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The Dr1/DRAP1 heterodimer is a global repressor of transcription *in vivo*

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ABSTRACT A general repressor extensively studied *in vitro* is the human Dr1/DRAP1 heterodimeric complex. To elucidate the function of Dr1 and DRAP1 *in vivo*, the yeast *Saccharomyces cerevisiae* Dr1/DRAP1 repressor complex was identified. The repressor complex is encoded by two essential genes, designated *YDR1* and *BUR6*. The inviability associated with deletion of the yeast genes can be overcome by expressing the human genes. However, the human corepressor DRAP1 functions in yeast only when human Dr1 is coexpressed. The yDr1/Bur6 complex represses transcription *in vitro* in a reconstituted RNA polymerase II transcription system. Repression of transcription could be overcome by increasing the concentration of TATA-element binding protein (TBP). Consistent with the *in vitro* results, overexpression of *YDR1 in vivo* resulted in decreased mRNA accumulation. Furthermore, *YDR1* overexpression impaired cell growth, an effect that could be rescued by overexpression of TBP. In agreement with our previous studies *in vitro*, we found that overexpression of Dr1 *in vivo* also affected the accumulation of RNA polymerase III transcripts, but not of RNA polymerase I transcripts. Our results demonstrate that Dr1 functions as a repressor of transcription *in vivo* and, moreover, directly targets TBP, a global regulator of transcription.

Initiation of transcription by RNA polymerase II (RNAPII) is an intricate process requiring different families of transcription factors operating at the promoter (1, 2). One family of factors, the so-called general transcription factors (GTFs), functions to deliver RNAPII to the promoter (for review see ref. 3). This process is initiated by association of the TATA-element binding protein (TBP) subunit of TFIID with the TATA motif. TBP recognizes the minor groove of the 8-bp TATA element (4–6), and the TATA element is molded to follow the curved β-sheet on the underside of the TBP saddle (6). As a result, the TATA sequence is partially unwound and bent in a smooth arc. The dramatic distortion of the TATA element by TBP allows TFIIB to interact with the phosphodiester backbone of DNA both upstream and downstream of the TATA sequence. The crystal structure of the TBP–TFIIB–DNA ternary complex (TB complex) illustrates how TFIIB recognizes the preformed TBP–DNA complex (7). As suggested by footprinting (8) and crosslinking (9) experiments, TFIIB binds underneath and on one face of the TBP–DNA complex where it interacts with TBP and DNA. TBP–TFIIB contacts are mainly between the basic amino-terminal repeat of TFIIB and the acidic carboxyl-terminal stirrup of TBP, in agreement with mutagenesis studies (10, 11). The TB complex provides the recognition site for entry of RNAPII, which is escorted to the promoter by TFIIF (1–3). The resulting DNA–protein complex (TBPolF) is recognized by TFIIE, providing the recognition site for entry of TFIIH (1–3), resulting in the formation of a transcription competent complex. An alternative model for the formation of transcription complexes has been suggested. In this model the RNAPII exists in a complex with most of the GTFs and other regulatory factors, such as an RNAPII "holoenzyme" complex (12).

Regardless of the pathway used to establish a transcription complex, the GTFs and RNAPII cannot access promoter sequences *in vivo*, where the GTFs are limiting and the DNA is in the form of chromatin. Under these conditions a second family of factors is required. These factors are sequence-specific DNA binding proteins that recognize a specific promoter element(s) present in different promoters (3). These regulatory factors also stimulate transcription by enhancing the formation/stability of preinitiation complex intermediates, which are kinetically not favorable (13).

Another family of factors operating on promoters are those that negatively regulate transcription. A large number of these factors have been described (for reviews see refs. 14 and 15). These factors repress transcription by different modes. Some are sequence-specific DNA binding proteins, which upon binding to specific promoters render the genes silent (15–17). Other gene specific repressors inhibit transcription by sequestering activators and preventing their translocation to the nucleus and/or preventing their association with promoter sequences (18, 19). Another growing family of repressors includes molecules that are tethered to promoters by interacting with sequence-specific DNA binding proteins and/or components of the basal transcription machinery. Factors in this category include the yeast Tup1/Ssn6 repressor complex, Mot1, Sin3, and Dr1 (20–23).

Human Dr1 was isolated as an activity that represses basal transcription (23). The activity was shown to reside in a single polypeptide of ≈20 kDa, which interacts with TBP and prevents the association of TFIIB with the TBP–TATA complex. Dr1 was shown to have three functionally important domains (24). A domain that interacts with TBP, which is required to tether Dr1 to the promoter. The TBP-binding domain is not sufficient for repression of transcription, rather, repression requires a domain rich in glutamine and alanine residues (QA-domain) located at the C terminus. The QA-domain is capable of repressing transcription when tethered to the promoter via a DNA binding domain (25). The third domain includes a histone-fold motif located at the N terminus of the protein, which is dispensable for Dr1-mediated repression of transcription *in vitro* (24). Subsequent studies demonstrated that the repressing activity of Dr1 is dramatically stimulated by a corepressor molecule, DRAP1 [also known as NC2α (26)], which also contains a histone-fold motif (26–28). DRAP1-mediated enhancement of transcriptional repression requires an association with Dr1, mediated through the respective

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Abbreviations: RNAPI, RNAPII, RNAPIII, RNA polymerases I, II, and III; GTFs, general transcription factors; TBP, TATA-element binding protein; GST, glutathione *S*-transferase.

histone-fold motifs (26–28), and Dr1 association with TBP through both the TBP-binding domain and the QA-domain (25). Antibodies to recombinant Dr1 defined the NC2 repressing activity as Dr1 (29, 30).

Since the Dr1 effect on transcription is manifested through TBP, and TBP is required for transcription by all three RNA polymerases, it was thought that Dr1 might repress all transcription. Studies *in vitro* showed that Dr1 does indeed repress transcription by RNAPIII, but not by RNAPI (31). This observation is consistent with the biochemical analysis establishing that Dr1 inhibits transcription of RNAPII by preventing the association of TFIIB with the TBP–TATA complex and that transcription by RNAPIII, but not by RNAPI, requires TFIIB-related factor, a factor structurally and functionally similar to TFIIB.

To analyze the function of the Dr1/DRAP1 complex *in vivo*, we have isolated the yeast counterpart of the Dr1/DRAP1 complex and studied its role *in vivo*. Results presented here establish the physiological significance of Dr1/DRAP1-mediated repression.

MATERIALS AND METHODS

Disruption of the *YDR1* and *BUR6* Genes. A single copy of the *YDR1* gene was disrupted by one-step gene disruption in a homozygous *his3 YDR1* diploid strain using PCR-amplified *HIS3* DNA that was generated using primers with *YDR1* sequences at their termini. A single copy of the *BUR6* gene was disrupted by gamma-transformation in a homozygous *trp1 BUR6* diploid strain using the *TRP1* vector pRS304 (32) carrying *Xho*I–*Kpn*I and *Aha*II–*Spe*I *BUR6* DNA fragments. Disruption of a single copy of *YDR1* and *BUR6* was confirmed by Southern blot analysis.

Yeast Strains. Strain YMH196 (*MAT***a** *ura3 leu2 his3 ydr1*::*HIS3* [*CEN-URA3-YDR1*]) is a plasmid shuffle strain carrying the essential *YDR1* gene on the *URA3* plasmid pM722. Strains YMH218 (*MAT***a** *ade2 ade3 his3 leu2 trp1 ura3 can1 bur6*::*TRP1* [pM724: *CEN-URA3-BUR6*] [pM765: *CEN-LEU2-MET25/hDRAP1*]) and YMH234 (*MAT*α *his3 ura3 leu2 trp1 ade2 ydr1*::*HIS3 bur6*::*TRP1* [pM724: *CEN-URA3-BUR6*] [pM750: *2um-LEU2-MET25/hDr1*]) are *bur6*::*TRP1* plasmid shuffle strains carrying the essential *BUR6* gene on the *URA3* plasmid pM724. The principal difference between these two strains is that YMH218 expresses wild-type *YDR1*, whereas YMH234 is deleted at the *YDR1* chromosomal locus (*ydr1*::*HIS3*), with the essential *YDR1* function provided by human *Dr1* expressed behind the *MET25* promoter (*MET25/hDr1*).

Overexpression of *YDR1*, *BUR6*, and *SPT15*. DNA fragments encompassing the *YDR1* and *BUR6* open reading frames were amplified by PCR and ligated behind the *GAL1* promoter in either p424 (*GAL/YDR1-TRP1*), p425 (*GAL/YDR1-LEU2*), or p426 (*GAL/BUR6-URA3)* (33). The *GAL/SPT15*-LEU2 plasmid (pSH277) expresses TBP from the *GAL* promoter (32). In Fig. 4*A*, yeast strain FY833 (34) was transformed with either the *GAL/YDR1* and *GAL/BUR6* constructs or control vectors. The resulting transformants were grown in omission medium containing 2% glucose, harvested by centrifugation, and transferred to omission medium containing either 2% glucose (Glc) or 2% galactose (Gal) to induce expression of *YDR1* and *BUR6*. Quantitative Western blot analyses indicates a 4- to 5-fold overexpression of Dr1 with respect to the uninduced cells (data not shown). Cells were harvested at the indicated times following transfer to glucose or galactose medium, and total RNA was prepared. Hybridization was carried out at 37°C for 12 hr with ^{32}P-labeled oligo[dT], or oligonucleotide probes complementary to intron sequences of rRNA and tRNA^{W} as described (35). S1 nuclease protection assays were carried out with 40 μg of total RNA and *ACT1* oligonucleotide probe as described (35). In Fig. 4*C*, strain FY833 (34) was transformed with the indicated combinations of *GAL/YDR1*, *GAL/SPT15*, or vector control plasmids. Transformants were subsequently streaked on -Leu, -Trp galactose medium to induce expression of *YDR1* and/or *SPT15*. All media were prepared as described (36, 37).

Protein Purification. Yeast whole cell extract was prepared as described (38). The whole cell extract was dialyzed against buffer E (20 mM Hepes·KOH, pH 7.6/10 mM magnesium acetate/5 mM EDTA/5 mM DTT/20% glycerol/0.01% Nonidet P-40 and protease inhibitors) containing 50 mM KOAc and loaded onto a 400-ml DE-52 column (Whatman) to remove nucleic acids. The flow through and the 1 M KOAc wash fractions were pooled and subjected to ammonium sulfate precipitation (60% saturation). The precipitate was resuspended in buffer E containing no KOAc (500 mg, 15 ml) and loaded onto an ACA44 gel filtration column (2.6 cm × 85 cm; Spectrum, Los Angeles), which was equilibrated with buffer E containing 1 M KOAc and 0.005% Triton X-100. The peak fractions containing polypeptides, which are recognized by α-yDr1 and α-Bur6 antibodies, were dialyzed against buffer T (buffer E with 10% glycerol) containing 0.1 M KOAc. The dialyzed sample was applied to a TSK–DEAE–5PW column (TOSO HAAS, Montgomeryville, PA) and eluted with a linear gradient of KOAc from 0.1 to 2 M in buffer T. The fractions were analyzed by Western blot analysis and the gel mobility-shift assay (39), monitoring their ability to form TBP-dependent DNA protein complexes. Fractions containing the yDr1/Bur6 complex (1.1 mg, 6 ml) were dialyzed against buffer T containing 1.5 M $(NH_4)_2SO_4$ and loaded onto a phenyl–superose HR5/5 column (Pharmacia). Proteins were eluted with a decreasing linear gradient of $(NH_4)_2SO_4$ (1.5–0 M) in buffer T. Active pool (0.1 mg, 3 ml) was dialyzed in buffer E containing 0.1 M KOAc and loaded onto a 1.5 ml glutathione *S*-transferase (GST)–yTBP column. The GST–yTBP and GST columns were prepared as described (40, 41). The amount of proteins immobilized on the columns was 1.8 mg of GST–yTBP and 1.6 mg of GST per ml of glutathione–Sepharose CL4B resin. The column was eluted by step-washes with buffer E containing 0.1, 0.5, 1.0, and 1.5 M KOAc. The fractions were dialyzed in buffer E containing 0.1 M KOAc and were assayed using the gel mobility-shift assay (39).

***In Vitro* Transcription and Immunoprecipitation Assays.** Transcription assays were reconstituted on the Ad-MLP promoter with ryTBP (5 ng), and human rTFIIB (5 ng), rTFIIE (15 ng), rTFIIF (23 ng), native TFIIH (500 ng, phenyl-superose fraction), and anti-carboxyl-terminal domain affinity-purified RNAPII (50 ng).

Immunoprecipitation experiments were performed as described (42) with modifications. Antibodies were affinity purified using recombinant polypeptides. Recombinant polypeptides [yDr1 (3.0 μg) + Bur6 (2.8 μg), hDr1 (3.0 μg) + Bur6 (2.8 μg), hDr1 (3 μg) + DRAP1 (3.4 μg)] were mixed and incubated on ice for 30 min. The different protein mixtures were then incubated with the specific antibodies ($\approx$1 μg), which were immobilized on protein A-agarose beads (Repligen). Immunoprecipitates were washed three times with buffer containing 20 mM Hepes–KOH (pH 7.9), 1 mM EDTA, 10% (vol/vol) glycerol, 0.5% (vol/vol) Nonidet P-40, 0.1% (vol/vol) Triton X-100, and 0.25 M NaCl, and resuspended in SDS/PAGE loading dye. After electrophoresis, proteins were transferred to nitrocellulose membranes and detected by Western blot analysis using the indicated antibodies.

RESULTS

The Yeast *YDR1* Gene Is Essential for Cell Viability. Comparison of the human Dr1 sequence with the protein data bases revealed significant similarity to an open reading frame from the yeast *Saccharomyces cerevisiae* (Fig. 1*A*). The human and yeast proteins are 37% identical (58% similar) with only a single gap required to maintain the alignment. Both proteins include a histone-fold motif near the N terminus. The yeast *YDR1* gene was amplified from genomic DNA by the PCR and

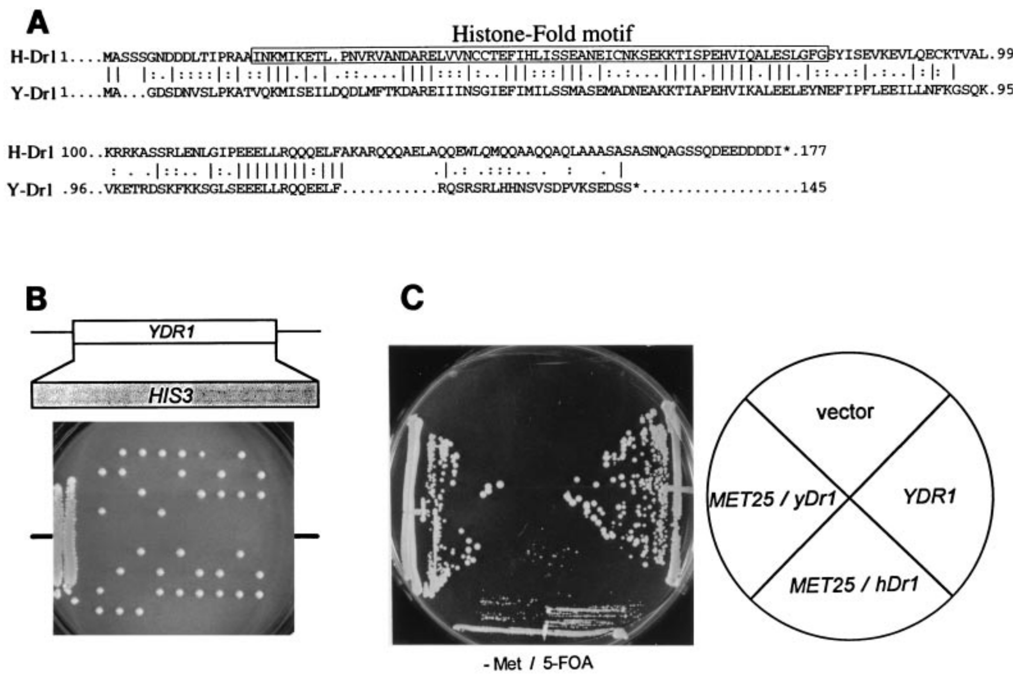

FIG. 1. The yeast *YDR1* gene encodes the homologue of the human Dr1 repressor. (*A*) Sequence alignment of the human (hDr1) and yeast (yDr1) proteins. Identical residues are denoted by |; similar residues are denoted by : or ·. A single gap (. . .) was introduced into the Ydr1 sequence to maintain the alignment. (*B*) *YDR1* is essential for cell viability. The *YDR1* coding region (open rectangle) was replaced by the *HIS3* gene and the resulting construct was used to disrupt a single copy of *YDR1* in a $his3^{-}/his3^{-}$ *YDR1*/*YDR1* diploid strain. Sporulation and dissection yielded 2 viable spores for each of 18 tetrads, all of which are phenotypically His^{-}. (*C*) Expression of human Dr1 restores viability to a *ydr1*::*HIS3* null mutant. Plasmid shuffle strain YMH196 (*ydr1*::*HIS3 leu2* [*YDR1-URA3*]), carrying either the *MET25-LEU2* plasmid p415 (vector), or its derivatives expressing *YDR1* behind its own promoter (*YDR1*), or human (*MET25*/*hDr1*), or yeast (*MET25*/*yDr1*) Dr1 behind the *MET25* promoter, were cured of *YDR1-URA3* under conditions that induce the *MET25* promoter (-Met/5-FOA).

cloned into low-copy-number yeast vectors for subsequent characterization.

One copy of the *YDR1* gene was disrupted in a diploid strain by replacement of the *YDR1* open reading frame with the *HIS3* gene (Fig. 1*B*). Upon sporulation and dissection, only two viable progeny were recovered from each tetrad, all of which were His^{-}. Visible inspection of inviable spores revealed that each had germinated and undergone 2-3 cell divisions. Four-spore viability was recovered when the same diploid strain was transformed with a plasmid carrying *YDR1* prior to dissection. Thus, the *YDR1* gene is essential for cell viability.

The Human Dr1 Gene Can Rescue the Inviability of a *ydr1* Null Mutant. To determine the relationship between human Dr1 and its yeast homologue, we asked if expression of human Dr1 could rescue the inviability of a *ydr1* null mutant. This was done using a plasmid shuffle assay (43). The human Dr1 gene, expressed behind the yeast *MET25* promoter, was introduced into strain YMH196 (*ydr1*::*HIS3* [*YDR1-URA3*]). The resulting transformants were streaked on synthetic complete (SC) medium containing 0.1% 5-fluoro-orotic acid (5-FOA), which counter-selects the *YDR1-URA3* plasmid. While the strain carrying vector alone failed to grow, the *MET25*/*hDr1* strain grew, albeit less well than either of the control strains expressing *YDR1* (Fig. 1*C*). These results establish that Ydr1 is the functional counterpart of human Dr1 and performs a function essential for cell growth.

The Yeast *BUR6* (*yDRAP1*) Gene Is Essential for Cell Viability. The human DRAP1 protein is a corepressor of Dr1 that enhances Dr1-mediated repression of transcription (26–28). A search for yeast sequences encoding a potential homologue of DRAP1 identified the *BUR6* gene, which was initially found in a genetic screen for transcriptional repressors (44). Sequence alignment indicated that the proteins are 37% identical (61% similar) with most of the homology centered in the histone-fold motif (Fig. 2*A*).

One copy of the *BUR6* gene was disrupted in a diploid strain by gamma-disruption (32) using *TRP1* as the marker (Fig. 2*B*). Upon sporulation and dissection, two viable progeny were recovered from each tetrad, all of which were Trp^{-}. Four-spore viability was restored by plasmid-borne *BUR6*. All nonviable spores germinated and underwent several cell divisions prior to cessation of growth. Thus, *BUR6*, like *YDR1*, is an essential gene.

The Association of Dr1 and DRAP1 Is Species-Specific. We determined whether Bur6 and human DRAP1 are functionally related by expression of DRAP1 in yeast. In this case, expression of DRAP1 behind the *MET25* promoter failed to complement a *bur6*::*TRP1* null mutation (Fig. 2*C*). Since human Dr1 and DRAP1 directly interact, we reasoned that failure of the DRAP1 gene to complement *bur6* might be due to defective human DRAP1–yeast Dr1 interaction, rather than DRAP1 and Bur6 being functionally distinct. We addressed this possibility by asking if DRAP1 would complement loss of Bur6 function in a strain expressing human Dr1 in place of yeast Ydr1. Indeed, expression of DRAP1 from the *MET25* promoter rescued the inviability of the *bur6* null mutation when human Dr1 was also expressed from the *MET25* promoter (Fig. 2*C*). The ability of DRAP1 to complement loss of Bur6 function in a Dr1-dependent manner establishes that DRAP1 is the functional counterpart of Bur6 and underscores the importance of the Dr1–DRAP1 interaction.

To further analyze whether yeast Dr1 and Bur6 form a complex and whether the human and yeast polypeptides interact, immunoprecipitation studies were performed (Fig. 2*D*). Antibodies against Bur6 immunoprecipitated yeast Dr1, as demonstrated by Western blot analysis using yeast Dr1 antibodies (lane 2). Immunoprecipitation of Dr1 by the Bur6 antibodies was lost if Bur6 was omitted from the protein mixture (lane 3), demonstrating that detection of Dr1 was due to coimmunoprecipitation. Next we analyzed whether human Dr1 and yeast Bur6 proteins interact. In agreement with the *in vivo* data (Fig. 2*C*), antibodies against

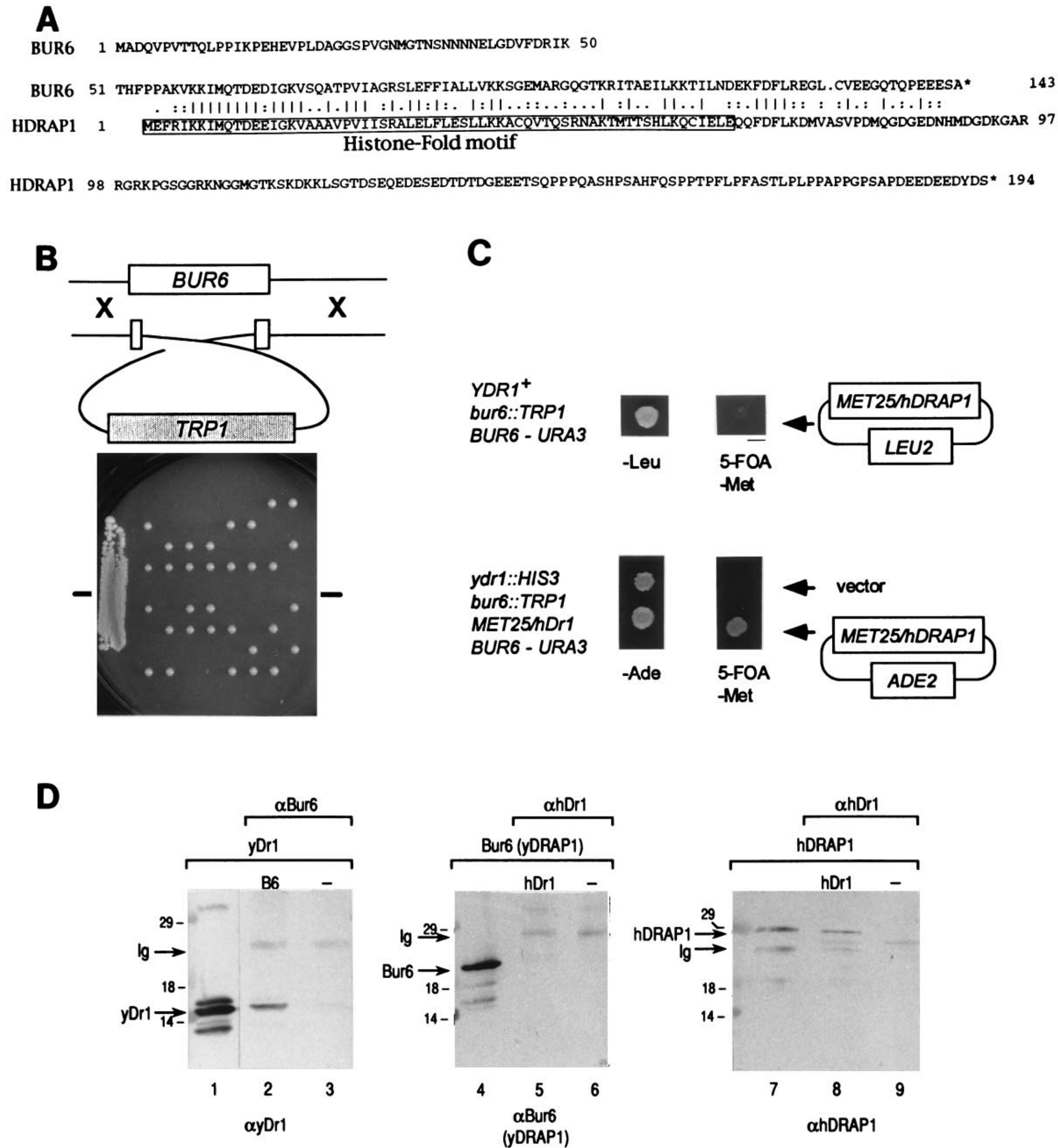

FIG. 2. The yeast *BUR6* gene encodes the homologue of the DRAP1 corepressor. (*A*) Sequence alignment of the human (DRAP1) and yeast (Bur6) proteins. Identical residues are denoted by |; similar residues are denoted by : or ·. (*B*) *BUR6* is essential for cell viability. Two fragments of the *BUR6* gene (open rectangles), corresponding to the indicated 5′ and 3′ regions of *BUR6*, were ligated into the *TRP1* integrating vector pRS304. The resulting plasmid was used to disrupt a single copy of *BUR6* in a *trp1*/*trp1 BUR6*/*BUR6* diploid strain. Sporulation and dissection yielded 2 viable spores for each of 16 tetrads, all of which were phenotypically Trp^-. (*C*) Expression of human DRAP1 complements a *bur6*::*TRP1* null mutation in a human Dr1-dependent manner. Strain YMH218 (*YDR1*$^+$ *bur6*::*TRP1 leu2* [*BUR6-URA3*]) was transformed with the *MET25*/*hDRAP1-LEU2* plasmid, selecting for Leu^+ transformants. When cured of the *BUR6-URA3* plasmid under conditions that induce expression from the *MET25* promoter (5-FOA/-Met) no growth was observed. However, strain YMH234 (*ydr1*::*HIS3 bur6*::*TRP1 ura3 leu2 ade2* [*MET25*/*hDr1-LEU2*] [*BUR6-URA3*]), which expresses human Dr1 rather than yeast *YDR1*, is viable when cured of the *BUR6-URA3* plasmid under conditions that induce expression of hDRAP1 (5-FOA/-Met). This effect is hDRAP1-dependent since the control strain carrying the *ADE2* vector alone failed to grow on the same medium. (*D*) The interaction between Dr1 and DRAP1 (*BUR6*) is species-specific. Protein mixtures containing recombinant polypeptides (yDr1 + Bur6, lanes 1–3; hDr1 + Bur6, lanes 4–6; hDr1 + DRAP1, lanes 7–9) were incubated with different antibodies as indicated at the top to coimmunoprecipitate yeast Dr1 (lanes 1–3), Bur6 (lanes 4–6), and DRAP1 (lanes 7–9). Antibodies used to detect the coimmunoprecipitated polypeptides in Western blot analysis are indicated at the bottom. Numbers on the left denote molecular weight markers, the immunoglobulin light chain (Ig), and the polypeptide analyzed in the coimmunoprecipitation.

human Dr1 failed to immunoprecipitate Bur6 from a protein mixture containing human Dr1 and Bur6 (lane 5). The inability to coimmunoprecipitate Bur6 was not due to a defect in the antibodies, as the replacement of yeast Bur6 by the human polypeptide resulted in effective coimmunoprecipitation of DRAP1 (lane 8). Similar results were observed using DRAP1 antibodies (data not shown). Thus, the interaction between Dr1 and DRAP1(Bur6) is species-specific.

Yeast Dr1 and Bur6 Physically Interact *in Vivo*. We extended the immunoprecipitation studies to analyze whether yeast Dr1 and Bur6 interact *in vivo*. We observed that the two polypeptides copurified through extensive chromatography (Fig. 3). Yeast Dr1 and Bur6 coeluted with an apparent mass of ≈45 kDa from a gel filtration column, as detected by Western blots using antibodies generated against recombinant yeast Dr1 and Bur6 (Fig. 3*A*). This analysis also revealed a population of Dr1 molecules that were free

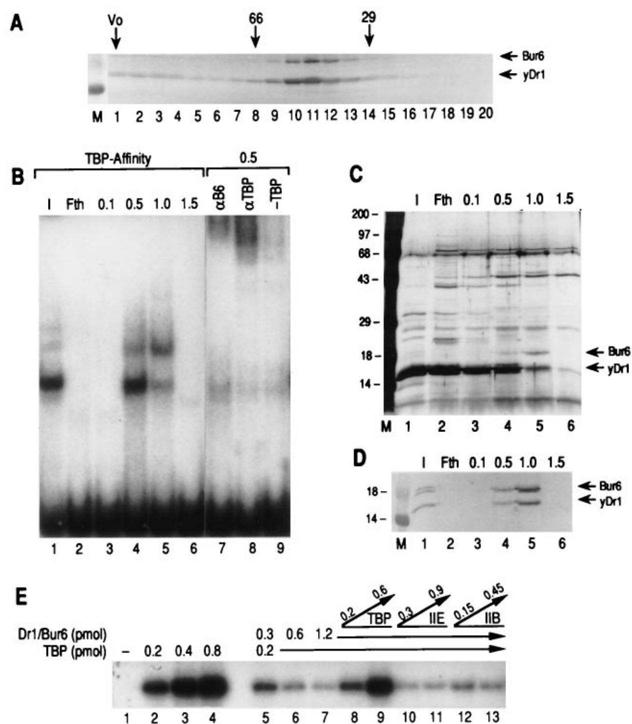

FIG. 3. Purification of the yeast Dr1/Bur6 complex. (*A*) The elution profile of the gel filtration column ACA44 was analyzed by Western blot analysis using affinity-purified polyclonal antibodies against both yDr1 and Bur6 polypeptides. The peak fractions of corresponding molecular weight standards (in kDa) are indicated at the top. Vo, void volume. (*B*) The ability of the yDr1/Bur6 complex isolated from yeast to form yTBP–yDr1–Bur6–DNA complexes was examined using the gel mobility shift assay. Lanes: 1, input (2 μl) to the GST–yTBP affinity column (I); 2, flow through (Fth, 4 μl) of the yeast TBP–affinity column; 3–6, fractions that were eluted from a GST–yTBP affinity column with 0.1, 0.5, 1.0, and 1.5 M KOAc (4 μl of each fraction). yTBP and a DNA probe containing the Ad-MLP sequences were added to all lanes. Affinity-purified polyclonal antibodies against Bur6 (lane 7) or yTBP (lane 8) were added to the preformed yTBP–yDr1–Bur6–DNA complexes formed with the 0.5-M fraction. (*C* and *D*) Fractions from GST–yTBP affinity column were analyzed by SDS/PAGE followed by silver staining (*C*) and Western blot (*D*) analysis. The corresponding polypeptides are indicated on the right and molecular weight markers are indicated on the left of each panel. (*E*) The yDr1/Bur6-mediated repression of basal transcription *in vitro* can be overcome by TBP, but not by TFIIB or TFIIE. Lanes: 1–4, titration of yTBP with amounts indicated at the top; 5–7, titration of purified yDr1/Bur6 complex (phenyl-superose fraction) with amounts indicated at the top [estimated by quantitative Western blot analysis using the Imagemaster system (Pharmacia)]; 8–13, addition of increasing amounts (in pmol) of yTBP (lanes 8 and 9), rTFIIE (lanes 10 and 11), and rTFIIB (lanes 12 and 13) in the presence of highest amount of yDr1/Bur6 complex.

of Bur6 and eluted in the high molecular weight range. Two Dr1 populations have also been observed in human cells (27). In agreement with the studies of the mammalian factors, we found that the yeast Dr1/Bur6 complex interacted with TBP, as defined by retention on a yeast TBP affinity column. This immobilized Dr1/Bur6 complex was eluted with high salt washes as detected by silver staining (Fig. 3*C*) and Western blot analysis (Fig. 3*D*). Moreover, the yeast Dr1/Bur6 complex interacted with TBP when TBP was bound to the TATA motif as defined by gel mobility shift assays (Fig. 3*B*, lanes 1–6). The shifted DNA–protein complexes were dependent on TBP (lane 9) and contained TBP and Bur6, as antibodies against these proteins supershifted the complexes (lanes 7 and 8). The association of the Dr1/Bur6 complex with the TBP–TATA complex was functional, because the addition of different amounts of the yeast Dr1/Bur6 complex to a reconstituted transcription assay resulted in repression. Repression could be overcome by increasing the concentration of TBP, but not that of TFIIE or TFIIB (Fig. 3*E*), thereby demonstrating that repression was specific and mediated through TBP. We therefore conclude that the yeast Dr1/Bur6 complex functions in repression of transcription in a manner analogous to the mammalian complex.

Overexpression of the Yeast Dr1/Bur6 Complex in Yeast Results in Toxicity in a TBP-Dependent Manner *in Vivo*. Most yeast genes can be overexpressed without apparent growth defects (45). However, a global repressor of transcription is a likely candidate to impair growth when overexpressed. We therefore asked if overexpression of *YDR1/BUR6* would affect mRNA accumulation and impair cell growth. Overexpression of *YDR1* from the *GAL1* promoter resulted in diminished accumulation of poly(A) RNA when cells were grown in the presence of galactose, whereas no effect was observed when the same strain was grown in glucose medium (Fig. 4*A*). This was further exemplified by analyzing the steady-state levels of a specific transcript, in this case *ACT1*, a relatively stable mRNA in yeast (Fig. 4*B*) (46). Furthermore, overexpression of *YDR1* from the *GAL1* promoter was found to impair cell growth (Fig. 4*C*). In contrast, overexpression of *BUR6* from the *GAL1* promoter was without effect. Because repression of transcription by Dr1 is dependent upon interaction with TBP, we asked if growth inhibition associated with *YDR1* overexpression could be compensated by overexpression of *SPT15*, the gene encoding yTBP. Indeed, cell growth was restored when both *YDR1* and *SPT15* were overexpressed. These effects can be attributed specifically to overexpression of yDr1 and TBP, since neither vector controls, nor overexpression of TBP alone, conferred growth phenotypes (Fig. 4*C*). These *in vivo* results demonstrate that Dr1 functions as a repressor and targets TBP.

TBP is required for transcription initiation by all three RNA polymerases and Dr1-mediated repression is manifest through TBP. We therefore asked if overexpression of *YDR1* would also inhibit transcription by RNAPI and -III. Indeed, overexpression of *YDR1* from the *GAL* promoter resulted in diminished accumulation of tRNA when cells were grown in the presence of galactose, whereas no effect was observed when the same strain was grown in glucose medium (Fig. 4*A*). This effect was specific, as the accumulation of rRNA, transcribed by RNAPI, was unaffected. Thus, yeast Dr1 represses transcription by RNAPII and -III *in vivo*, a result consistent with the effects of human Dr1 *in vitro* (31).

DISCUSSION

Previous results demonstrated that the Dr1/DRAP1 (NC2) complex is a general repressor of transcription that targets TBP, thereby blocking formation of the transcription preinitiation complex (24–26). Our studies establish that the Dr1/DRAP1 complex is a global transcriptional repressor operating *in vivo*. Moreover, Dr1/DRAP1-mediated repression is a critical cellular function since both *YDR1* and *BUR6* genes, which encode the yeast homologues of human Dr1 and DRAP1, respectively, are essential for cell viability.

Several lines of evidence suggest that Dr1 alone confers a function independent of the corepressor DRAP1 (Bur6). First, Dr1 alone is capable of mediating repression of transcription *in vitro*, albeit less efficiently than the Dr1/DRAP1 complex (27). Second, DRAP1 is undetectable in actively dividing cells, but is present at higher levels in differentiated cells with a low mitotic index (27). Third, human Dr1 can functionally replace *YDR1 in vivo* (Fig. 1*C*), yet human DRAP1 does not interact with yeast Dr1, either *in vivo* or *in vitro* (Fig. 2 *B* and *C*).

Transcriptional repression has emerged as an important regulatory mechanism for controlling gene expression. Several mechanisms have been described to account for transcriptional repression (for review see ref. 15). The Dr1/DRAP1 repressor is unique in that it directly targets TBP. Although similar to the Mot1 repressor in targeting the general machinery, there are clear

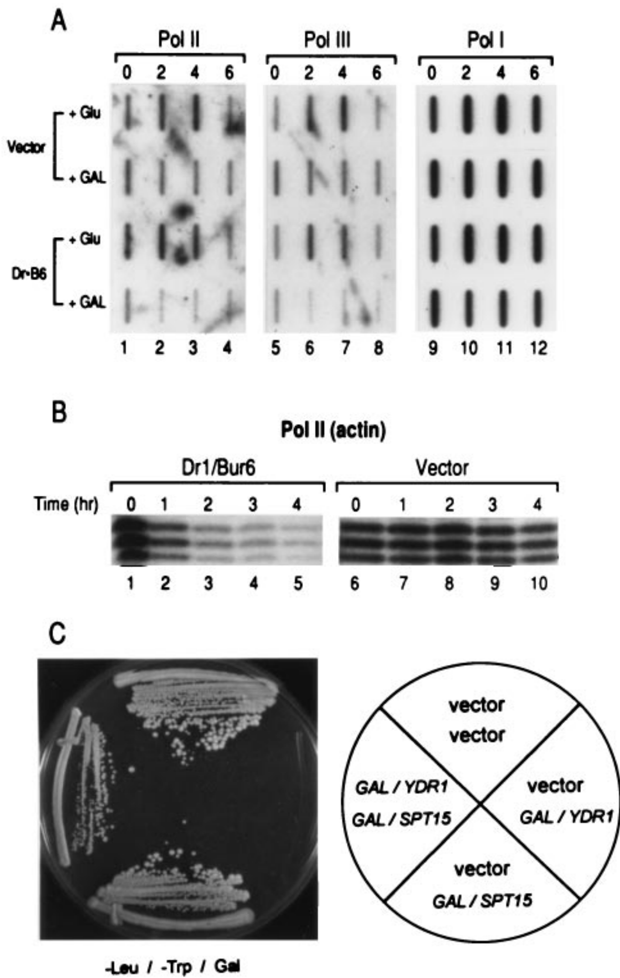

FIG. 4. Effects of yDr1 overexpression *in vivo*. (*A* and *B*) mRNA levels decline by overexpression of yDr1 and Bur6. RNA samples were prepared from galactose-induced (Gal) or uninduced (Glu) cultures of the indicated yeast strains, which contain either the *YDR1* and *BUR6* genes (Dr·B6) or vector controls. Each lane corresponds to the time points (in hours) at which aliquots of cells were taken from the culture following galactose induction. (*A*) Amounts of Pol II, Pol III, and Pol I transcript were determined by probing RNA samples obtained at various time points with labeled oligo(dT) (lanes 1–4), $tRNA^{W}$ (lanes 5–8), or rRNA (lanes 9–12). Row 1, uninduced culture of the control strain containing vectors only; row 2, induced culture of the strain containing vectors only; row 3, uninduced culture of the strain containing *YDR1* and *BUR6* constructs; row 4, induced culture of strain containing *YDR1* and *BUR6* constructs. Under these conditions Dr1 was overexpressed $\approx$5-fold with respect to the wild-type strain, as determine using quantitative Western blots. (*B*) Equivalent amounts of RNA were hybridized with ^{32}P-labeled oligonucleotide complementary to *ACT1* mRNA. After digestion with S1 nuclease, samples were subjected to denaturing PAGE. Lanes: 1–5, RNA samples from induced culture of the strain containing *YDR1* and *BUR6* constructs; 6–10, RNA samples from induced culture of the strain containing vectors only. (*C*) Toxicity of *YDR1* overexpression is rescued by overexpression of *SPT15* (TBP). Elevated expression of *YDR1* from the *GAL* promoter in the presence of galactose as the sole carbon source impaired cell growth (vector, *GAL/YDR1*). This effect was rescued, resulting in near normal growth, by elevated expression of *SPT15* from the *GAL* promoter (*GAL/YDR1*, *GAL/SPT15*). There is no growth phenotype associated with the presence of either vectors (vector, vector) or *SPT15* alone (vector, *GAL/SPT15*) under inducing conditions. We wish to note that the toxicity associated with *YDR1* overexpression was variable, ranging from minor effects on cell growth to nearly complete inhibition.

mechanistic distinctions. Whereas Mot1 promotes the ATP-dependent displacement of TBP from the promoter, Dr1/DRAP1 specifically targets TBP, either preventing the subsequent binding of TFIIB or displacing TFIIB from the TATA–TBP–TFIIB complex (23, 29). Interestingly, Dr1/DRAP1 represses transcription initiation by both RNAPII and -III, but not by RNAPI (Fig. 4*A*) (31), even though all three polymerases are TBP dependent. It is important to determine how class I promoters evade repression by Dr1/DRAP1 and what the regulatory significance of this effect might be. The isolation of the yeast genes encoding Dr1 and Bur6 provides the means to define the precise function of Dr1 and DRAP1 (BUR6) *in vivo*.

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