

Genetic Analysis of the Ydr1-Bur6 Repressor Complex Reveals an Intricate Balance among Transcriptional Regulatory Proteins in Yeast

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A transcriptional repressor complex encoded by two essential genes, *YDR1* and *BUR6*, was isolated from *Saccharomyces cerevisiae* and shown to be the functional counterpart of the human repressor complex Dr1-DRAP1. To elucidate the mechanism of repression by this complex, altered forms of Ydr1 and Bur6 were studied in vitro and in vivo. Deletion of the C-terminal 41 amino acids of Ydr1 resulted in loss of repressor activity and a growth defect, suggesting that the C-terminal domain of Ydr1 functions as a potent transcriptional repressor. A screen for extragenic suppressors of a cold-sensitive *ydr1* (*ydr1*^{cs}) mutant led to the identification of recessive mutations in the *SIN4* gene, which encodes a component of the SRB-MED complex. The *sin4* alleles suppressed not only *ydr1*^{cs} mutations but also *bur6*^{cs} mutations. In contrast, deletion of the *gal11* gene, whose product is also a member of the SRB-MED complex, failed to suppress *ydr1*^{cs} and *bur6*^{cs} mutations, indicating that suppression is not due to general defects in the SRB-MED complex. Moreover, one of the *sin4* alleles, but not the *sin4* deletion, was found to specifically suppress the inviability of a *ydr1* deletion, demonstrating that the essential function of Ydr1 becomes dispensable in a *sin4* mutant background. Biochemical analysis of the SRB-MED complex from the *sin4* suppressor strain revealed a structurally distinct form of the SRB-MED complex that lacks a subset of mediator subunits. These results define a delicate balance between positive and negative regulators of transcription operating through the Ydr1-Bur6 repressor complex.

Regulation of transcription in eukaryotes requires an intricate network of both positive and negative factors to maintain optimal expression of target genes (16; for details, see <http://www.wi.mit.edu/young/expression.html>). The identification of an additional class of regulators referred to as coactivators and corepressors underscores the complexity of transcriptional regulatory networks in eukaryotes (for reviews, see references 12 and 13). These factors, some of which are components of multi-protein complexes including RNA polymerase II (RNAPII) (the so-called “RNAPII holoenzyme” [33]), provide specific interaction sites for positive and negative regulators (14).

A large number of proteins that negatively regulate transcription have been described (for reviews, see references 15, 21, and 30). One family of repressors includes proteins that are tethered to promoters by interacting with sequence-specific DNA binding proteins and/or components of the basal transcription machinery. These include, among others, Tup1-Ssn6 (22), Mot1 (1), Sin3 (2), and Dr1-DRAP1 (17). A repressor complex from the yeast *Saccharomyces cerevisiae* which is encoded by two essential genes (*YDR1* and *BUR6*) was identified as the functional counterpart of the human Dr1-DRAP1 (NC2) complex (9, 10, 24, 36). The transcriptional repressor activity of this yeast complex was demonstrated in vitro by biochemical studies and in vivo by genetic studies (9, 10, 24, 36). Amino acid sequence comparison of yeast, drosophila, and human Dr1 polypeptides revealed two highly conserved domains located at the N and C termini of the protein. The C-terminal domain of human Dr1 includes a transferable transcriptional repressor domain (45). Dr1-DRAP1 heterodimer formation occurs via the N-terminal domains of both proteins. These domains contain a histone fold motif that is crucial for

DRAP1-mediated enhancement of transcriptional repression by Dr1 (11, 23, 31). Mutations in the histone fold motifs of Ydr1 or Bur6 were found to inhibit repressor function and to suppress an *srb4* temperature-sensitive (*ts*⁻) phenotype (9, 26).

Characterization of the RNAPII holoenzyme has identified mechanisms by which this complex mediates transcriptional regulation. The RNAPII holoenzyme from *S. cerevisiae* consists of core RNAPII, a set of general transcription factors, Srb proteins (SRB), mediator proteins (MED), and several other polypeptides identified previously as both positive and negative transcriptional regulators (33, 34). Taken together, the results of several studies suggest the presence of modular subcomplexes that associate with RNAPII to mediate the response to physiological or developmental cues from specific transcription factors (for a review, see reference 13). One of these subcomplexes, the Gal11 subcomplex, contains the Gal11, Sin4, Med3 (Hrs1), and Med2 proteins and interacts physically with the Rgr1 protein (27). *SIN4* (*TSF3*) was identified as a negative regulator of *HO* transcription (19) and has been implicated in the transcriptional activation and repression of a broad spectrum of genes (6, 7, 18, 19, 20). It has been suggested that the Sin4-containing Gal11 module functions as an input port for signals from a subset of gene-specific transcriptional regulators (14).

Nearly all of what we know regarding the functions of Dr1-DRAP1 has been gleaned from in vitro studies. To investigate the role of the Ydr1-Bur6 repressor complex in vivo, we have isolated and characterized extragenic suppressors of the cold-sensitive (*cs*⁻) phenotype of a *ydr1* mutant. Here we report the identification of a *sin4* allele as a suppressor of *ydr1*^{cs}. These results define a genetic relationship between positive and negative transcriptional regulators. We also describe a possible mechanism of suppression in terms of the subunit composition of the RNAPII holoenzyme, emphasizing the importance of the delicate balance between positive and negative transcriptional regulators.

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MATERIALS AND METHODS

Preparation of recombinant Ydr1 and Bur6 polypeptides. Full-length Ydr1 (FL-Ydr1), FL-Bur6, and two truncated Ydr1 polypeptides were expressed in *Escherichia coli* using the pET21-a plasmid and purified through a Ni-nitrilotriacetic acid (NTA) column (Qiagen) under denaturing conditions. After mixing of FL-Ydr1 and two truncated versions of Ydr1 with FL-Bur6 in an equal molar ratio, renaturation was carried out overnight at room temperature in buffer G (30 mM Tris-HCl [pH 7.5], 150 mM KCl, 10% glycerol, 5 mM dithiothreitol). The Ydr1-Bur6 heterodimer was further purified by S-200 gel filtration chromatography.

In vitro transcription and I.P. Transcription reactions were carried out using highly purified preparations of human transcription factors (24). Immunoprecipitation (I.P.) experiments were performed as described previously (24).

Genetic manipulation. The yeast strains used in this study are listed in Table 1. Details of the strains and plasmid constructions are available upon request. Strain DY1717 was a generous gift from David Stillman, and strain MCY2253 was from Marian Carlson. DY1717 was described previously (18). Yeast media were prepared as previously described (41). Yeast transformations were performed by a lithium acetate procedure (39). The plasmid shuffle method was performed as previously described (3), using 5-fluoroorotic acid (FOA).

Isolation of conditional mutations by error-prone PCR was done as described elsewhere (32), with the following modifications. The gapped plasmids for both *ydr1* and *bur6* were constructed by removing most of the open reading frames by restriction digestion. For efficient mutagenesis, the mutagenizing deoxyribose nucleoside triphosphate concentration was less than 30 μ M. The FOA-resistant candidates were tested for the cs^- phenotype by growth at 11°C for 14 days. The sequences of the mutant alleles were determined by sequencing the plasmid DNA isolated from mutant cells by standard methods (43).

To establish allelism between the *ydr1* suppressors and the cloned *SIN4* gene, a *URA3*-tagged *ydr1* deletion strain (YSK075) was constructed by transforming YSK013 with the linearized YIp-*SIN4-URA3* construct and selecting for *Ura*⁺ transformants. A diploid strain was generated by mating YSK075 with each suppressor strain, followed by sporulation and tetrad analysis using standard procedures (42). The suppressor was scored as ts^- in the *ydr1* background, whereas *SIN4* was scored by the *Ura*⁺ phenotype.

Suppressor alleles were cloned from genomic DNA by gap repair (35) and sequenced.

Suppressor screening. Strain YSK027 (*ydr1*^{cs}) was grown overnight at 30°C in 10 ml of yeast extract-peptone-dextrose (YPD) medium, plated on YPD medium at a cell density of 10⁶, and incubated at 11°C for 14 to 21 days. Spontaneous revertants were obtained at a frequency of 10⁻⁶. Each colony was purified by subcloning and rescored at 11°C. Five cs^+ revertants (YSK041 to YSK045) were subsequently found to be ts^- . To determine whether the mutations in the revertants were dominant or recessive, the five ts^- revertants were crossed with YMH200 and the resulting diploid strains were scored for cold and temperature sensitivity.

In order to clone the suppressor gene from the revertants, a yeast genomic DNA library (37) obtained from the American Type Culture Collection was introduced into each suppressor strain and *Ura*⁺ transformants were selected and scored for complementation of ts^- at 37°C. Library DNAs were isolated from ts^+ transformants, and sequenced. DNA manipulations and PCR amplifications were performed as previously described (38).

Purification of SRB-MED complexes. Strain VM02 (His-tagged *SRB5*) was a generous gift from Rick Young. Large-scale cultures of yeast strains VM02 and YSK149 were grown in the fermentation facility at the Waksman Institute. Yeast whole-cell extracts were prepared as previously described (40). The SRB-MED complex was purified from 1 kg of cells of each strain using Bio-Rex70 (Bio-Rad), DEAE-Sephacel (Pharmacia), Bio-Gel-HP hydroxyapatite (Bio-Rad), and MonoQ HR 10/10 (Pharmacia) as described previously (25). The 1 M potassium acetate eluent from the MonoQ column was dialyzed against buffer I (20 mM Tris-acetate [pH 7.9], 10% glycerol, 10 mM imidazole, 0.02% NP-40, 5 μ M β -mercaptoethanol) and loaded onto a Ni-NTA column. After extensive washing with buffer I containing 0.8 M potassium acetate, the bound material was eluted with a buffer containing 400 mM imidazole and subjected to Western blot analysis.

RESULTS

The C-terminal conserved domain of Ydr1 is critical for repression and growth. Comparison of the human (17) and yeast (24) Dr1 proteins revealed a conserved 12-amino-acid sequence near the C termini (Fig. 1). This region of human Dr1 functions as a transferable repression domain in vitro and in transfected cells (44). In order to further characterize this region of Ydr1, we performed studies with FL-Ydr1 and two truncated Ydr1 derivatives in vitro and in vivo (Fig. 1). In the in vitro experiments, we tested the ability of the truncated Ydr1 polypeptides to heterodimerize with Bur6, bind to the

yeast TATA binding protein (TBP), and repress transcription in a reconstituted human system (Fig. 2A to C). These activities were correlated with the ability of the two derivatives to support cell viability (Fig. 2D). The two Ydr1 derivatives are C-terminal truncations that retain the N-terminal 130 amino acids (C130) or 105 amino acids (C105) (Fig. 1). C130 and C105 are able to form heterodimers with FL-Bur6 in vitro, as determined by co-I.P. experiments (Fig. 2A). These two forms of Ydr1 also retain the ability to interact with TBP, although the affinity of the Ydr1-TBP interaction appears to be diminished relative to that of FL-Ydr1 (Fig. 2B and data not shown). The C130 protein retains the ability to repress transcription; however, C105 fails to repress transcription (Fig. 2C). The C130 derivative fully supports cell growth in the absence of normal Ydr1, whereas the C105 derivative is nearly inviable (Fig. 2D; summarized in Fig. 3). These results are consistent with the previous characterization of human Dr1 which demonstrated that the transferable repressor domain includes residues that have been deleted from C105 but are present in C130 (45). Thus, the essential function of Ydr1 in vivo correlates with its ability to repress transcription in vitro, rather than its interaction with Bur6.

Isolation of suppressors of *ydr1-13*. In order to extend our understanding of Ydr1 function in vivo, we sought to define the genetic relationship between Ydr1 and other transcriptional regulatory proteins. As an initial step toward this goal, we generated cs^- *ydr1* mutants by PCR-mediated mutagenesis. Some of the cs^- mutations mapped within the 25-amino-acid C-terminal conserved domain of Ydr1 (Fig. 4A). One mutant (YSK027) encodes replacement of phenylalanine with serine at position 106 (*ydr1-13*; F106S) and exhibits a marked cs^- phenotype at 11°C (Fig. 4B; *ydr1-13 SIN4*). From a total of 10⁸ cells plated, ~100 independent, spontaneous cs^+ revertants were selected on rich medium at 11°C (YSK041 to YSK045; Table 1). In an effort to identify potential pleiotropic phenotypes associated with these suppressors, cs^+ revertants were screened for ts^- growth defects at 37°C. Five revertants exhibited distinct ts^- phenotypes and were further characterized (Fig. 4B and data not shown).

The five cs^+ ts^- revertants were crossed with strain YMH200 (*ydr1 Δ ::HIS3 [YDR1-URA3]*). The resulting diploid strains were cured of the *YDR1-URA3* plasmid and subsequently scored for the cs^- and ts^- phenotypes. All five diploid strains were phenotypically cs^- and ts^+ , indicating that the revertant phenotypes were due to recessive mutations (data not shown). Plasmid-borne *YDR1* was unable to complement the ts^- phenotype of the haploid revertants, indicating that the suppressors are not allelic to *YDR1*. We tentatively designated this suppressor gene(s) *scd*, for suppressor of cold-sensitive *ydr1-13*.

The allele specificity of each suppressor was determined by plasmid shuffle using two other *ydr1* cs^- alleles. The *ydr1-11* allele is the result of a nonsense mutation, encoding a truncated form of Ydr1 at position 122, whereas *ydr1-16* encodes F124S and R130S replacements (Fig. 4A). These alleles were introduced into strains YMH201 (*ydr1 Δ ::HIS3 SCD [YDR1-URA3]*) and YSK125 (*ydr1 Δ ::HIS3 scd [YDR1-URA3]*), and the wild-type *YDR1* gene was counterscreened on FOA medium. The resulting strains were then scored for growth at 11°C. All three *ydr1* mutants grew well in the *scd* background, compared to the marked cs^- phenotype in the *SCD* background. Thus, suppression by the *scd* allele of strain YSK125 is not specific to the *ydr1-13* allele, but instead, *scd* is able to suppress the cs^- growth phenotype associated with three different *ydr1* alleles.

Identification of *sin4* as a suppressor of *ydr1-13*. Since all *scd* suppressor strains were isolated as spontaneous cs^+ revertants,

TABLE 1. Yeast strains used in this study

Strain	Genotype
YMH200	<i>MATa ura3-52 leu2-7 his3-11 trp1-1 ade2-1 ydr1Δ::HIS3 [CEN-URA3-YDR1]</i>
YMH201	<i>MATα ura3-52 leu2-7 his3-11 trp1-1 ade2-1 ydr1Δ::HIS3 [CEN-URA3-YDR1]</i>
YMH202	<i>MATα ura3-52 leu2-7 his3-11 trp1-1 ade2-1 bur6Δ::HIS3 [CEN-URA3-BUR6]</i>
YMH203	<i>MATα ura3-52 leu2-7 his3-11 trp1-1 ade2-1 bur6Δ::HIS3 [CEN-URA3-BUR6]</i>
YSK013 ^a	<i>MATa ydr1Δ::HIS3 [CEN-URA3-YDR1] [CEN-TRP1-YDR1]</i>
YSK014 ^a	<i>MATa ydr1Δ::HIS3 [CEN-URA3-YDR1] [CEN-TRP1]</i>
YSK025 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-11]</i>
YSK027 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13]</i>
YSK028 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-16]</i>
YSK033 ^b	<i>MATα bur6Δ::HIS3 [CEN-LEU2-bur6-42]</i>
YSK034 ^b	<i>MATα bur6Δ::HIS3 [CEN-LEU2-bur6-43]</i>
YSK035 ^b	<i>MATα bur6Δ::HIS3 [CEN-LEU2-bur6-47]</i>
YSK041 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-42</i>
YSK042 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-54</i>
YSK043 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-61</i>
YSK044 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-121</i>
YSK045 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-168</i>
YSK054 ^a	<i>MATα ydr1Δ::HIS3 [CEN-TRP1-MET25]</i>
YSK055 ^a	<i>MATα ydr1Δ::HIS3 [CEN-TRP1-MET25-YDR1]</i>
YSK056 ^a	<i>MATα ydr1Δ::HIS3 [CEN-TRP1-MET25-ydr1C130]</i>
YSK057 ^a	<i>MATα ydr1Δ::HIS3 [CEN-TRP1-MET25-ydr1C105]</i>
YSK069 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-54 [CEN-URA3-SIN4]</i>
YSK070 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-168 [CEN-URA3-SIN4]</i>
YSK071 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-54 [CEN-URA3-YTP1]</i>
YSK072 ^a	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4-168 [CEN-URA3-YTP1]</i>
YSK075 ^a	<i>MATa ydr1Δ::HIS3 [CEN-TRP1-YDR1] SIN4::[SIN4-URA3]</i>
DY1717	<i>MATα ade2-1 his3-11 leu2-7 lys2 trp1-1 ura3-52 sin4Δ::TRP1</i>
MCY2253	<i>MATα ade2-101 ura3-52 spt13-10Δ::TnLUK</i>
YSK084 ^a	<i>MATa ydr1Δ::HIS3 [CEN-URA3-YDR1] sin4Δ::TRP1</i>
YSK085 ^a	<i>MATa ydr1Δ::HIS3 [CEN-LEU2-ydr1-11] sin4Δ::TRP1</i>
YSK086 ^a	<i>MATa ydr1Δ::HIS3 [CEN-LEU2-ydr1-13] sin4Δ::TRP1</i>
YSK087 ^a	<i>MATa ydr1Δ::HIS3 [CEN-LEU2-ydr1-16] sin4Δ::TRP1</i>
YSK093 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] sin4Δ::TRP1</i>
YSK095 ^b	<i>MATa bur6Δ::HIS3 [CEN-LEU-bur6-42] sin4Δ::TRP1</i>
YSK096 ^b	<i>MATa bur6Δ::HIS3 [CEN-LEU2-bur6-43] sin4Δ::TRP1</i>
YSK097 ^b	<i>MATa bur6Δ::HIS3 [CEN-LEU2-bur6-47] sin4Δ::TRP1</i>
YSK103 ^c	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-11]</i>
YSK104 ^c	<i>MATα ydr1Δ::HIS3 [CEN-LEU2-ydr1-13]</i>
YSK105 ^c	<i>MATα ydr1Δ::HIS3 [CEN-LEU-ydr1-16]</i>
YSK108 ^c	<i>MATa bur6Δ::HIS3 [CEN-LEU2-bur6-42]</i>
YSK109 ^c	<i>MATa bur6Δ::HIS3 [CEN-LEU2-bur6-43]</i>
YSK110 ^c	<i>MATa bur6Δ::HIS3 [CEN-LEU2-bur6-47]</i>
YSK124 ^a	<i>MATα ydr1Δ::HIS3 [CEN-URA3-YDR1] sin4-168</i>
YSK125 ^a	<i>MATα ydr1Δ::HIS3 [CEN-URA3-YDR1] sin4-54</i>
YSK126 ^a	<i>MATα ydr1Δ::HIS3 [CEN-URA3-YDR1] [CEN-TRP1-YDR1] sin4-54</i>
YSK127 ^a	<i>MATα ydr1Δ::HIS3 [CEN-URA3-YDR1] [CEN-TRP1] sin4-54</i>
YSK128 ^a	<i>MATα ydr1Δ::HIS3 [CEN-URA3-YDR1] [CEN-TRP1-YDR1] sin4-168</i>
YSK129 ^a	<i>MATα ydr1Δ::HIS3 [CEN-URA3-YDR1] [CEN-TRP1] sin4-168</i>
YSK130 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] sin4-54</i>
YSK131 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] sin4-168</i>
YSK132 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] [CEN-TRP1-BUR6] sin4-54</i>
YSK133 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] [CEN-TRP1] sin4-54</i>
YSK134 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] [CEN-TRP1-BUR6] sin4-168</i>
YSK135 ^b	<i>MATa bur6Δ::HIS3 [CEN-URA3-BUR6] [CEN-TRP1] sin4-168</i>
YSK140 ^a	<i>MATa ydr1Δ::HIS3 sin4-54 [CEN-TRP1-SIN4]</i>
YSK141 ^a	<i>MATα ydr1Δ::HIS3 sin4-54 [CEN-TRP1]</i>
VM02	<i>MATα ade2 leu2 ura3 HA-6His-tagged SRB5</i>
YSK149	<i>MATα ade2 leu2 ura3 HA-6His-tagged SRB5 sin4-54</i>

^a Derived from YMH200 or YMH201.

^b Derived from YMH202 or YMH203.

^c Derived from MCY2253.

we made the tentative assumption that the *ts*⁻ phenotype of these revertants is a pleiotropic phenotype associated with the *scd* mutations. Accordingly, we exploited the *ts*⁻ phenotype to clone the wild-type allele. Strain YSK042 (*ydr1-13 scd1*) was transformed with the YCp50 library and selected for Ura⁺

transformants at 37°C. Several Ura⁺ *ts*⁺ transformants were obtained. Plasmid DNA was isolated from one transformant and reintroduced into YSK042. In this case, all of the scored transformants were *ts*⁺, indicating that the *ts*⁺ phenotype is due to plasmid DNA rather than strain reversion. Restriction

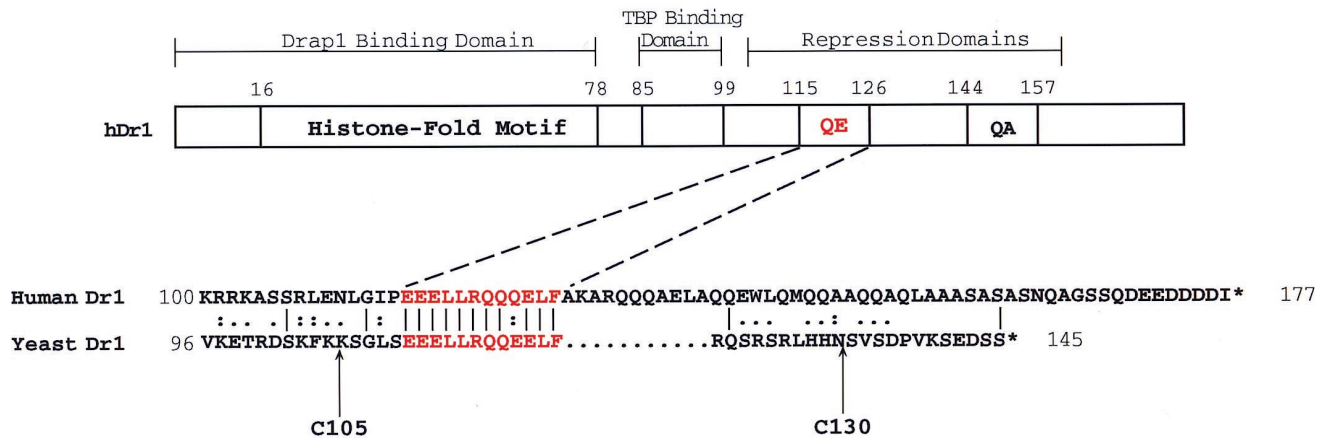


FIG. 1. Sequence alignment of the C termini of human and yeast Dr1 polypeptides. The amino acid numbers are shown at the ends of the sequences. A conserved 12-amino-acid sequence is in red. Two truncated Ydr1 derivatives are indicated by arrows at the bottom of the yeast Dr1 sequence. A schematic representation of the human Dr1 polypeptide is shown at the top. QE, QE-rich domain; QA, QA-rich domain. For details, see reference 45.

analysis of this clone identified a 5.65-kb insert. Sequence analysis using primers that annealed immediately adjacent to the insert identified the *YTP1* and *SIN4* genes. Both genes were cloned individually into CEN plasmids and reintroduced into both YSK042 and YSK045. Plasmid DNA containing the *SIN4* gene (YSK069 and YSK070), but not *YTP1* (YSK071 and YSK072), fully complemented the ts^- growth defect. Furthermore, all ts^+ transformants were cs^- , demonstrating that *SIN4* complements both the pleiotropic ts^- and suppressor cs^+ phenotypes.

Allelism between the *scd1* suppressor and *SIN4* was tested with two different suppressor strains (YSK042 and YSK043). YSK042 and YSK043 (*ydr1-13 scd*) were crossed with strain YSK075 (*ydr1-13 SIN4-URA3*), which contains the wild-type *SIN4* gene tagged with *URA3*. Diploid strains were sporulated and dissected, and the resulting progeny were scored for uracil auxotrophy and temperature sensitivity at 37°C. Among 20 tetrads derived from the YSK042 × YSK075 cross, the Ura^+ : Ura^- and ts^+ : ts^- phenotypes segregated 2:2. Moreover, all Ura^+ segregants were ts^+ and all Ura^- segregants were ts^- . Similar results were obtained with the YSK043 × YSK075 cross (data not shown). Thus, the ts^- phenotype conferred by the *scd1* mutation segregates opposite to *SIN4*, thereby establishing that *scd1* is allelic to *SIN4*. We now refer to the *scd1* suppressor as *sin4-54*.

The cloned wild-type *SIN4* gene was introduced into each of the other four *scd* suppressor strains. The resulting transformants were all ts^+ , indicating that wild-type *SIN4* complements all *scd* suppressors. Thus, each of the other four *scd* suppressors is likely to be allelic to *SIN4*. Moreover, as defined below, *SIN4* DNA was cloned from two of the five suppressors (*sin4-54* and *sin4-168*) and found to contain specific mutations.

Characterization of *sin4* suppressors. Genomic DNA encompassing the *sin4-54* and *sin4-168* alleles was cloned by gap repair from strains YSK042 and YSK045, respectively. Sequence analyses showed that both alleles encode truncated forms of Sin4: *sin4-54* contains a nonsense mutation at codon 672, whereas *sin4-168* contains a frameshift mutation at codon 388 (Fig. 4C).

The nature of the *sin4* alleles suggested that suppression is due to loss of Sin4 function. We tested this possibility by determining whether a *sin4* deletion would also suppress the *ydr1* mutations. We constructed a *ydr1* plasmid shuffle strain using *sin4Δ::TRP1* deletion strain DY1717 (18). The *ydr1-11*,

ydr1-13, and *ydr1-16* alleles were introduced into the resulting strain, YSK084, and the wild-type *YDR1* plasmid was counterselected on FOA medium. In contrast to the *ydr1 SIN4* strains (YSK025, YSK027, and YSK028), all three *ydr1 sin4Δ* strains (YSK085, YSK086, and YSK087) grew well at 11°C (Fig. 5A), indicating that the deletion of *SIN4* is sufficient to suppress all three *ydr1* cs mutations.

Suppression of *bur6* alleles by *sin4* mutations. Because of the functional relationship between Ydr1 and Bur6, we analyzed the potential effects of the *sin4* alleles on *bur6* mutations. Three *bur6* alleles were generated by error-prone PCR as described above for *ydr1* alleles. The *bur6-42*, *bur6-43*, and *bur6-47* alleles of strains YSK033, YSK034, and YSK035 each encode two or more amino acid replacements (Fig. 5B) and confer cs^- growth defects (Fig. 5C). The *sin4-54* and *sin4-168* mutations suppress the cs^- phenotypes of all three *bur6* alleles (data not shown). Furthermore, the *sin4Δ* deletion fully restored growth at 11°C in all three *bur6* backgrounds (YSK095, YSK096, and YSK097) (Fig. 5C). These results, in combination with the *ydr1 sin4* data, indicate that deletion of *SIN4* compensates for diminished Ydr1-Bur6 function.

Neither *ydr1* nor *bur6* alleles are suppressed by *gal11*. The *SIN4* gene was identified originally as a negative regulator of *HO* transcription (19) and was later recovered in a screen for genes that relieve repression of the *GAL1* and *GAL10* genes (6). It was proposed that *SIN4* plays both positive and negative roles in transcriptional regulation (20). More recently, Sin4 was identified as a component of the SRB-MED transcriptional regulatory complex that associates with RNAPII to form the so-called holoenzyme complex (29).

Gal11 is another component of the SRB-MED complex and is found in a subcomplex with Sin4 (27, 29). Moreover, *sin4* and *gal11* mutants have certain genetic similarities, including transcriptional defects (5, 8, 20). This prompted us to examine whether a mutation in the *GAL11* gene can suppress the *ydr1* and *bur6* alleles. The *YDR1* and *BUR6* plasmid shuffle systems were set up in a *gal11Δ* deletion strain and transformed individually with each of the three distinct cs^- *ydr1* and *bur6* alleles described above to generate strains YSK103, YSK104, and YSK105 (*ydr1 gal11*), and YSK108, YSK109, and YSK110 (*bur6 gal11*). Surprisingly, none of the *ydr1* or *bur6* alleles were suppressed by *gal11Δ* deletion (Fig. 5). Because we observed no genetic suppression of *ydr1^{cs}* or *bur6^{cs}* by *gal11Δ*, we conclude that suppression of *ydr1^{cs}* and *bur6^{cs}* is not common to

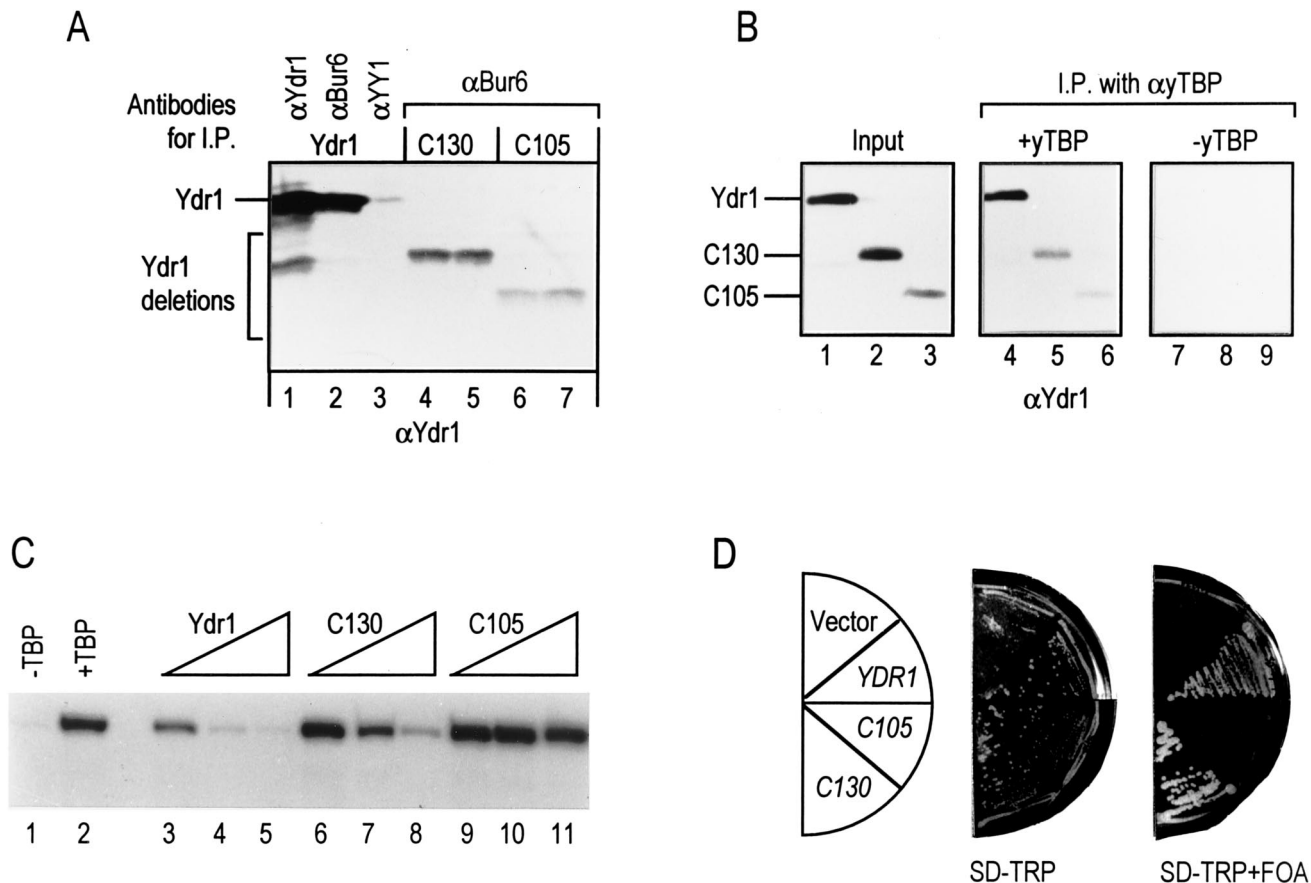


FIG. 2. The C-terminal conserved domain of Ydr1 is critical for transcriptional repression and cell growth. (A) The ability of Ydr1 derivatives to form a heterodimer with Bur6 was examined by co-I.P. Ydr1, FL-Ydr1 (lanes 1 to 3); C130 and C105, two truncated forms of Ydr1 (lanes 4 and 5 and 6 and 7, respectively). Lanes 4 and 5 are duplicates of C130 under the same conditions; lanes 6 and 7 are the same as for C105. Antibodies for I.P. are indicated at the top. α-Ydr1, antibody against Ydr1; α-Bur6, antibody against Bur6; α-YY1, antibody against YY1 (control). The antibody used for Western blot analysis is indicated at the bottom. (B) The interaction between yeast TBP and the Ydr1-Bur6 complex was monitored in a co-I.P. experiment (24). α-yTBP antibody-beads were preincubated with (+yTBP, lanes 4 to 6) or without (-yTBP, lanes 7 to 9) yeast TBP, and then each Ydr1-Bur6 heterodimer was added. Input, 1/10 of each Ydr1-Bur6 heterodimer that was added to α-yTBP-beads (lanes 1 to 3). The polyclonal antibody against Ydr1 recognizes C105, yet its affinity for this truncated polypeptide is approximately 2.5-fold less than its affinity for FL and C130 polypeptides. This differential affinity of the antibodies is important in the interpretation of the results shown in lanes 5 and 6. The overall decrease in binding of C130 and C105 to TBP remains constant at approximately 30% of the binding observed with the FL-Ydr1 polypeptide. (C) Transcriptional repressor activity was measured in vitro using yeast TBP and a highly purified human transcription system. Ydr1, FL-Ydr1 (lanes 3 to 5); C130 and C105, C-terminal truncation of Ydr1 to amino acids 130 (lanes 6 to 8) and 105 (lanes 9 to 11), respectively. (D) Each truncated form of the *ydr1* gene in the CEN plasmid was transformed into a *YDR1* plasmid shuffle strain. The viability of each transformant was examined on a plate containing FOA (SD-TRP+FOA). The genotypes of the strains are shown in the semicircular diagram on the left.

these two components of the Gal11 subcomplex but is a specific feature of *sin4*.

The *sin4-54* allele renders *YDR1*, but not *BUR6*, dispensable for cell viability. The genetic relationship between *ydr1* or *bur6* and *sin4* defined in this study suggested that the activities of the

Ydr1-Bur6 repressor and the Sin4 component of the SRB-MED complex exist in a delicate balance to regulate gene expression. This prompted us to examine whether *YDR1* and *BUR6*, both essential genes in a wild-type background, are dispensable for cell viability in *sin4* genetic backgrounds. It is

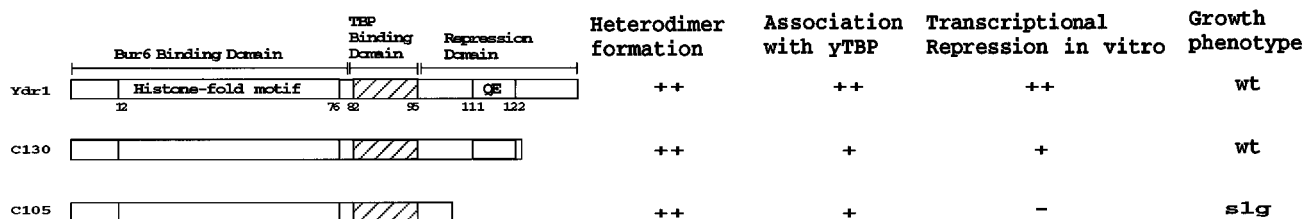


FIG. 3. Summary of the characterization of Ydr1 derivatives. Ydr1 truncations are represented schematically by bars. The various biochemical activities of Ydr1 were measured in vitro using Ydr1-Bur6 heterodimers, and the effects on growth are summarized at the right. TBP Binding Domain, putative TBP binding domain; Repression Domain, assigned repression domain based on human Dr1 studies (44); QE, QE-rich domain; wt, wild-type growth; slg, slow growth. ++, same as FL-Ydr1; +, slightly reduced; -, significantly reduced.

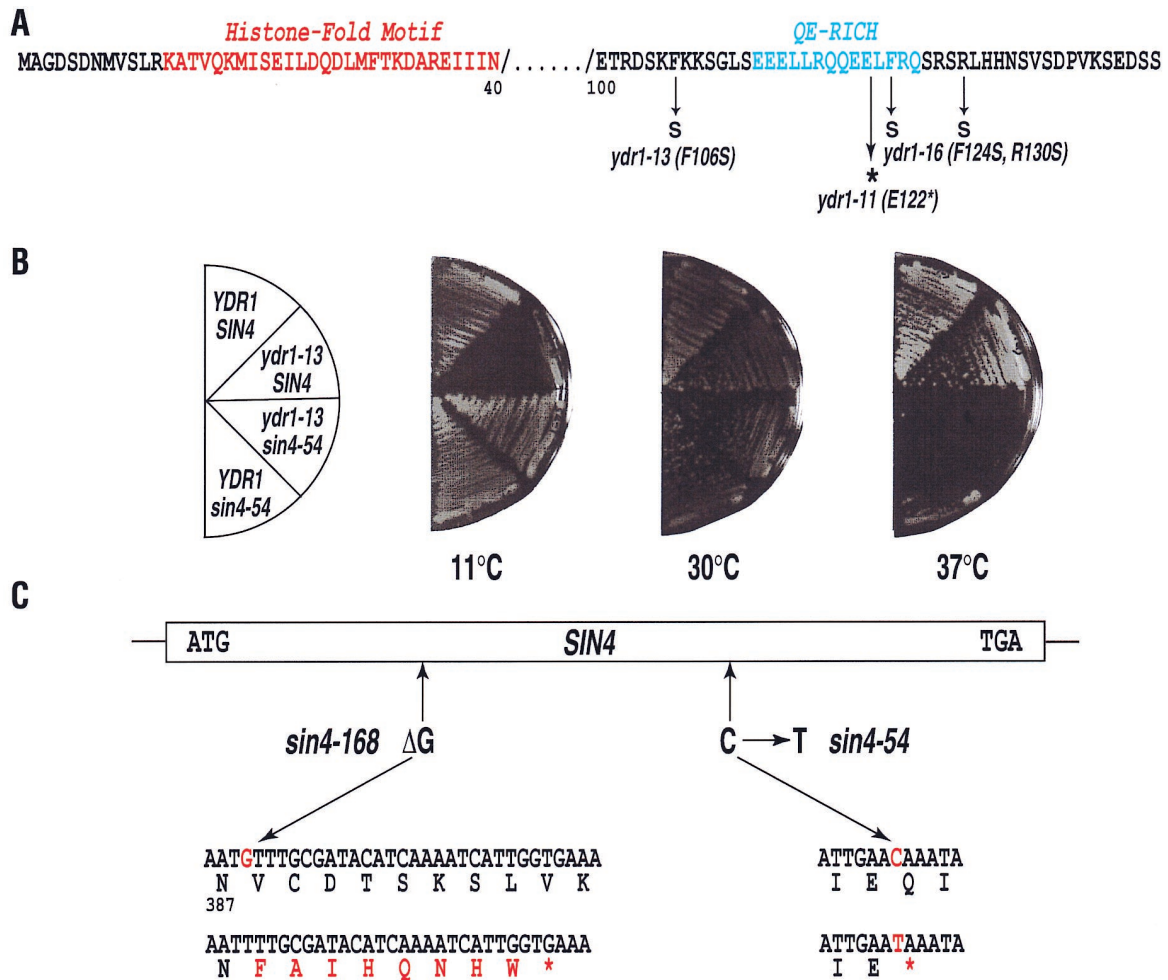


FIG. 4. Suppression of the cs^- phenotype of the *ydr1* mutant allele by recessive mutation in the *SIN4* gene. (A) Point mutations of cs^- *ydr1* mutant alleles. The histone fold motif (in red) and the conserved QE-rich domain (in blue) are indicated on the Ydr1 amino acid sequence. Amino acid substitutions for each mutation are indicated below the sequence. (B) Four different isogenic strains (genotypes are indicated on the left) were compared for the ability to grow at various temperatures on YPD medium. (C) Sequence analyses of two different *sin4* suppressor mutations. Genomic DNA segments containing the *sin4* alleles were retrieved from each suppressor strain by the gap repair method. The amino acid sequences changed by the *sin4* mutations are indicated at the bottom in red.

conceivable that loss of Ydr1-Bur6 function eliminates an essential balance between positive and negative transcriptional regulatory mechanisms, with this balance apparently restored by the loss of Sin4 function.

We tested this possibility by investigating if the *sin4* suppressors render *YDR1* dispensable for cell viability. This was done by a *YDR1* plasmid shuffle assay in the wild-type *SIN4* (YSK013 and YSK014), *sin4-54* (YSK126 and YSK127), and *sin4-168* (YSK128 and YSK129) genetic backgrounds. Results are shown in Fig. 6A. When cured of the *YDR1-URA3* plasmid, the wild-type strain failed to grow on FOA medium, consistent with *YDR1*'s being an essential gene. However, the *sin4-54* strain grew in the absence of *YDR1*, albeit more slowly than the wild-type *SIN4* strain. To confirm that viability of the *ydr1Δ sin4-54* mutant is due to *sin4-54*, rather than to an undefined mutation, we investigated if plasmid-borne *SIN4* would render the double mutant inviable. Accordingly, the YSK125 (*ydr1Δ [YDR1-URA3] sin4-54*) strain was transformed with plasmid DNA containing either wild-type *SIN4* (YSK140) or the vector alone (YSK141). Accordingly, plasmid-borne *SIN4* will compensate the recessive *sin4-54* allele. As expected, the strain containing plasmid DNA alone remained viable on the FOA

medium but the strain containing *SIN4* became sensitive to FOA (Fig. 6C). Thus, complementation of *sin4-54* by wild-type *SIN4* restored the essential Ydr1 requirement. This effect is specific to the *sin4-54* allele, because *sin4-168* and *sin4Δ* (data not shown) failed to grow when cured of *YDR1*. These results demonstrate that *sin4-54* alone is able to suppress the inviability of *ydr1Δ*. We conclude that the *sin4-54* suppressor mutation is able to bypass the essential requirement for *YDR1* in vivo. Nonetheless, the *ydr1Δ sin4-54* strain grew more slowly than the *YDR1 sin4-54* strain (Fig. 6A) and a growth difference was not observed in the *ydr1-13 sin4-54* strain relative to the *YDR1 sin4-54* strain (Fig. 4B). This difference is due to the partial retention of Ydr1 function encoded by the partially functional *ydr1-13* allele. Therefore, although *sin4-54* renders *YDR1* dispensable for cell viability, the normal rate of cell growth remains Ydr1 dependent. Interestingly, this effect is specific to *YDR1* since neither *sin4-54*, *sin4-168*, nor *sin4Δ* rendered *BUR6* dispensable in a similar assay (Fig. 6B). The inability of either suppressor to render *BUR6* dispensable suggests that Bur6 affects another cellular process(es) independent of Ydr1.

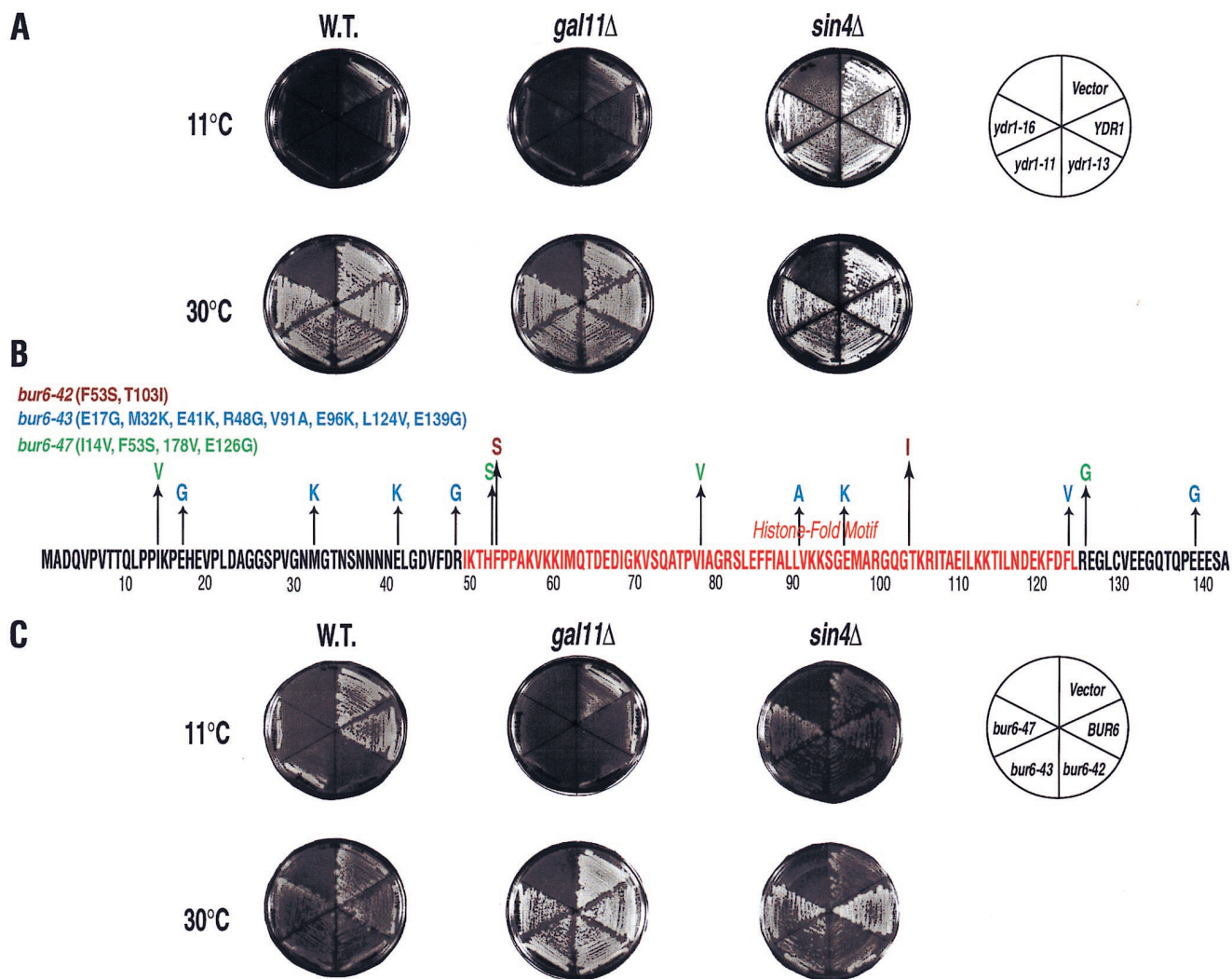


FIG. 5. Suppression of *cs⁻ ydr1* and *bur6* mutants by the *sin4* deletion mutation but not by the *gal11* deletion mutation. (A) Different *ydr1* mutant alleles in plasmid DNA were transformed into either a *sin4* deletion strain (*sin4Δ*), a *gal11* deletion strain (*gal11Δ*), or the wild-type strain (W.T.). The *cs⁻* phenotype of each strain was examined by streaking onto a YPD plate and incubation at either 30 or 11°C. The genotypes of the strains are shown in the circular diagram. Vector, strain YMH200 containing wild-type *YDR1* and an empty *LEU2* marker plasmid (control). (B) Point mutations of the *bur6* *cs⁻* mutant alleles. The histone fold motif is in red. Amino acid substitutions for each mutation are indicated as follows: *bur6-42*, brown; *bur6-43*, blue; *bur6-47*, green. (C) Different *bur6* mutant alleles in plasmid DNA were transformed into either a *sin4* deletion strain, a *gal11* deletion strain, or the wild-type strain. The *cs⁻* phenotype of each strain was examined by streaking onto a YPD plate and incubation at either 30 or 11°C. The genotypes of the strains are shown in the circular diagram. Vector, strain YMH203 containing wild-type *BUR6* and an empty *LEU2* marker plasmid (control).

The *sin4-54* suppressor alters the composition of the SRB-MED complex. In order to elucidate both the specific effect of the *sin4-54* mutation and the possible mechanism of *sin4*-mediated suppression, we characterized the effect of *sin4* suppressor mutations on the composition of the SRB-MED complex. Deletion of *SIN4* causes the loss of specific subunits from the SRB-MED complex (28, 29). Therefore, SRB-MED complexes were purified from both a wild-type *SIN4* strain (VM02) and the *sin4-54* suppressor strain (YSK149) and their subunit compositions were compared. We observed two interesting features of the complex isolated from the *sin4-54* suppressor strain. First, Western blot analysis (Fig. 7) showed a significant reduction in the amounts of the Med9, Med10, and Med11 polypeptides. The loss of specific subunits of the SRB-MED complex suggests that the *sin4* suppressor mutation weakens the association of a unique set of mediator proteins and causes a defect in transcriptional activation by certain activators in

vivo. Secondly, in contrast to the effect of the total deletion of *sin4*, which causes the loss of all subunits of the Gal11 module except Rgr1 (29), the SRB-MED complex from the *sin4-54* strain retained all of the subunits of the Gal11 module (Med2 was not determined). On the basis of these results, we conclude that *sin4-54* has a unique effect on the SRB-MED complex, possibly affecting the interaction of the Gal11 subcomplex containing the Sin4 subunit with the subcomplex containing the Med9, Med10, and Med11 subunits.

DISCUSSION

We have exploited the power of yeast genetics to further define the role of the Ydr1-Bur6 transcriptional repressor in vivo. A previously unrecognized relationship between the Ydr1-Bur6 complex and the Sin4 component of the SRB-MED complex was uncovered. These results underscore the positive

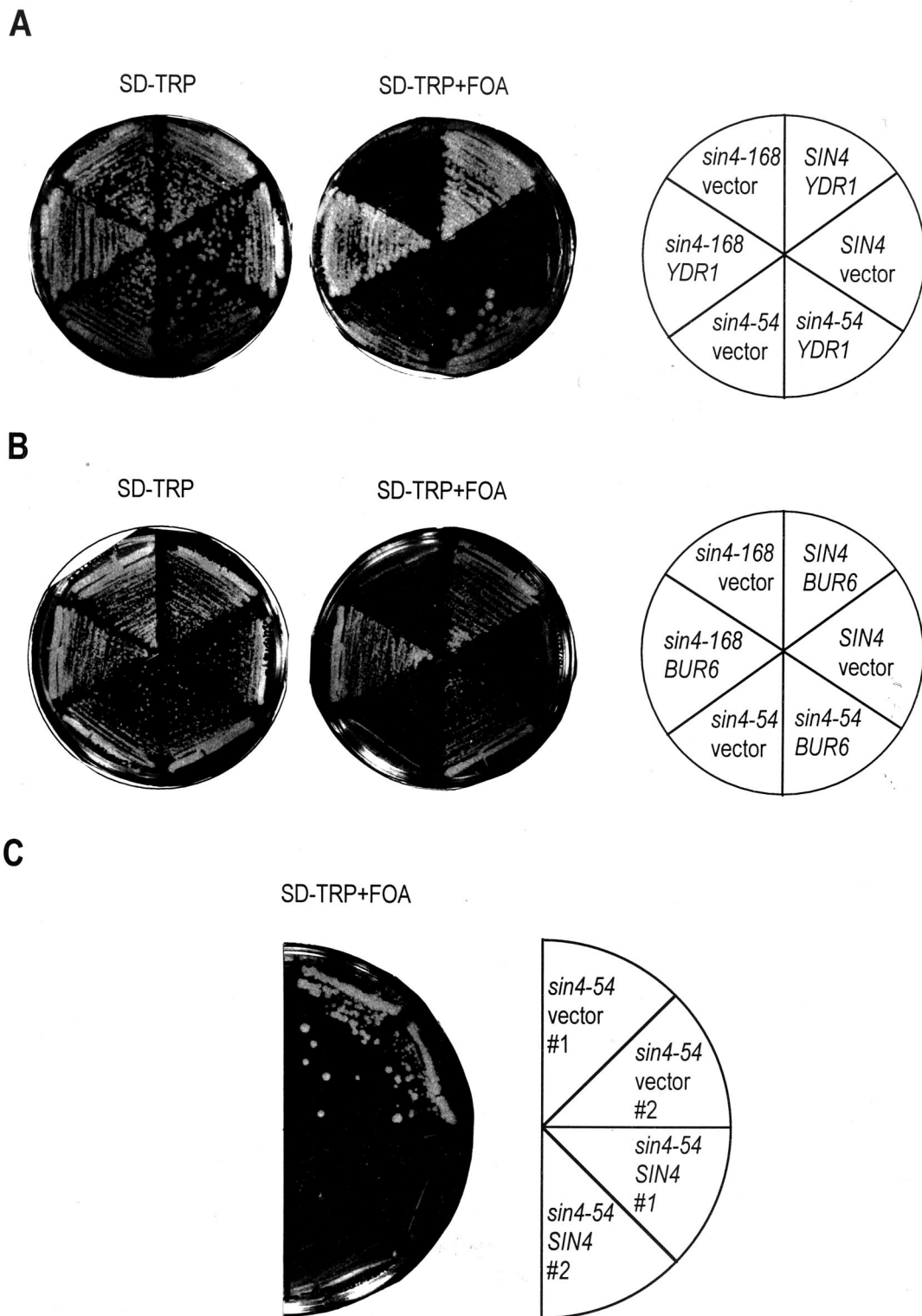


FIG. 6. *sin4* suppressor allele *sin4-54* makes *YDR1*, but not *BUR6*, dispensable for cell viability. The genotypes of the strains are shown in the circular diagram. (A) Cell viability of *ydr1* deletion strains in different *sin4* allele backgrounds was determined by scoring growth on a plate containing FOA (SD-TRP+FOA). Each strain (*SIN4*, *sin4-54*, or *sin4-168*) was transformed either with the vector containing wild-type *YDR1* (*SIN4-YDR1*, *sin4-54-YDR1*, or *sin4-168-YDR1*) or the vector alone (*SIN4-vector*, *sin4-54-vector*, or *sin4-168-vector*) to generate a pair of isogenic strains. (B) Cell viability of a *bur6* deletion strain in different *sin4* allele backgrounds was determined as described for panel A, except that *BUR6* was substituted for *YDR1*. (C) The specificity of lethality overcome by the *sin4-54* allele was demonstrated by converting an FOA-resistant *ydr1*-negative strain (*sin4-54-vector*; two independent isolates are indicated as #1 and #2) into an FOA-sensitive strain by introducing wild-type *SIN4* (*sin4-54-SIN4*; two independent isolates are indicated as #1 and #2).

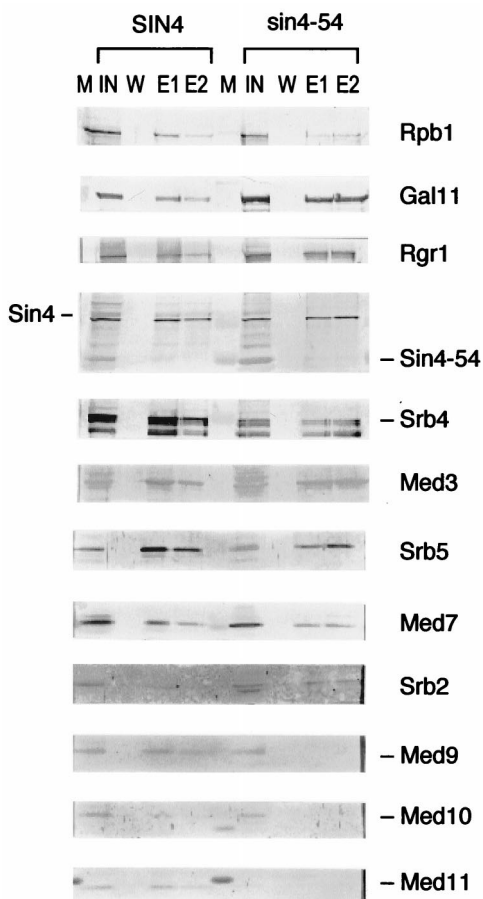


FIG. 7. The composition of the SRB-MED complex from the *sin4* suppressor strain differs from that of the complex isolated from the wild-type strain. Western blot analyses of Ni-NTA column-purified SRB-MED complexes isolated from the wild-type *SIN4* strain (VM02) or the *sin4-54* suppressor strain (YSK149). An isogenic *sin4-54* suppressor strain was generated by recombination-mediated truncation of the C terminus of the *SIN4* gene using an *SRB5*-tagged strain (VM02). M, molecular size marker; IN, input; W, wash; E1 and E2, elution fractions (first and second lanes). The FL-Sin4 polypeptide from the wild-type strain is indicated at the left. The truncated Sin4-54 polypeptide from the *sin4-54* strain is indicated at the right.

role of Sin4 in transcription and define a delicate balance between positive and negative effectors of gene regulation *in vivo*.

We began the studies described here by analyzing the *in vivo* and *in vitro* functions of the C terminus of Ydr1, which in the human polypeptide contains a transferable repressor domain. This domain is conserved in the yeast Dr1 polypeptide. From these analyses, important implications were derived. First, deletion of the repression domain (C105) did not affect the ability of Ydr1 to interact with Bur6. This truncated polypeptide also interacted with TBP, although with approximately 30% efficiency compared to the wild-type Ydr1 polypeptide. This reduction in TBP binding is consistent with previous studies demonstrating that the C terminus of human Dr1 (residues 101 to 165) contains a low-affinity TBP binding site (46). This truncated form of Ydr1 completely lost its ability to repress transcription *in vitro*. Another C-terminal truncation which left the repression domain intact (C130) was capable of repressing transcription *in vitro*, although its ability to interact with TBP was affected to approximately the same extent as with C105. We conclude that the ability of Ydr1 to interact with TBP,

while necessary and important for repression, is not sufficient but also requires the highly conserved QE-rich domain (Fig. 4). Second, the inability of C105 to repress transcription correlates with the extremely slow growth phenotype. In contrast to previous studies (10), the C-terminal domain of Ydr1, which is distinct from the histone fold motif and the TBP binding domain, is essential not only for transcriptional repression but also for normal cell growth.

We expanded the truncation analyses by introducing specific substitutions within the C terminus of Ydr1. The discovery of cs^- phenotypes associated with several *ydr1* mutations in the repressor domain led us to postulate that the defects caused by these mutations may be linked directly to the observed transcriptional defects. Suppressor mutations that compensate for these specific defects were expected to identify gene products that function in transcriptional activation. This expectation was borne out by the identification of *sin4* alleles as suppressors of *ydr1* mutations. Our analysis indicates that suppression does not involve a direct protein interaction between Sin4 and Ydr1-Bur6. This interpretation does not imply that the *sin4*-mediated suppression is nonspecific. The observation that the *gal11* deletion was not able to suppress the cs^- *ydr1* mutations indicates that suppression by the *sin4* mutant alleles resulted not from the disruption of the Gal11 subcomplex but from specific alterations of the Sin4 molecule. Importantly, a correlation between Ydr1-Bur6 and the RNAPII holoenzyme established in these studies is in agreement with previous findings of Young and coworkers (9, 26). Their studies, analyzing an allele of the *SRB4* gene, *srb4-138*, which encodes another component of the SRB-MED complex, identified alleles of *YDR1* and *BUR6*, as well as other negative regulators, including *NOT1* and *MOT1*. Taken together, these studies point to a delicate balance between positive and negative regulators of transcription and indicate that loss of one function can be compensated for by loss of a reciprocal function *in vivo*.

Although the physiological role of Sin4 is not fully understood, its function has been correlated with both positive and negative regulation of gene expression. Based on the general repressive effect of Ydr1-Bur6 on transcription, our results suggest that the positive regulatory function of Sin4 has broad effects on gene expression. These results do not exclude the participation of Sin4 in transcriptional repression but suggest that negative regulation is a secondary or even indirect function of Sin4.

The reciprocal relationship between Ydr1-Bur6 and Sin4 is not subtle. This is most evident in the ability of *sin4-54* to render the otherwise essential *YDR1* gene dispensable for cell viability. This is a dramatic effect but is not without precedent. For example, in a study by Zhao et al. (46), a mutation in the *SML1* gene allowed cell growth in the absence of two otherwise essential checkpoint genes, *MEC1* and *RAD53*. The authors suggested that Mec1 and Rad53 may be no longer required if there is a defect in an inhibitory function of the Sml1 protein. Our findings may be related to those of Zhao et al. and have two important implications. First, the defect caused by the *ydr1* deletion is likely confined to a transcriptional defect. Importantly, only the *sin4-54* allele, but not *sin4-168* or the deletion of *sin4*, was capable of negating the *YDR1* requirement for cell viability. Second, the C terminus of the Sin4 polypeptide (amino acids 672 to 974, those deleted in *sin4-54*) may have an as yet uncharacterized role(s) *in vivo*. Truncation of the C terminus of Sin4 does not appear to expose a cryptic transcriptional repression domain because *sin4-54* did not repress *lacZ* expression under the control of different promoters (data not shown). Moreover, complete deletion of *SIN4* will also suppress *Ydr1* cs^- alleles. Rather, the removal of this particular region of Sin4

appears to alter the subunit composition of the RNAPII holoenzyme in a specific manner. While deletion of *SIN4* causes the dissociation of most of the Gal11 subcomplex from the RNAPII holoenzyme (29), the *sin4-54* mutation gave rise to the loss of a subset of mediator components, specifically, Med9, Med10, and Med11. Med9 and Med10 specifically mediate transcription signals from the BAS1-BAS2, Gcn4, and Gal4 transcriptional activators, respectively (14).

It is also important to recognize that whereas *sin4-54*, *sin4-168*, or the deletion of *sin4* can suppress the cs^- phenotype associated with *ydr1* and *bur6*, *sin4-54* renders *YDR1*, but not *BUR6*, dispensable for cell viability. This observation is likely related to our previous studies with the mammalian Dr1-DRAP1 repressor complex. In those studies, we analyzed the expression pattern of Dr1 and DRAP1 in the developing mouse and found that all cells expressed Dr1, yet only highly differentiated cells expressed DRAP1 (31; R. Iratni and D. Reinberg, unpublished data). In light of these findings, we proposed that there are at least two mechanisms by which Dr1 represses transcription: a low-affinity mechanism which is independent of DRAP1 (*Bur6*) and a high-affinity mode dependent on DRAP1. Recent studies mapped residues in TBP that define the interaction with the Ydr1-Bur6 complex (4). These residues are clustered in a previously undefined domain of TBP adjacent to the transcription factor IIB (TFIIB) binding site. The TBP mutational analysis provides a clear explanation of previous findings demonstrating that the association of Dr1 with TBP prevented the entry of TFIIB into the transcription complex. However, the mutational analysis of TBP does not provide a logical explanation for the finding that TFIIA can overcome Dr1-mediated repression of transcription. TFIIA and TFIIB-Dr1 bind to diametrically opposed surfaces of TBP. In light of these findings, we recently proposed that Ydr1-Bur6 either induces a conformational change in TBP that affects TFIIA binding or alters the structure of the TBP-DNA complex (30). Dr1-mediated repression is likely to involve two mechanisms, one directly blocking the assembly of TFIIB, the other inducing a TBP conformational change that alters TFIIA binding. This latter mechanism may be dependent on the presence of DRAP1 (*Bur6*), yet this speculation does not provide an explanation for the findings described above with the *sin4-54* allele, which allows viability in the absence of *YDR1* but not in the absence of *BUR6*. The most logical interpretation of this finding is that *Bur6* has functions that are independent of Ydr1.

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