SPO13*::*lacZ* recipients, restored decreased levels of β-galactosidase activity and *SPO13* mRNA. The cloned sequences direct homologous recombination of an integrative plasmid to the *ume4* locus, demonstrating that they contain *UME4* (see Materials and Methods).

Four lines of evidence demonstrate that *RPD1* and *UME4* are the same gene. (i) *ume4-1* maps to the same genetic location previously reported for *rpd1* (57): a DNA fragment containing *UME4* hybridized to chromosome XV by trans-

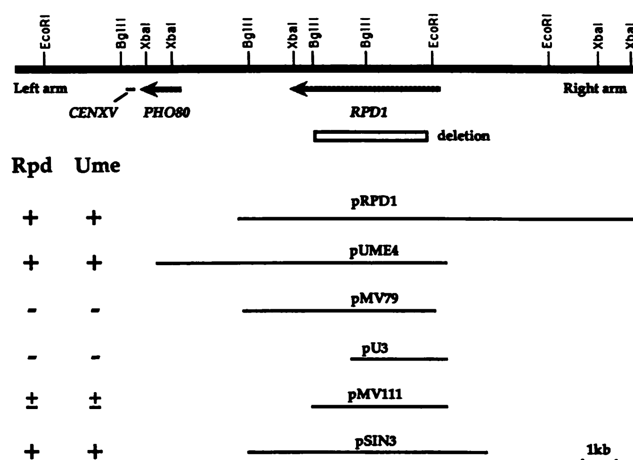


FIG. 1. Restriction map of the *RPD1* locus. The top line represents an abbreviated restriction map of the *RPD1* region. The relative positions of *PHO80* and *RPD1* are based on restriction map comparisons of four plasmids, pC1C2-PHO80, pCEN15 (15), pRPD1, and pUME4. This physical assignment is consistent with the previously identified genetic map positions of *pho80*, *rpd1*, and *CENXV* (34, 57). The arrows represent the ORFs and direction of transcription of *PHO80* (54) and *RPD1*. The open box represents the genomic sequence, between the *Eco*RI and *Bgl*II sites, deleted in the *rpd1*Δ mutation. The *Eco*RI site is located 24 nucleotides downstream of the potential translation initiator ATG in the *RPD1* ORF. The lines below represent *RPD1* clones obtained independently in three cloning experiments (the data on pSIN3 are from reference 60, no. 389) and subclone fragments that delimit the borders of the gene. Each clone was tested in two genetic backgrounds: (i) the Rpd phenotype was assayed in an *rpd1-41 trk1*Δ mutant strain (a plus sign indicates growth on cycloheximide and no growth on low-K$^+$ media), and (ii) the Ume phenotype was assayed in a *ume4-1* mutant strain (a plus sign indicates absence of β-galactosidase activity exhibited by a *SPO13:lacZ* fusion and ability to grow on 5-FOA plates in the presence of a *SPO13:URA3* fusion).

verse alternative-field electrophoresis analysis of separated yeast chromosomes (data not shown) and tetrad analysis indicated that *ume4-1* is tightly centromere linked (23 FDS:0 SDS). (ii) Single-copy *RPD1* and *UME4* clones complement *ume4* and *rpd1* mutations, respectively (Fig. 1): monocopy plasmid pRPD1 conferred decreased β-galactosidase activity in a *ume4-1 SPO13::lacZ* recipient, and plasmid pUME4 suppressed the Cyh^{hs} and Trk$^+$ phenotypes in *rpd1-41 trk1*Δ *TRK2* mutants. (iii) *RPD1* and *UME4* clones share common restriction fragments: restriction digests of the cloned DNA carried by the pRPD1 and pUME4 plasmids yielded comigrating fragments consistent with an overlap of about 6 kb (Fig. 1). (iv) *ume4-1* and *rpd1-41* mutations fail to complement each other: a *ume4/rpd1* diploid obtained by mating a *MAT*α *rpd1* strain (R1689 [Table 1]) with a *MAT***a** *ume4-1* strain (RSY143 [Table 1]) exhibited the Cyh^{hs} phenotype of the haploid parents.

Sequence analysis of *RPD1* (*UME4*) (see below) indicated identity with another gene. Comparison of the single ORF with other protein sequences within a data base containing unpublished sequences (8a) revealed 99.5% identity between RPD1 (UME4) and the protein encoded by *SIN3* (60). Two additional observations are consistent with the identity between *SIN3* and *RPD1* (*UME4*). (i) A centromeric plasmid carrying *SIN3* fully complements the *rpd1-41* mutation (Fig. 1), and (ii) *sin3* mutations have been recently mapped to the same location as *rpd1* (*ume4*) (60). These data led us to conclude that *rpd1*, *ume4*, and *sin3* (also allelic to *sdi1* [61]) are mutant alleles of the same gene.

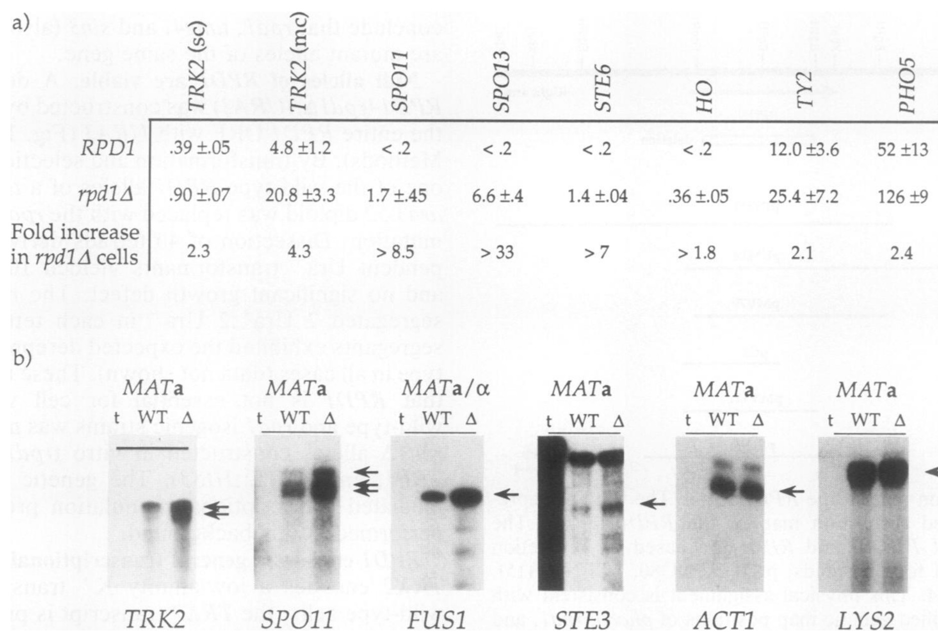
Null alleles of *RPD1* are viable. A deletion mutation in *RPD1* (*rpd1*Δ*::URA3*) was constructed by replacing virtually the entire *RPD1* ORF with *URA3* (Fig. 1 and Materials and Methods). By transformation and selection for Ura$^+$ clones, one of the wild-type *RPD1* alleles of a *trk1*Δ*/trk1*Δ *ura3-52/ura3-52* diploid was replaced with the *rpd1*Δ*::URA3* deletion mutation. Dissection of 40 tetrads derived from four independent Ura$^+$ transformants yielded 100% spore viability and no significant growth defect. The *rpd1*Δ*::URA3* allele segregated 2 Ura$^+$:2 Ura$^-$ in each tetrad, and the Ura$^+$ segregants exhibited the expected derepressed *TRK2* phenotype in all cases (data not shown). These results demonstrate that *RPD1* is not essential for cell viability. A set of wild-type and *rpd1* isogenic strains was made by using three *rpd1*Δ alleles constructed in vitro (*rpd1*Δ*::URA3*, *rpd1*Δ*::TRP1*, and *rpd1*Δ*::HIS3*). The genetic analysis of *RPD1*-mediated transcriptional modulation presented below was performed in this background.

***RPD1* encodes a general transcriptional negative regulator.** *TRK2* encodes a low-affinity K$^+$ transporter (24, 25). In wild-type cells, the *TRK2* transcript is present at low levels because of the presence of an upstream repression sequence in the promoter region (58). Loss of RPD1 function results in an increase in the V_{max} of low-affinity K$^+$ transport, allowing *trk1*Δ *TRK2* cells to grow on low K$^+$ medium. *trk2* mutations are epistatic to this phenotype, indicating that the effect of the *rpd1* mutation is mediated through *TRK2* (57). *TRK2* transcripts are virtually undetectable in the wild type, *rpd1* mutants, and cells containing only a single copy of the transporter gene. However, when expressed from a multicopy plasmid, *TRK2* transcripts were observed to increase two- to threefold in *rpd1* cells compared with isogenic wild-type cells (Fig. 2). In addition, β-galactosidase activity derived from *TRK2::lacZ* fusions in which *lacZ* transcription is under control of the *TRK2* promoter was correspondingly higher in *rpd1* cells than in wild-type cells (Fig. 2). We conclude that *RPD1* is required for normal repression of *TRK2*.

Entry of diploid cells into meiosis is under the control of both cell type and nutritional status. The *SPO11*, *SPO13*, and *SPO16* genes, normally expressed only in *MAT***a**/*MAT*α cells starved for glucose and nitrogen, are coordinately repressed by *RPD1* (*UME4*) during vegetative growth (52). The phenotype of the *rpd1*Δ mutant was examined further by assaying β-galactosidase activity from *SPO11::lacZ* and *SPO13::lacZ* fusions and by measuring *SPO11* mRNA levels. A significant increase (>8.5- and >33-fold, respectively) in expression of these genes was observed in *rpd1*Δ mutant cells compared with wild-type *RPD1* cells (Fig. 2a and b). Additional studies of mRNA turnover demonstrated that increased message accumulation of *SPO11* and *SPO13* in *rpd1* cells is due to enhanced transcription initiation and not to changes in transcript stability (53).

Finally, work from other laboratories revealed that *RPD1* (*SIN3*) is required for repression of *HO* in daughter cells (37, 50). Thus, the transcription of genes as apparently unrelated as those involved in K$^+$ transport, cell differentiation, and cell type are, at least in part, regulated by the product of the same regulatory gene.

***RPD1* is required for repression of cell type-specific genes: (i) Mating type-specific genes.** Mating type specificity in *S. cerevisiae* depends on appropriate expression of two sets of cell type-specific genes (**a**- and α-specific genes) (reviewed in reference 12). Mutations in genes essential for transcrip-



a)

	TRK2 (sc)	TRK2 (mc)	SPO11	SPO13	STE6	HO	TY2	PHO5
RPD1	.39 ±.05	4.8 ±1.2	< .2	< .2	< .2	< .2	12.0 ±3.6	52 ±13
rpd1Δ	.90 ±.07	20.8 ±3.3	1.7 ±.45	6.6 ±.4	1.4 ±.04	.36 ±.05	25.4 ±7.2	126 ±9
Fold increase in *rpd1Δ* cells	2.3	4.3	> 8.5	> 33	> 7	> 1.8	2.1	2.4

FIG. 2. Transcriptional repression in *rpd1* mutants. (a) β-Galactosidase specific activities exhibited by *RPD1* and *rpd1* cells under repression conditions. *TRK2*(sc), *TRK2:lacZ* fusion integrated into the yeast genome at the *URA3* locus; *TRK2*(mc), *TRK2:lacZ* fusion in a multicopy plasmid; *SPO11*, *SPO11:lacZ* fusion in a centromeric plasmid during mitosis; *SPO13*, *SPO13:lacZ* fusion in a centromeric plasmid during mitosis; *STE6*, *cyc1* promoter deleted of its endogenous UAS element (9) and containing the **a**-specific UAS element (19) fused to *lacZ* in a multicopy plasmid in *MATα* cells; *HO*, *HO:lacZ* fusion in a multicopy plasmid in diploid strains; *TY*, *TY2:lacZ* fusion in a centromeric plasmid transformed in diploid strains; *PHO5*, *PHO5:lacZ* fusion in a centromeric plasmid at a high phosphate concentration. (b) RNA quantitation. The riboprobes used for the various S1 protection experiments are listed below the panels. The cell type from which the RNA was isolated is given above each panel. The arrows indicate the bands, resulting from the mRNA protected probe. From 20 to 40 μg of total RNA was used in each lane, except for *FUS1*, in which 10 μg of poly(A)$^+$ RNA was used. *TRK2* RNA was isolated from a strain containing a high-copy *TRK2:lacZ* fusion plasmid to amplify the *TRK2* mRNA signal. t, tRNA used as a negative control. The tRNA lane in the *STE3* panel was overexposed to detect the low-abundance *STE3* message.

tional regulation of cell type-specific genes can result in a sterility or semisterility phenotype. *MAT***a** *rpd1* and *MATα rpd1* strains mate poorly. The effect is minor when *rpd1* cells are crossed with *RPD1* cells but easily detectable in homozygous *rpd1* × *rpd1* crosses (Fig. 3a). In the latter case, mating efficiency is reduced approximately 10-fold (see Materials and Methods). Mating of *MAT***a** *RPD1* and *MAT***a** *rpd1Δ* strains with a *MATα* strain incapable of mating (*matα2*) is shown in Fig. 3a for comparison.

To determine whether the semisterility phenotype of *rpd1* cells is due to a decrease in pheromone production, we examined the amount of α-factor secreted by *MATα rpd1* cells. As shown in Fig. 3b, the level of extracellular α-factor was reduced, compared with that of an isogenic *MATα RPD1* strain. Paradoxically, expression of *MFα1*, the major gene that codes for α-factor (28), was only slightly lower in *rpd1* cells, a decrease that is insufficient to account for the total reduction of α-factor in these cells (data not shown). It was therefore of interest to determine whether aberrant expression of *BAR1* (also called *SST1*), an **a**-specific gene that encodes an α-factor protease normally repressed in *MATα* cells (14, 30), is involved in the reduction of α-factor in *MATα rpd1* cells. This was tested by measuring the level of α-factor in a set of strains containing both *bar1* and *rpd1* mutations. The *bar1Δ* mutation significantly increased the ability of *MATα rpd1* cells to produce α-factor (Fig. 3b), suggesting that *RPD1* is a negative regulator of *BAR1*.

Expression of **a**-specific genes is regulated by a well-defined *cis* element present in their promoters; repression of **a**-specific genes in *MATα* cells is mediated by an upstream repression sequence overlapping an upstream activation sequence (UAS), both of which are contained in this element (27). This element, when inserted into a *CYC1* promoter deleted for its own UAS [*cyc1*::UAS (*STE6*)::*lacZ*], is sufficient for **a**-specific expression of the chimeric promoter (19). Mutant *rpd1 MATα* cells containing the *cyc1*::UAS(*STE6*)::*lacZ* plasmid exhibited sevenfold higher β-galactosidase levels than the wild type *RPD1 MATα* cells (Fig. 2a). Expression of an α-specific gene, *STE3* (49), in *MAT***a** cells was also reproducibly altered, although precise quantitation was difficult as the basal level of *STE3* in *MAT***a** cells is extremely low (Fig. 2b). These results demonstrate that accurate repression of at least a subset of cell type-specific genes in haploid *MATα* and *MAT***a** cells is dependent on *RPD1*.

(ii) Haploid-specific genes. In addition to the **a**- and α-specific cell type genes described above, we also examined the regulation of haploid-specific cell type genes, i.e., genes normally expressed in both *MAT***a** and *MATα* cells but repressed in **a**/α diploids. Meiosis and spore formation in **a**/α diploid cells are dependent on the repression of such haploid-specific genes (reviewed in reference 13). Typically, wild-type (*RPD1*/*RPD1*) or heterozygous (*RPD1*/*rpd1Δ*) diploid cells produce approximately 70% four-spored asci when induced to undergo sporulation, whereas this frequency falls to less than 0.01% in *rpd1*/*rpd1* homozygous diploids; no asci were observed in over 50,000 cells microscopically examined.

By analogy with the semisterility phenotype that results at least in part from the aberrant derepression of **a**-specific

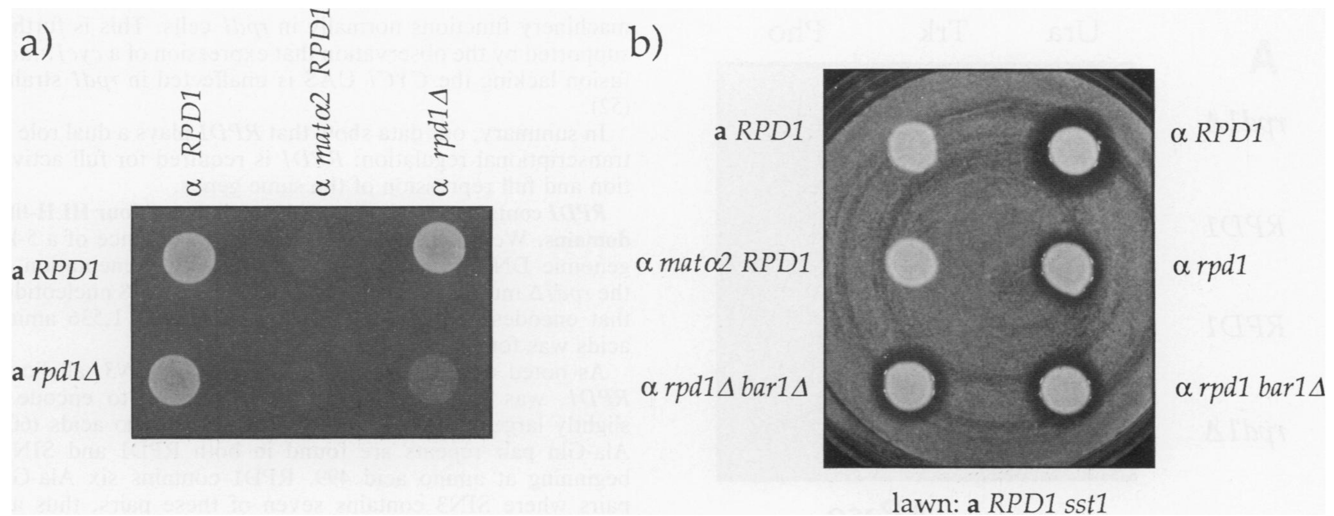


FIG. 3. Mating defect of *rpd1* cells. (a) Semisterility phenotype. For each mating, equal numbers of cells were mixed, incubated for 3 h, diluted, and plated on medium selective for diploids. The strains used are listed in Table 1: KT43 (*MATα RPD1*), KT53 (*matα2 RPD1*), M664 (*MATα rpd1Δ*), M513 (*MAT***a** *RPD1*), and M613 (*MAT***a** *rpd1Δ*). (b) α-Factor production. For each strain tested, a suspension of 10^5 cells was spotted on a lawn of approximately 10^4 cells of the α-factor-hypersensitive tester strain. The strains used are listed in Table 1: KT43 (*MATα RPD1*), KT53 (*matα2 RPD1*), M664 (*MATα rpd1Δ*), KT146 (*MAT***a** *RPD1*), M708 and M709 (two independent transformants; *MATα rpd1Δ bar1Δ*), and RC634 (*MAT***a** *RPD1* sst1).

genes in *MATα rpd1* cells, we suspected that derepression of haploid-specific genes in *rpd1/rpd1* diploid cells might be responsible for their Spo⁻ phenotype. For example, *RME1*, a repressor of meiosis expressed in haploid cells and repressed in **a**/α diploid cells blocks meiotic induction if overexpressed in **a**/α diploids (32). To determine whether aberrant derepression of *RME1* is associated with the Spo⁻ phenotype of *rpd1/rpd1* homozygous diploids, we examined the effect of *rme1* mutations on sporulation in these cells. We observed 50 to 100 four-spored asci among approximately 50,000 *rpd1/rpd1 rme1/rme1* homozygous diploid cells, demonstrating that the ability to sporulate is increased by at least an order of magnitude in these cells.

Partial suppression of the Spo⁻ phenotype of *rpd1/rpd1* cells by mutations at *RME1* indicated that *RPD1* might be required for full repression of haploid-specific genes in diploids. This view was further tested by experiments in which reporter gene activities or mRNA levels of three other haploid-specific genes (*HO*, *TY2*, and *FUS1*) were measured in wild-type and *rpd1/rpd1* diploid cells. In each case, an increase in expression was detected in *rpd1/rpd1* diploids (Fig. 2). On the basis of these results, we conclude that accurate repression of haploid-specific genes in diploid cells is dependent on RPD1.

***RPD1* is required for repression of *PHO5*.** APase in wild-type yeast cells is encoded by at least three genes (*PHO3*, *PHO5*, and *PHO11*) (38). Expression of *PHO5* and *PHO11* responds to external changes in phosphate concentration: they are transcriptionally repressed in the presence of high phosphate concentrations (26). The *rpd1Δ* mutation increased APase activity (Pho⁺ phenotype) in cells grown in phosphate concentrations that normally confer repression (Fig. 4A). The Pho⁺ phenotype is recessive (data not shown) and cosegregates with other *rpd1*-dependent phenotypes in the meiotic progeny of a heterozygous *rpd1/RPD1* diploid (Fig. 4A). Quantitation of APase activity demonstrated a twofold increase in *rpd1* cells (Fig. 4B).

pho5 rpd1 double mutants did not exhibit a Pho⁺ phenotype (data not shown), suggesting that aberrant expression of *PHO5* is responsible for the *rpd1*-dependent Pho⁺ phenotype. This was further tested by measuring the activity of the *PHO5* promoter fused to the *lacZ* gene. A threefold increase in β-galactosidase activity occurred in *rpd1* cells compared with wild-type cells under conditions of phosphate repression (Fig. 2a). On the basis of these results, we conclude that *RPD1* is required for proper repression of *PHO5* in the presence of high phosphate concentrations.

PHO80 has been demonstrated to be a repressor of *PHO5* expression (38) in the *PHO5* system described above. The possibility that *RPD1* functions independently of this repressor was tested by generating *rpd1 pho80* double mutants and assaying their Pho phenotypes. The Pho phenotypes of recombinant progeny obtained from an *RPD1/rpd1 PHO80/pho80* diploid are shown in Fig. 4B. Aberrant derepression of APase activity in the *rpd1 pho80* double mutant is about 20% higher than in the *pho80* single mutants, demonstrating additivity between the *rpd1* and *pho80* mutations. This additivity suggests that *RPD1* does not function simply to control *PHO80* levels.

***RPD1* is required for transcriptional activation.** The results described thus far implicate *RPD1* as a negative regulator of a variety of genes when these genes are assayed under repression conditions. To assess the regulatory role of *RPD1* further, we examined the effect of the *rpd1Δ* mutation on the expression of many of these genes under conditions of activation.

(i) Normally, *PHO5* is derepressed during phosphate starvation (38). To examine the effect of the *rpd1Δ* mutation on *PHO5* expression under activation conditions, β-galactosidase expression from the *PHO5::lacZ* fusion was measured in low-phosphate medium (see Materials and Methods). The results demonstrated a twofold reduction of *PHO5* activation in *rpd1* mutants (Fig. 5).

(ii) **a**-specific genes are repressed in *MATα* cells and derepressed in *MAT***a** cells (13). Measurements of β-galactosidase activity in wild-type and *rpd1 MAT***a** cells trans-

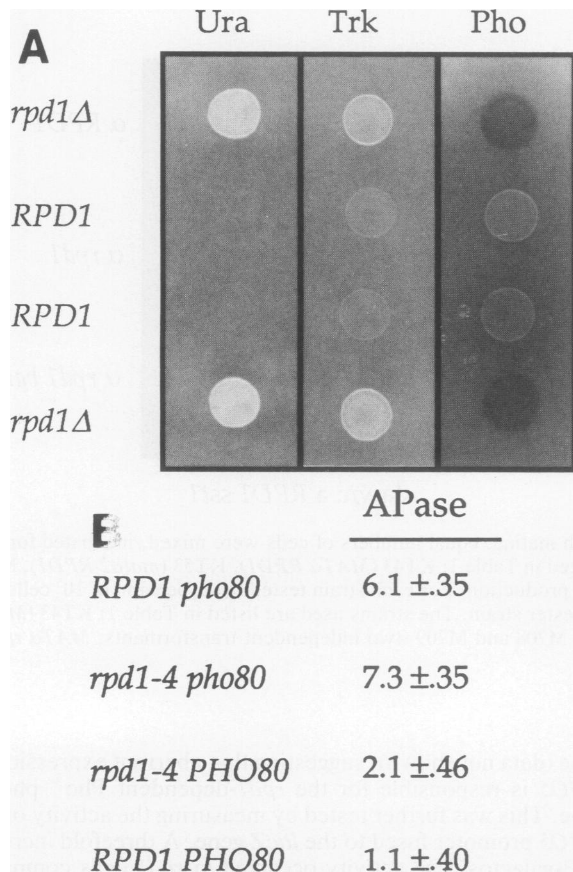


FIG. 4. *rpd1*Δ cells exhibit a Pho phenotype. (A) One of the *RPD1* loci in diploid strain R1224 (*ura3-52/ura3-52 trk1*Δ*/trk1*Δ) (8) was transplaced with a deletion allele, *rpd1*Δ*::URA3*, generated in vitro. A typical tetrad, among over 50 such tetrads analyzed from this diploid, is shown above. The *rpd1*Δ*::URA3* spores are Ura$^+$, Trk$^+$, and Pho$^+$ (see Materials and Methods). (B) Results of APase assays performed on cultures derived from a tetratype tetrad from a *rpd1-4 PHO80* × *RPD1 pho80* cross.

formed with a *cyc1::lacZ* fusion in which the endogenous *CYC1* UAS have been replaced with the *STE6* UAS *cyc1::*UAS(*STE6*)*::lacZ* fusion (Fig. 5a) indicated that activation of the **a**-specific UAS element was fivefold lower in *MAT***a** *rpd1* cells than in wild-type *MAT***a** *RPD1* cells.

(iii) Expression of a *TY2::lacZ* fusion, used to determine the effect of *rpd1* on haploid-specific activation (13), was decreased fourfold in *rpd1* cells (Fig. 5a).

(iv) S1 analysis, using specific probes for *STE2*, *STE6*, *STE3*, and *FUS1* hybridized to total RNA isolated from *rpd1* and wild-type cells of the appropriate mating type, demonstrated that transcription of these genes was decreased in *rpd1* cells. The amount of mRNA transcribed from these cell type-specific genes was reduced 4- to 10-fold in *rpd1* cells (Fig. 5b).

Although representatives of cell type-specific, differentiation-specific, and metabolically regulated genes are under *RPD1* regulation, mutations in *RPD1* do not affect the expression of all genes. *LYS2* and *ACT1* message levels, for example, are identical in *rpd1*Δ and wild-type cells (Fig. 2b). Since *ACT1* expression is under the control of a constitutive promoter, the equivalent levels of transcription of this gene in *rpd1* and *RPD1* cells indicate that the general transcription machinery functions normally in *rpd1* cells. This is further supported by the observation that expression of a *cyc1::lacZ* fusion lacking the *CYC1* UAS is unaffected in *rpd1* strains (52).

In summary, our data show that *RPD1* plays a dual role in transcriptional regulation: *RPD1* is required for full activation and full repression of the same genes.

***RPD1* contains transactivatorlike regions and four HLH-like domains.** We determined the nucleotide sequence of a 5-kb genomic DNA fragment sufficient for complementation of the *rpd1*Δ mutation. A single large ORF of 4,608 nucleotides that encodes a predicted 175-kDa protein of 1,536 amino acids was found (data not shown).

As noted earlier, the DNA sequence of *SIN3*, allelic to *RPD1*, was recently determined and found to encode a slightly larger predicted protein of 1,538 amino acids (60). Ala-Gln pair repeats are found in both RPD1 and SIN3, beginning at amino acid 499. RPD1 contains six Ala-Gln pairs where SIN3 contains seven of these pairs, thus accounting for the slight difference in the sizes of the two proteins. RPD1 contains nine amino acid substitutions and a two-amino-acid deletion compared with SIN3. Four of the RPD1 substitutions are conservative and replace SIN3 residues Ser-33 with Thr, Ala-400 with Val, Glu-442 with Asp, and Arg-1169 with Lys. Six RPD1 substitutions are not conservative and replace SIN3 residues Gln-442 with Glu, His-485 with Arg, Ile-579 with Met, Phe-1028 with Leu, and Asp-1220 with Gly. *SIN3* and *RPD1* were cloned from libraries constructed from *S. cerevisiae* strains of independent origins. Since the *RPD1* and *SIN3* clones both suppress several of the *rpd1* phenotypes, it appears that the sequence polymorphisms between these genes do not significantly alter protein function.

The inferred amino acid sequence of RPD1 contains four regions that share significant primary sequence similarity with each other (Fig. 6). Each of these domains could form two amphipathic helices separated by a stretch of about 20 amino acids containing residues predicted to disrupt α-helical secondary structures (60). This structural motif has been previously described for two families of proteins, the TPR (tetratricopeptide)-repeat containing proteins (16, 47) and the HLH proteins similar to *myc* (36). The HLH motif was demonstrated to be required for protein dimerization of transcriptional regulators (35), and the presence or absence of basic residues adjoining it appears to dictate whether or not proteins can bind DNA via these domains. The regions immediately adjacent to the amino-terminal side of the putative HLH-like domains of RPD1 contain only one to four basic residues (Fig. 6B). Thus, if RPD1 undergoes dimerization with other proteins containing paired amphipathic helices, it is not likely that such multimers would be competent to bind DNA via these domains. In this regard, although not related by primary amino acid sequence, the HLH-like domains of RPD1 are structurally reminiscent of the HLH domains found in Id and Emc, proteins thought to regulate HLH-containing *trans* activators negatively through formation of DNA-binding-incompetent dimers (see Discussion).

That RPD1 may modulate transcriptional regulation through protein-protein interactions is suggested by additional structural features. RPD1 contains several regions similar to activating domains found in transcriptional activators (reviewed in reference 33), including (i) a region of 49 amino acids (Glu-166 to Asp-215) that contains 15 acidic residues giving rise to a net charge of −12, (ii) a region of 137 amino acids (Gln-480 to Gln-617) that contains 25% glu-

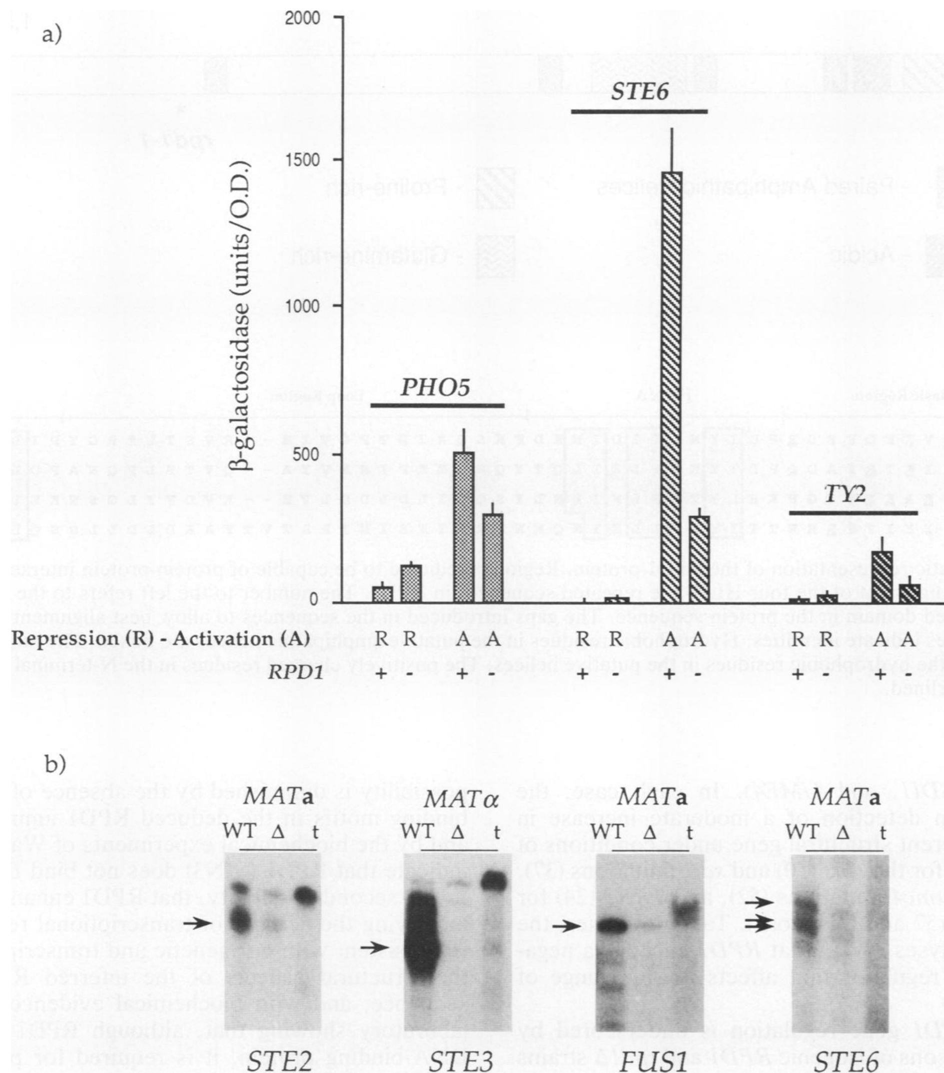


FIG. 5. Activation and repression in *rpd1* mutants. (a) β-Galactosidase specific activities exhibited by wild-type *RPD1* (+) and *rpd1* mutant (−) cells. *PHO5*, *PHO5:lacZ* fusion in a centromeric plasmid under repression (R) and activation (A) conditions (i.e., high and low phosphate levels, respectively); *STE6*, *cyc1*:UAS(*STE6*):*lacZ* in a multicopy plasmid in *MAT*α (R) and *MAT***a** (A) cells; *TY*, *TY2:lacZ* fusion in a centromeric plasmid in diploids (R) or haploids (A). (b) RNA quantitation. The riboprobes used for the various S1 protection exp iments are listed below the panels. The cell type from which the RNA was isolated is given above each panel. The arrows indicate the banus resulting from the mRNA protected probes. A 20-μg sample of total RNA was used in each lane. t, tRNA used as a negative control.

tamine residues, and (iii) a region of 67 amino acids (Pro-85 to Pro-152) that contains 19% proline residues (Fig. 6A).

The carboxy-terminal 276 residues of RPD1 are dispensable for repression. We obtained a mutant that was able to derepress *TRK2* conditionally by selecting for growth of *trk1*Δ *TRK2* cells on low-potassium medium at 37°C. From among many such mutants obtained, complementation tests with an *rpd1*Δ strain indicated that many of the mutants harbored temperature-sensitive *rpd1* alleles. One of these, *rpd1-1*, was cloned and its sequence was determined. The *rpd1-1* mutation was determined to be an A→T transversion that produces a premature translation termination codon at position 1262. Since the *rpd1-1* mutation confers a wild-type (Trk^-) phenotype on *trk1*Δ *TRK2* cells at the permissive temperature (22°C), we conclude that the 267 carboxy-terminal amino acids of RPD1 are not essential for RPD1-mediated repression of *TRK2*. Other relevant phenotypes, including cycloheximide sensitivity, sporulation of homozygous diploids, and mating ability, are also indistinguishable from the wild type at 22°C, an indication that repression of multiple *RPD*-regulated genes is essentially normal in cells harboring the truncated protein. Although the *rpd1-1* mutation should delete a sizable region of the protein, each of the putative protein-protein interaction domains is still present. Other, uncharacterized *rpd1* mutations exhibit specific subsets of the different phenotypes observed in the *rpd1*Δ mutant (7a), suggesting that different domains are responsible for mediating transcriptional effects on different genes.

DISCUSSION

We have established that several independent genetic screens or selections, designed to identify yeast transcriptional regulators, have detected the same locus, *RPD1* (also

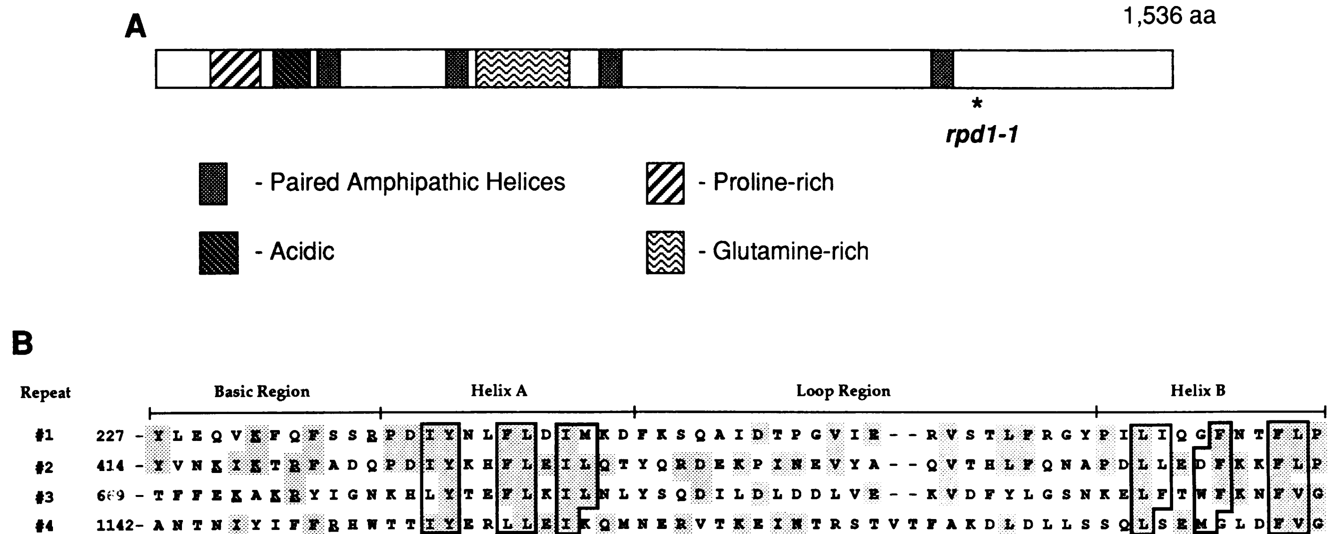


FIG. 6. (A) Schematic representation of the RPD1 protein. Regions predicted to be capable of protein-protein interactions are indicated. aa, amino acids. (B) Alignment of the four HLH-like repeated sequences in RPD1. The number to the left refers to the position of the first residue of each repeated domain in the protein sequence. The gaps introduced in the sequences to allow best alignment are represented by dashes. Shaded residues indicate identities. Hydrophobic residues in the putative amphipathic helices are boxed. The most highly conserved regions correspond to the hydrophobic residues in the putative helices. The positively charged residues in the N-terminal region, labelled the basic region, are underlined.

known as *SIN3*, *SDI1*, and *UME4*). In each case, the strategy relied upon detection of a moderate increase in expression of a different structural gene under conditions of repression: *HO* (51) for the *sin3* (50) and *sdi1* mutations (37), *SPO13* (63) for the *ume4* mutations (52), and *TRK2* (24) for the *rpd1* mutations (57 and this work). Taken together, the results of these analyses reveal that *RPD1* encodes a negative transcriptional regulator that affects a wide range of target genes.

The range of *RPD1* gene regulation is underscored by phenotypic comparisons of isogenic *RPD1* and *rpd1*Δ strains in which seemingly unrelated functions, including ion transport, mating, sporulation, and APase activity, were found to be altered in the mutant cells. On the basis of a series of epistasis tests, expression assays for promoter-reporter fusions, and direct quantitation of mRNA levels, the expression of a wide variety of genes, including *PHO5*, *STE6*, *TY2*, *STE2*, *STE3*, *SPO11*, *SPO13*, *TRK2*, *FUS1*, and *HO*, appears to be under *RPD1* regulation. The expression of several of these was measured under both repression and activation conditions. Our results show that *RPD1* is required for maximal transcriptional response by these genes: *rpd1* mutants exhibit higher levels of expression under repression conditions and lower levels of expression under activation conditions.

Possible mechanisms for the transcriptional role of *RPD1*. Several possibilities can account for the observed effects of *rpd1*Δ mutations on transcriptional regulation: (i) RPD1 could be a DNA-binding protein that directly affects the regulation of specific target genes or their regulators; (ii) RPD1 could alter the activity of *trans*-acting regulatory factors through specific protein-protein interactions, either with transactivators-repressors themselves or with factors that regulate them; or (iii) RPD1 could be a determinant in general chromatin structure, being required for normal efficiency of regulation but not having specific effects on individual *trans*-acting regulators. The likelihood of the first possibility is diminished by the absence of canonical DNA-binding motifs in the deduced RPD1 amino acid sequence and by the biochemical experiments of Wang et al. (60) that indicate that RPD1 (SIN3) does not bind DNA.

The second possibility, that RPD1 enhances regulation by modifying the activity of transcriptional regulatory factors, is consistent with our genetic and transcriptional data, with the structural aspects of the inferred RPD1 amino acid sequence, and with biochemical evidence from Stillman's laboratory showing that, although RPD1 (SIN3) is not a DNA-binding protein, it is required for proper binding of specific DNA-binding proteins (61, 62). Our genetic and transcriptional experiments revealed that deletion of *RPD1* reduces the normal extent of both activation and repression. Although many genes fall under *RPD1* regulation, this regulation exhibits gene specificity. For example, in vegetatively growing haploid *MAT***a** cells, *RPD1* simultaneously ensures maximal expression of **a**-specific gene *STE6* and minimal expression of meiosis-specific gene *SPO13*. Such specificity in opposite directions under a single set of cell type and growth conditions supports a model in which RPD1 specifically interacts with transcriptional regulatory proteins at some level.

The third possibility, a model in which RPD1 determines some aspect of chromatin structure, is favored by the observation that *rpd1*Δ mutations affect many, seemingly unrelated, genes. The observation that not all genes appear to be regulated by *RPD1* could be explained by local differences in chromatin structure such as the register of nucleosomes or the occurrence of higher-order structures. Such a model would require that the *RPD1*-dependent aspect of chromatin enhance the activity of DNA-binding proteins and thus enhance transcriptional regulation. This is plausible in light of recent experiments by Durrin et al. (7), which showed that mutations in the amino-terminal region of histone H4 result in incomplete transcriptional activation of *GAL1* and *PHO5*. Thus, chromatin, already known to act in

a negative manner on transcription, is required for complete transcriptional activation of some genes.

The biochemical evidence that RPD1 (SIN3) itself is not a DNA-binding protein but is required for proper binding of specific DNA-binding proteins (61, 62) is consistent with either of the two models proposed, since changes in chromatin structure or changes in proteins that regulate the activity of *trans* activators could result in aberrant DNA-binding activities.

Analysis of the predicted RPD1 protein structure suggests the presence of an array of protein-protein interaction motifs that could account for its global yet specific regulatory activity. In addition to acidic, Gln-rich, and Pro-rich domains, RPD1 contains four related but not identical domains likely to adopt a secondary structure resembling the previously described HLH domains (36). Thus, whether RPD1 affects chromatin structure or specific *trans*-acting factors, the heterogeneity and number of putative protein-protein interaction domains in RPD1 may reflect the heterogeneity of the factors with which it interacts to confer specific transcriptional effects. This is consistent with our observations that some mutant alleles of *RPD1* exhibit different subsets of the phenotypes observed in *rpd1*Δ cells (7a).

A biological role for the RPD effect? Diverse regulatory mechanisms, dependent on different types of signals (differentiation, external stimuli, cell type, etc.), have evolved to ensure that transcription levels of particular genes are maintained at low levels under conditions in which the gene products are not required and high levels under conditions in which they are needed. Despite the wide differences in their primary regulatory signals, for many yeast genes, the difference between repressed and derepressed transcription levels is increased by RPD1 and RPD3 (59). Hence, the RPD proteins increase the efficiency of transcriptional regulation for these genes. One might expect that this type of regulation would provide a significant selective advantage to the organism, since apparently only a few factors are required to enhance the regulation of many different genes.

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