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Saccharomyces cerevisiae BUR6 Encodes a DRAP1/NC2α Homolog That Has both Positive and Negative Roles in Transcription In Vivo

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***BUR3* and *BUR6* were identified previously by selecting for mutations that increase transcription from an upstream activating sequence (UAS)-less promoter in *Saccharomyces cerevisiae*. The *bur3-1* and *bur6-1* mutations are recessive, increase transcription from a *suc2*Δ*uas* allele, and cause other mutant phenotypes, suggesting that Bur3p and Bur6p function as general repressors of the basal transcriptional machinery. The molecular cloning and characterization of *BUR3* and *BUR6* are presented here. *BUR3* is identical to *MOT1*, a previously characterized essential gene that encodes an ATP-dependent inhibitor of the TATA box-binding protein. Cloning and nucleotide sequence analysis reveals that *BUR6* encodes a homolog of DRAP1 (also called NC2α), a mammalian repressor of basal transcription. Strains that contain a *bur6* null allele are viable but grow extremely poorly, demonstrating that *BUR6* is critical for normal cell growth in yeast. The Bur6p histone fold domain is required for function; an extensive nonoverlapping set of deletion alleles throughout the histone fold domain impairs *BUR6* function in vivo, whereas mutations in the amino- and carboxy-terminal tails have no detectable effect. *BUR6* and *BUR3*/*MOT1* have different functions depending on promoter context: although the *bur3-1* and *bur6-1* mutations increase transcription from Δ*uas* promoters, they result in reduced transcription from the wild-type *GAL1* and *GAL10* promoters. This transcriptional defect is due to the inability of the *GAL10* UAS to function in *bur6-1* strains. The similar phenotypes of *bur6* and *bur3* (*mot1*) mutations suggest that Bur6p and Mot1p have related, but not identical, functions in modulating the activity of the general transcription machinery in vivo.**

Basal transcription of most, if not all, protein-encoding genes requires the activity of RNA polymerase II and the general transcription factors (GTFs) TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (66). Highly purified preparations of RNA polymerase II and the GTFs are both necessary and sufficient to direct promoter-specific basal transcription at the correct initiation sites on a number of model templates in vitro. The levels of transcription that are established by this basal machinery are then regulated either positively or negatively in a promoter-specific manner mediated through *cis*-acting regulatory elements. The mechanism by which regulatory factors influence the activities of the GTFs remains an area of intense interest, because transcription initiation is usually the major regulatory step in differential gene expression and thus performs critical roles in normal cell growth and in the development and differentiation of multicellular organisms.

Regulators of the general transcription machinery can be grouped into two main classes. The best-characterized class of regulators contains the site-specific DNA-binding proteins that recognize *cis*-acting regulatory elements known as upstream activating sequences (UASs) in *Saccharomyces cerevisiae* or enhancers in larger eukaryotes (40, 55). A second class of transcriptional regulators affects the general transcription machinery in a UAS-independent manner. UAS-less promoters are transcribed by RNA polymerase II and the GTFs in vitro yet are often transcribed very poorly, if at all, in vivo. The identification of recessive mutations that increase transcription from UAS-less promoters in the yeast *S. cerevisiae* (7, 11, 13, 28, 36, 43, 47) indicates that basal promoter elements are capable of higher levels of transcription in vivo but are normally repressed in wild-type strains. A number of recent studies have begun to identify at least two classes of proteins that repress the GTFs by distinctly different mechanisms in vivo.

One class of general transcriptional repression activity in vivo is due to the assembly of the DNA template into nucleosomes (20). Nucleosomes inhibit the recognition of regulatory elements by some UAS-binding proteins (45, 58), thereby reducing activated transcription, but nucleosomes can also affect basal transcription by inhibiting the function of GTFs (33, 64). For example, assembly of the adenovirus major late promoter into nucleosomes represses transcription in vitro but not if the template is incubated with TFIID prior to assembly (64). This result suggests that nucleosomes inhibit binding of TFIID to the TATA box. Recent studies utilizing purified recombinant TATA-binding protein (TBP) demonstrated that nucleosomes directly inhibit binding of TBP, confirming and extending the results obtained with the crude TFIID fraction (24). The inhibitory effects of nucleosomes on basal transcription have also been demonstrated in vivo; depletion of nucleosomes in yeast results in increased constitutive expression from the *PHO5*, *CYC1*, *GAL1*, *CUP1*, and *HIS3* basal promoters fused to *lacZ* (15, 21). Furthermore, mutations in the genes encoding histones H2A and H2B alter transcription from promoters that contain insertions of Ty elements (Spt^- phenotype) and increase transcription from a *suc2*Δ*uas* promoter (9, 22). Nucleosomal repression is likely to require more factors than just the core histones, and genetic selections in yeast have identified additional factors that are likely to inhibit transcription through chromatin effects (8, 48, 54, 62).

Another class of repressors directly inhibits the activity of the GTFs in a chromatin-independent manner. Proteins such

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as Mot1, Dr1 (also called NC2), and NC1 (2, 25, 37) were all identified as having biochemical activities that inhibit the function of the TBP subunit of TFIID in vitro. Mot1p displaces TBP from the TATA box in an ATP-dependent manner (2), while NC1 and Dr1/NC2 allow TBP to bind DNA but inhibit subsequent interactions between TBP and TFIIA and/or TFIIB (25, 32). The in vivo role of each of these TBP inhibitors is not yet clear, but the phenotype of *mot1* mutations is consistent with its proposed repressor activity, since *mot1* mutations cause increased transcription from ΔUAS promoters in vivo (13). An important issue raised by these studies is whether analogous activities that specifically inhibit other components of the basal transcription machinery exist.

We have used a genetic selection designed to identify mutations that affect basal transcription in *S. cerevisiae* (47), with the expectation that such mutations would identify additional chromatin-dependent and chromatin-independent repressors of the basal machinery. The promoter used for this selection was the *suc2*Δ*uas*(*-1900*/*-390*) allele (51), which produces barely detectable levels of transcripts, thus causing an inability to grow on media that contain sucrose as the carbon source (Suc^- phenotype). A selection for Suc^+ suppressors of *suc2*Δ*uas*(*-1900*/*-390*) identified many mutations in previously characterized *SPT* genes, including those that encode histones H2A and H2B. However, six other genes were also identified by this selection and were designated *BUR1* through *BUR6* (for bypass UAS requirement) (47). In addition to suppressing *suc2*Δ*uas*, each of the *bur* mutations causes other mutant phenotypes, indicating that they have more general effects and also that they are likely to affect expression from some wild-type promoters. Further evidence that the *BUR* gene products have important general roles in transcription is provided by the ability of some *bur* mutations to suppress transcriptional defects caused by an *snf5* mutation (47). Snf5p is a component of the highly conserved SNF-SWI complex, which is required for transcription at many diversely regulated promoters in vivo (42, 44). Genetic and biochemical evidence indicates that the SNF-SWI complex helps TBP and site-specific activators bind to nucleosomal DNA (12, 23, 24, 34). Based on their distinct sets of unselected mutant phenotypes, including the ability to suppress *snf5* mutations, it was proposed (47) that the *bur* mutations comprise two groups that suppress *suc2*Δ*uas* by different mechanisms. One group contains *BUR1*, *BUR2*, *BUR4*, and *BUR5*, while the other group consists of *BUR3* and *BUR6*. Since *BUR5* encodes histone H3 (47), the first group of *BUR* genes was proposed to suppress *suc2*Δ*uas* via nucleosomal repression while the second group was proposed to suppress *suc2*Δ*uas* by a nucleosome-independent mechanism, perhaps by directly repressing the basal transcription factors. The results presented here support this hypothesis: *BUR3* is identical to *MOT1* (13), which encodes an inhibitor of the TBP, while *BUR6* encodes a histone fold-containing protein with significant sequence similarity to the mammalian repressor DRAP1 (also called NC2α). Surprisingly, analysis of transcriptional defects in *bur3-1* and *bur6-1* mutant strains suggests that *MOT1* and *BUR6* may also have positive roles at some promoters in vivo.

MATERIALS AND METHODS

Media and genetic methods. *S. cerevisiae* strains used in this study are shown in Table 1. All strains used in this study were derived from FY2 (*MAT*α *ura3-52*), a *GAL2*$^+$ derivative of S288C (63). All media used, including rich medium (YPD), synthetic complete drop-put medium (for example, SC-Ura), minimal medium (SD), and sporulation medium, were made as described previously (50). YPSuc plates contained YEP, 2% sucrose, and 1 μg of antimycin A (Sigma) per ml. YPGal plates contained YEP, 2% galactose, and 1 μg of antimycin A per ml. Standard genetic methods for mating, sporulation, and tetrad analysis (50) were used throughout this study. Yeast cells were transformed by the lithium acetate method (27).

TABLE 1. *S. cerevisiae* strains

Strain	Genotype
GY139	*MAT***a**/α *his4-912*δ/*his4-912*δ *lys2-128*δ/*lys2-128*δ *suc2*Δ*uas*(*-1900*/*-390*)/*suc2*Δ*uas*(*-1900*/*-390*) *ura3-52*/*ura3-52 trp1*Δ*63*/*trp1*Δ*63 LEU2*$^+$/*leu2*Δ*1*
GY215	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 trp1*Δ*63 bur6-1*
GY218	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 ade8 bur6-1*
GY236	*MAT*α *his4-912*δ *lys2-128*δ *ura3-52 leu2*Δ*1 mot1-301*
GY315	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 trp1*Δ*63*
GY319	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 ade8*
GY561	*MAT***a** *his4-912*δ *lys2-128*δ *suc2*Δ*uas*(*-1900*/*-390*) *ura3-52 leu2*Δ*1 bur6-1*
GY562	*MAT***a**/α *his4-912*δ/*his4-912*δ *lys2-128*δ/*lys2-128*δ *suc2*Δ*uas*(*-1900*/*-390*)/*suc2*Δ*uas*(*-1900*/*-390*) *ura3-52*/*ura3-52 trp1*Δ*63*/*trp1*Δ*63 LEU2*$^+$/*leu2*Δ*1 BUR6*$^+$/*bur6*Δ*2*::*TRP1*
GY568	*MAT***a** *his4-912*δ *lys2-128*δ *suc2*Δ*uas*(*-1900*/*-390*) *ura3-52 trp1*Δ*63 leu2*Δ*1 bur6*Δ*2*::*TRP1* (*CEN URA3 BUR6*$^+$)

Plasmids. pGP256, the original *BUR6*$^+$ plasmid that was isolated from a YCp50-based yeast genomic DNA library (49), contains a 20,007-bp *Sau*3A insert at the *Bam*HI site of YCp50. A 2,393-bp *Bgl*II fragment that contains *BUR6* was subcloned from pGP256 into the *Bam*HI site of pRS416 to create pGP258. To construct a *bur6* null allele, an 820-bp *Eco*RI-*Stu*I fragment of *TRP1* was used to replace the *Nde*I-*Kpn*I fragment of *BUR6*, generating the *bur6*Δ*2*::*TRP1* null allele in pGP267. To integrate this null allele at the *BUR6* genomic locus, a 2.4-kb *Bgl*II fragment of pGP267 containing *bur6*Δ*2*::*TRP1* was transformed into the diploid strain GY139 and Trp^+ transformants were selected. Integration at the *BUR6* locus was confirmed by Southern blotting. The same 2.4-kb *Bgl*II fragment was also cloned into the *Bam*HI site of pRS416 to create pGP273. Plasmids containing the *ho*Δ*uas-lacZ* reporter (p740), *HO-lacZ* (p1701), and the *cyc1*Δ*uas-lacZ* reporter (pLGΔ−178) were obtained from David Stillman, while the $GAL10_{UAS}$-*CYC1p-lacZ* (pLGSD5) and $GAL4_{BS}$-*CYC1p-lacZ* (pSV14) plasmids were obtained from Karen Arndt.

RNA analysis. Cells were grown to 1×10^7 to 2×10^7 cells per ml, and RNA was isolated as described previously (6). RNA was separated in a 1% formaldehyde agarose gel. Blotting and hybridization to DNA probes were performed as described previously (56). The probes used were pDE32-1 (*SPT15*) and an ~1.8-kb *Eco*RI fragment from p4812 (*GAL1* and *GAL10*). RNA was cross-linked to GeneScreen (New England Nuclear) with the auto cross-link mode in a Stratalinker 1800 (Stratagene). Probes were radiolabeled with ^{32}P with a random priming kit from Boehringer Mannheim according to the manufacturer's directions.

β-Galactosidase assays. Five-milliliter yeast cultures were grown to a density of 1×10^7 to 2×10^7 cells per ml in SC-Ura medium to select for reporter plasmids. Cells were harvested by centrifugation and were resuspended in 250 μl of breaking buffer (0.1 M Tris [pH 8.0], 20% glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). Cells were disrupted by vortexing six times for 1 min at 4°C in the presence of an equal volume of glass beads. More breaking buffer (250 μl) was added, the tubes were vortexed again briefly, and the liquid was transferred to a 1.5-ml tube and centrifuged for 15 min at 4°C to remove cellular debris. The supernatants were assayed for β-galactosidase activity as described previously (39). Protein levels were determined by the method of Bradford (5a) with bovine serum albumin as the protein standard. All reported β-galactosidase levels are the means from at least three independent transformants with standard errors of <20%.

Construction of *bur6* deletion alleles. Deletion alleles of *bur6* were created by oligonucleotide-directed mutagenesis of plasmid pGP258. pGP258 contains *BUR6* on a 2.4-kb *Bgl*II fragment cloned into the *Bam*HI site of pRS416. pGP258 was transformed into the *dut1 ung1* mutant *Escherichia coli* strain RZ1032, and single-stranded DNA was prepared after infection with the M13 helper phage K07. The single-stranded DNA was mutagenized with a Mut-a-gene kit from Bio-Rad Laboratories according to the manufacturer's directions. All deletion mutations were verified by DNA sequencing of the entire *BUR6* open reading frame.

Western analysis. Cells were grown to mid-log phase in 10 ml of dropout medium, harvested, and lysed by vortexing in the presence of glass beads in a buffer that contained 50 mM Tris (pH 7.5), 150 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol, 200 μg of aprotinin per ml, 100 μg of peptstatin A per ml, and 50 μg of leupeptin per ml. Debris from cell lysates was pelleted for 15 min at 16,000 × *g*. Twenty micrograms of protein from each extract was separated in a sodium dodecyl sulfate–15% polyacrylamide gel. Proteins were transferred to Immobilon P (Mil-

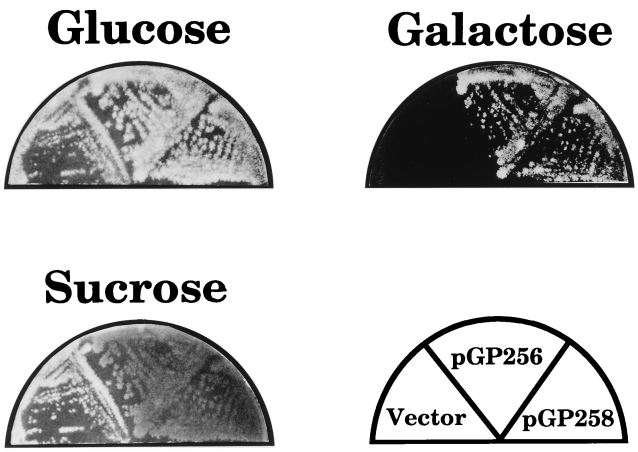

FIG. 1. Complementation of *bur6-1* phenotypes by *BUR6*$^+$ clones. Yeast strain GY561 (relevant genotype, *bur6-1 suc2Δuas ura3-52*) was transformed with pRS416 (the CEN vector), pGP256 (the original CEN *BUR6*$^+$ plasmid), or pGP258 (a CEN *BUR6*$^+$ subclone; see Fig. 2 for insert details), and transformants were selected on SC-Ura plates. A single transformant with each plasmid was restreaked onto an SC-Ura plate and then was replica plated onto SC-Ura (glucose), SC-Ura plus galactose plus antimycin A (galactose), and SC-Ura plus sucrose plus antimycin A (sucrose).

lipore), and the filter was probed with an anti-Bur6 antibody (gift of E. Gadbois and R. Young). Primary antibody was washed with phosphate-buffered saline plus 0.1% Tween 20, and the filter was then probed with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (Amersham). Final detection was done with an enhanced chemiluminescence kit (Amersham) according to the manufacturer's instructions.

RESULTS

***BUR3* is identical to *MOT1*.** The Bur selection identified mutations in six genes that appear to comprise two phenotypic classes. One class consists of *BUR1*, *BUR2*, *BUR4*, and *BUR5*, while the other class consists of *BUR3* and *BUR6*. Mutations in *BUR3* and *BUR6* are similar in that they are the only *bur* mutations that cause Gal$^-$ and weak Spt$^-$ phenotypes and are also unable to suppress an *snf5*Δ allele. Before attempting to clone *BUR3* by standard plasmid complementation of the recessive *bur3-1* allele, we first tested whether *BUR3* might be identical to any previously cloned genes that cause similar mutant phenotypes. The *bur3-1* mutation was not complemented by plasmids that contained *SIN3*, *SIN4*, *GAL11*, *RPB1*, *RPB2*, or any of the previously characterized *SPT* genes. In contrast, a *MOT1* CEN plasmid complemented all the phenotypes of *bur3-1*, including the Bur$^-$, Ts$^-$, Gal$^-$, and slow-growth phenotypes. To determine whether *bur3-1* was actually a mutation of *MOT1*, complementation and linkage tests were performed. The *bur3-1* and *mot1-1* mutations were unable to complement each other in heterozygous diploids and were tightly linked, displaying no recombination in 20 four-spored tetrads. *BUR3* is therefore identical to *MOT1*, an essential gene previously shown to encode an inhibitor of TBP (3). The *bur3-1* allele will hereinafter be referred to as *mot1-301*.

Molecular cloning and sequence analysis of *BUR6*. The finding that *BUR3* is identical to *MOT1* prompted further characterization of *BUR6* at the molecular level, since the *bur6-1* and *mot1-301* mutations cause similar mutant phenotypes. The *BUR6* gene was cloned by transforming a *bur6-1* strain with a yeast genomic DNA library in a centromeric vector (49) and by screening for plasmids that complemented the *bur6-1* Gal$^-$ phenotype. A single plasmid was obtained and designated pGP256. In addition to complementing the Gal$^-$ phenotype of *bur6-1*, pGP256 also complemented the Bur$^-$ and slow-growth phenotypes (Fig. 1).

To identify the location of *BUR6* within the plasmid insert, the chromosomal region contained in pGP256 was characterized by sequencing the ends of the pGP256 insert. A comparison of this nucleotide sequence with sequences in the GenBank database revealed that pGP256 contained portions of the *SPT2* and *BEM2* open reading frames on either end. The nucleotide sequence of the *SPT2-BEM2* region of chromosome V was kindly provided by F. Dietrich, revealing that in addition to the amino terminus of *BEM2* and the carboxy terminus of *SPT2*, pGP256 also contained a Ty element, a *tRNA*^{Arg} gene, and five previously unidentified open reading frames (Fig. 2). A 2.4-kb subclone (pGP258) that contained only one open reading frame and the *tRNA*^{Arg} gene was able to fully complement all of the phenotypes caused by *bur6-1*, indicating that *BUR6* was contained within that 2.4-kb fragment (Fig. 1). To determine whether complementation was due to the open reading frame or the *tRNA*^{Arg} gene, a deletion allele that re-

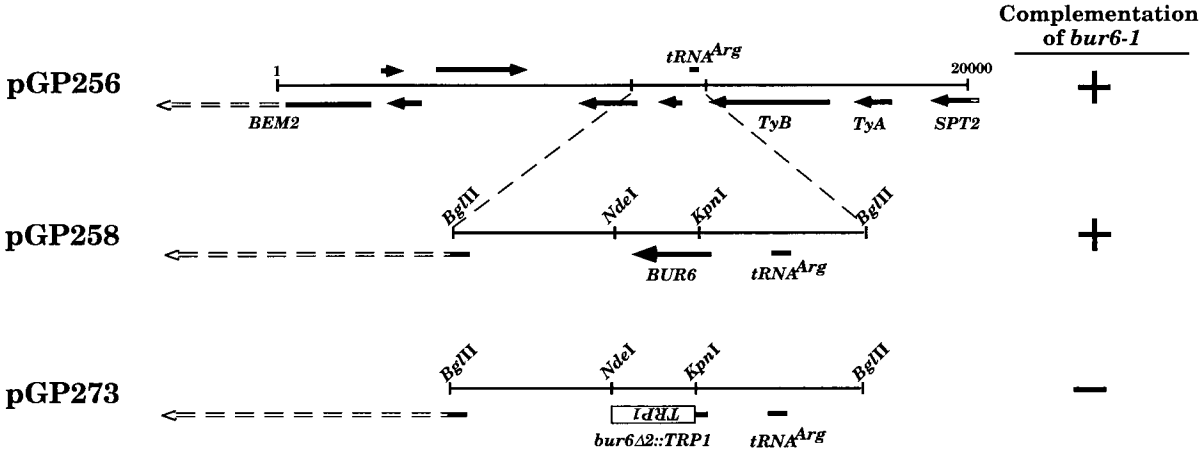

FIG. 2. Identification of the *BUR6* open reading frame. Plasmid pGP256 is the original *BUR6*$^+$ complementing plasmid isolated from a yeast genomic CEN library. The locations of open reading frames on the 20-kb insert are shown as filled arrows, with the approximate extents of the interrupted *SPT2* and *BEM2* open reading frames indicated by broken open arrows. The location of the *tRNA*^{Arg} gene is shown as a filled box. pGP258 contains a 2.4-kb subclone from pGP256 that encodes only the *BUR6* open reading frame and the *tRNA*^{Arg} gene. pGP273 is identical to pGP258, except that most of the *BUR6* open reading frame has been replaced by the *TRP1* gene. The ability of each plasmid to complement *bur6-1* is depicted to the right.

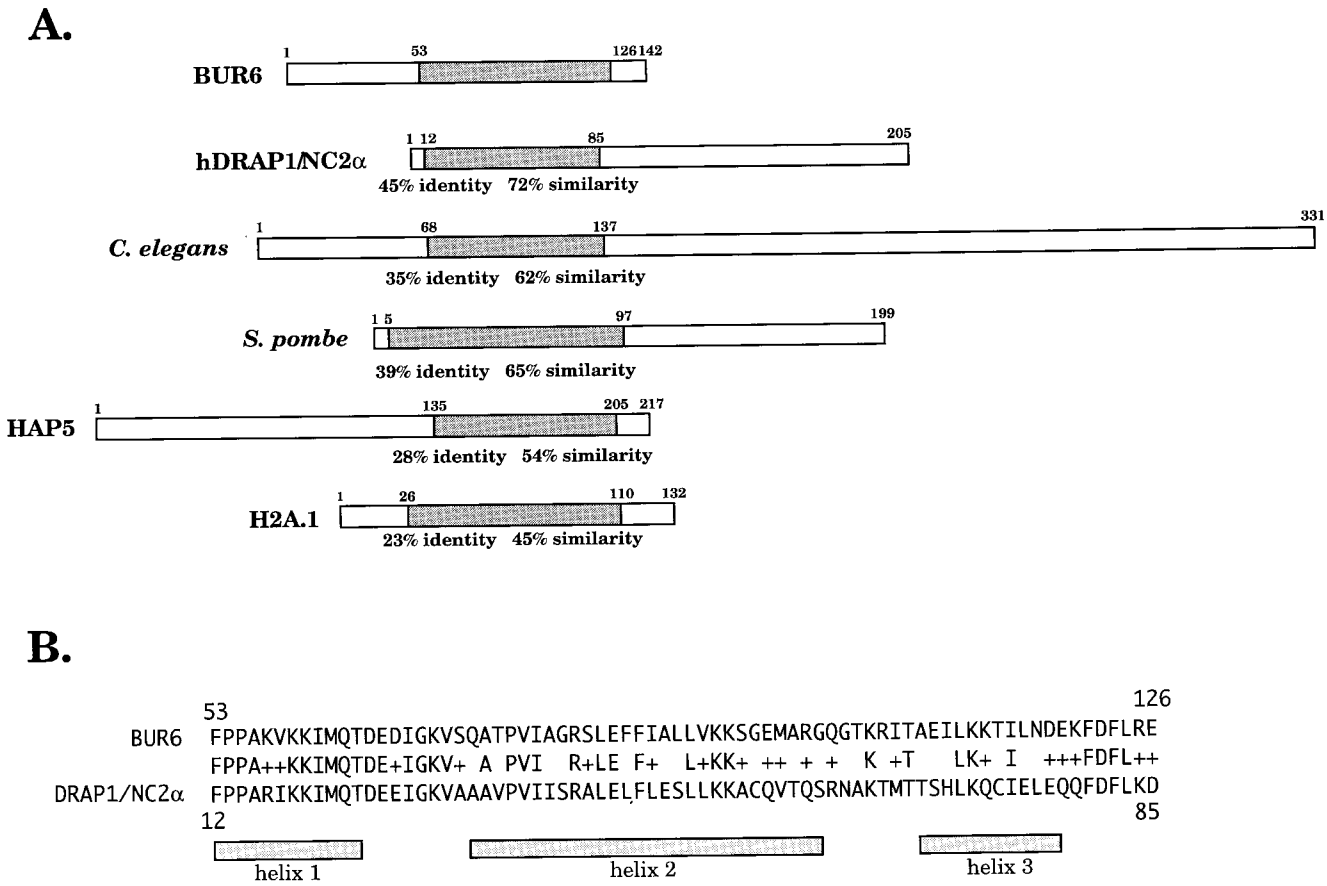

FIG. 3. Similarities between Bur6p and selected histone fold proteins. (A) The *BUR6* coding region is aligned with the five histone fold proteins that gave the highest similarity scores from a BLAST search of sequences in the current GenBank database. The region of similarity is shaded for each protein, and the percentages of amino acid identity and similarity are shown below the shaded histone fold domains. Amino acid residues are numbered above each protein. The accession numbers for the indicated sequences are as follows: *BUR6*, U32274; hDRAP1, U41843; a *Caenorhabditis elegans* open reading frame, Z70753; a *Schizosaccharomyces pombe* open reading frame, Z69795; *HAP5*, U19932; and *S. cerevisiae HTA1*, P04911. (B) Alignment of similar amino acids between human DRAP1/NC2α and Bur6p. The proposed locations of the helices that comprise the histone fold are shown below the alignment.

placed nearly all of the open reading frame with *TRP1*, yet left the *tRNA*^{Arg} gene intact, was created. This *bur6Δ2*::*TRP1* allele was unable to complement *bur6-1* when it was present on a CEN plasmid, indicating that this short open reading frame is responsible for the plasmid-borne complementation activity. Linkage analysis confirmed that pGP258 contains authentic *BUR6* and not a dosage-dependent suppressor of *bur6-1* (data not shown).

A BLAST comparison of the *BUR6* nucleotide sequence with sequences in the current GenBank database revealed that *BUR6* encodes a 142-amino-acid protein with significant sequence similarity to histone H2A and other members of the histone fold family of proteins (Fig. 3). The histone fold is a structural motif of approximately 65 amino acids in length originally identified from crystallographic analysis of the histone octamer (1), but this domain has since been predicted (4) and demonstrated (65) to occur in a number of nonhistone proteins. The histone fold constitutes an interface for specific and direct interactions between two histone fold proteins. For example, the histone fold domains are responsible for extensive direct interactions between histones H2A and H2B, between histones H3 and H4 (1), between HAP3/CBF-A and HAP5/CBF-C (53), and between *Drosophila* $TAF_{II}42$ ($dTAF_{II}42$) and $dTAF_{II}62$ (65). The score showing highest similarity to *BUR6* was that of DRAP1/NC2α, a human protein recently identified as a transcriptional repressor that inhibits interactions between TBP and the GTFs TFIIA and/or TFIIB in vitro (19, 38). Inhibition of TBP by DRAP1/NC2α occurs through a heterodimeric complex of DRAP1/NC2α and Dr1/NC2β. The similarity between Bur6p and DRAP1/NC2α is highly significant (45% identity and 72% similarity over 79 amino acids) and extends slightly beyond the carboxy-terminal end of the histone fold domain. Deletion analysis (described below) demonstrates the importance of the DRAP1/NC2α-homologous domain for Bur6p function in vivo. Furthermore, recent biochemical studies (17, 18, 31) indicate that Bur6p directly interacts with a yeast Dr1 homolog and that the Bur6p-yDr1 complex represses transcription in vitro. Bur6p thus encodes a yeast homolog of DRAP1/NC2α.

***bur6* null phenotype.** To determine the effects of a complete loss of *BUR6* function, a *bur6* null allele was constructed and integrated into the genome of a diploid yeast strain, replacing one of the wild-type *BUR6* alleles. When the heterozygous *BUR6*$^{+}$/*bur6Δ2*::*TRP1* diploids were sporulated and the tetrads were dissected, the resulting tetrads produced two healthy colonies and two microcolonies that were easily visible with the naked eye only after 7 to 10 days of growth (Fig. 4). Although the *bur6Δ2*::*TRP1* cells were viable, their remarkably poor growth and relatively high frequency of reversion prohibited further analysis of the *bur6Δ* strains. The growth defect was

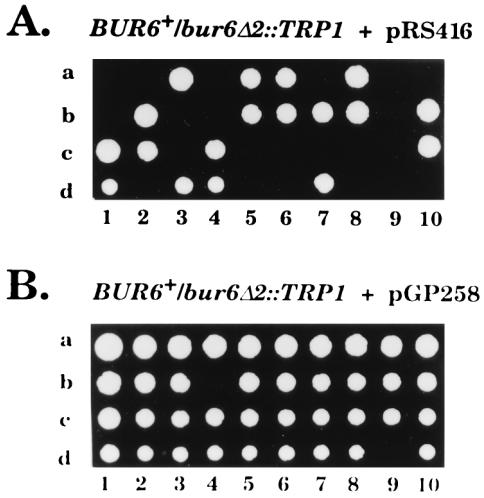

FIG. 4. *BUR6* is essential for normal cell growth. (A) Yeast strain GY562 (*BUR6*/*bur6Δ2*::*TRP1*) was transformed with a *URA3* vector and sporulated, and 10 tetrads were dissected. Colonies derived from the individual spores are labeled a to d. The dissection plate was photographed on day 3, when *bur6Δ* colonies were visible only with the aid of a microscope. Identical results were obtained when GY562 was dissected in the absence of the vector. (B) The same strain (GY562) was transformed with pGP258 (CEN *BUR6*$^+$) prior to sporulation, and tetrads were dissected and photographed as described for panel A.

directly attributable to *bur6Δ2*::*TRP1*, since poor growth and the Trp$^+$ phenotype cosegregated precisely in more than 100 tetrads. Furthermore, the growth defect can be rescued by transforming the heterozygous diploid strain with a *BUR6*$^+$ *URA3* plasmid prior to sporulation; after tetrad dissection, viable and healthy *bur6Δ2*::*TRP1* colonies were obtained (Fig. 4) and all the Trp$^+$ colonies were also Ura$^+$, indicating that the poor growth of the *bur6Δ2*::*TRP1* strain is rescued by a *BUR6*$^+$ plasmid. Continued viability was dependent upon the *URA3* *BUR6*$^+$ plasmid, since all Trp$^+$ colonies were 5-fluoroorotic acid (5-FOA) sensitive. Combined, these results indicate that although *BUR6* function is not absolutely required for viability, it is clearly critical for normal cell growth.

***BUR6* deletion analysis.** To determine whether the histone fold similarity was functionally significant and to delimit the regions of *BUR6* that are required for function in vivo, an extensive series of nonoverlapping *bur6* deletion alleles was constructed and tested for function in vivo (Fig. 5A). *BUR6* function was assayed in two ways, first, by plasmid complementation of the slow-growth, Bur$^-$, and Gal$^-$ phenotypes of the chromosomal *bur6-1* mutation and second, by testing the phenotypes of the plasmid-borne *bur6* deletion alleles in a chromosomal *bur6* null background. The results from the deletion analysis are striking; five different alleles that each have 10 amino acids deleted between residues 51 and 100 are completely nonfunctional for *BUR6* function by these assays; they do not complement *bur6-1* and are inviable in a *bur6* null strain. Four other alleles have partial complementing activity; *Δ41-50*, in which residues directly amino terminal to the histone fold domain are deleted, and three alleles that, combined, have amino acids 101 through 130 deleted each show reduced complementation of *bur6-1* and also have Bur$^-$ and Gal$^-$ phenotypes in a chromosomal *bur6* null strain. In contrast, all other deletions within the amino-terminal and carboxy-terminal tails of *BUR6* have no detectable effect on *BUR6* function. The region of *BUR6* that is absolutely essential for function (amino acids 50 through 100) thus corresponds well to the most conserved part of the histone fold, where amino acids 53 to 93 of Bur6p are 66% identical and 83% similar to human DRAP1 (Fig. 3). To determine whether redundancy within the amino-terminal tail accounts for the lack of phenotype by the N-terminal deletions, the entire N-terminal tail from residues 2 through 40 was deleted, with no apparent effect on *BUR6* function. Western analysis (Fig. 5B) indicated that the nonfunctional alleles produce detectable amounts of the mutant proteins. In fact, the nonfunctional deletion alleles generally produced higher levels of Bur6 protein than functional deletion derivatives, suggesting that *BUR6* may be regulated by a negative feedback loop. These results demonstrate the importance of the *BUR6* histone fold domain in vivo and suggest that this domain may also be sufficient for function, since the amino- and carboxy-terminal tails of *BUR6* appear to be dispensable by these in vivo assays.

***bur6-1* increases expression of other ΔUAS reporter genes.** Because *bur6-1* increased transcription from the *suc2Δuas* promoter and caused other mutant phenotypes, it was proposed (47) that Bur6p might function as a repressor of the general transcription machinery. If this is true, then *bur6-1* might be expected to increase expression from promoters other than *suc2Δuas*, as has previously been shown for the *mot1-1* mutation (13). To test this prediction, the effects of the *bur6-1* mutation on expression from the *cyc1Δuas* and the *hoΔuas* core

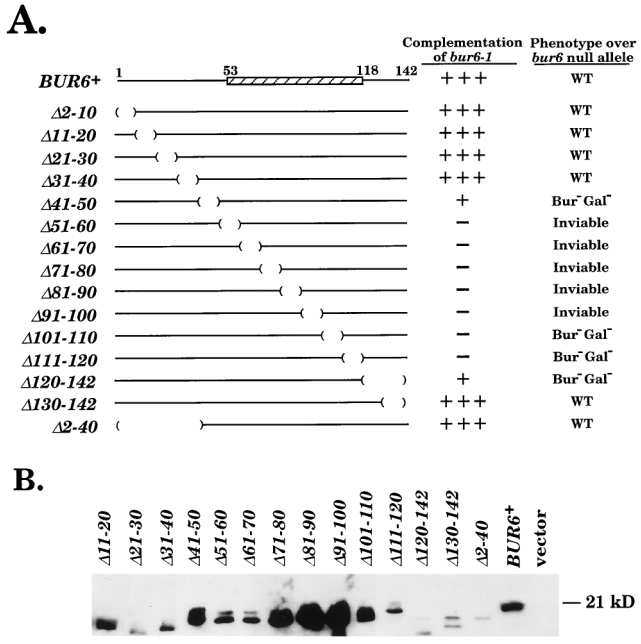

FIG. 5. *BUR6* deletion analysis. *BUR6* deletion alleles were constructed by oligonucleotide-directed mutagenesis by deleting the amino acid residues listed on the left. The regions deleted in each allele are also represented schematically by the parentheses to show alignment with the wild-type *BUR6* histone fold domain. The wild-type (WT) *BUR6* coding region is shown at the top, with the histone fold domain, extending approximately from amino acids 53 to 118, being depicted by the hatched box. (A) CEN plasmids containing the deletion alleles were transformed into GY561 (*bur6-1*) and were examined for complementation of the *bur6-1* Bur$^-$, Gal$^-$, and slow-growth phenotypes. +++ indicates complete complementation, + indicates weak complementation, and − indicates lack of complementation. *LEU2* CEN plasmids containing the deletion alleles were also transformed into GY568 (*bur6Δ2*::*TRP1* [*CEN BUR6*$^+$ *URA3*]) and streaked onto 5-FOA plates to assess the plasmid phenotypes in a *bur6Δ* background (right column). (B) Western analysis of *bur6* deletion alleles. CEN plasmids containing the deletion alleles shown above the lanes were transformed into GY561 (*bur6-1*), and extracts prepared from these strains were probed with polyclonal anti-Bur6 antibody (a gift of E. Gadbois and R. Young). The position of the 21-kDa molecular mass marker is shown on the right.

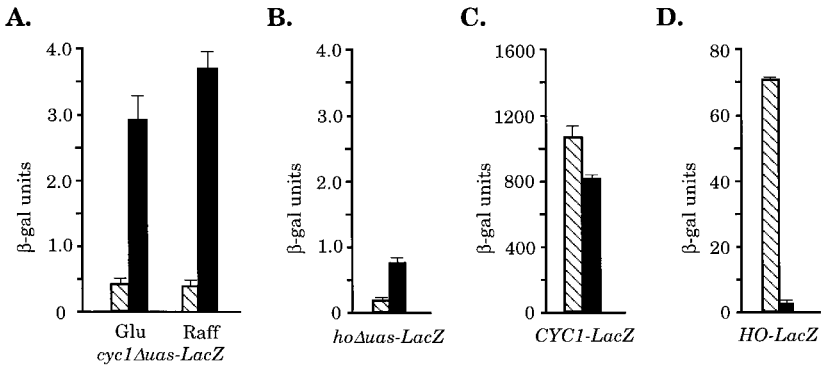

FIG. 6. *bur6-1* causes increased expression from two other basal promoters. (A) Yeast strains GY218 (*bur6-1*) and GY319 (*BUR6*$^+$) were transformed with a *cyc1Δuas* plasmid (pLGΔ178). Individual transformants were grown in either SC-Ura plus raffinose (Raff) or SC-Ura plus glucose (Glu). Extracts were prepared, and β-galactosidase (β-gal) activities were quantitated as described previously (39). Levels from GY218 (*bur6-1*) are represented by filled boxes, while levels from GY319 (*BUR6*$^+$) are represented by hatched boxes. (B) GY218 (*bur6-1*) and GY319 (*BUR6*$^+$) were transformed with an *hoΔuas* plasmid (M740), grown in SC-Ura medium, and assayed for β-galactosidase activity as described for panel A. (C) GY215 (*bur6-1*) and GY315 (*BUR6*$^+$) were transformed with a *CYC1-lacZ* plasmid (pLG669Z), grown in SC-Ura plus raffinose medium, and assayed for β-galactosidase activity as described for panel A. (D) GY215 (*bur6-1*) and GY315 (*BUR6*$^+$) were transformed with an *HO-lacZ* plasmid (p1701), grown in SC-Ura medium, and assayed for β-galactosidase activity as described for panel A. β-Galactosidase levels are the means from at least three experiments using independent transformants, with standard errors (T bars) of <20%.

promoters were determined. Plasmids containing *cyc1Δuas-lacZ* and *hoΔuas-lacZ* were transformed into *BUR*$^+$ and *bur6-1* strains, and β-galactosidase levels were quantitated. Both reporter plasmids reproducibly directed higher levels of expression of *lacZ* in the *bur6-1* mutant strain relative to that of a *BUR*$^+$ strain (Fig. 6A and B), indicating that the effects of *bur6-1* are not limited to *suc2Δuas*. For the *cyc1Δuas-lacZ* plasmid, expression levels were relatively equivalent under both repressing and derepressing conditions for wild-type *CYC1* expression, as expected due to the absence of the *CYC1* UAS. Similar constitutively increased expression from *cyc1Δuas* has been observed in *mot1-1*, *sin4*Δ, and *gal11*Δ strains and in strains that have been depleted of histone H4 (7, 13, 21, 28). To determine whether *BUR6* has any effect on the intact *CYC1* and *HO* promoters, plasmids that express wild-type *CYC1-lacZ* (pLG669Z) or *HO-lacZ* (p1701) were transformed into *BUR6*$^+$ and *bur6-1* strains, and expression of β-galactosidase was assayed. *bur6-1* caused a slight (less than twofold) reduction of expression of *CYC1-lacZ* but caused a dramatic reduction of expression of *HO-lacZ* (Fig. 6C and D). Combined with the results described below, this indicates that *BUR6* affects expression from some intact promoters and is not limited to ΔUAS promoters.

***BUR6* is required for full induction of *GAL1* and *GAL10* transcription.** Among the unselected phenotypes caused by *bur6-1* and *mot1-301*, the Gal$^-$ phenotype was particularly interesting, because it suggested that some genes involved in galactose metabolism might not be properly induced in these mutant strains. This would be intriguing in light of previous biochemical characterization of Mot1p and DRAP1, since it implies that *BUR6* and *MOT1* may have positive roles at some promoters in vivo and negative transcriptional roles at other promoters. To investigate this possibility, *BUR*$^+$, *bur6-1*, and *mot1-301* strains were inoculated into media that contained either raffinose (nonrepressing and noninducing) or galactose (inducing) as the carbon source. RNA was prepared from these cultures at three time points after galactose induction, and Northern blots were used to detect the levels of expression from the *GAL1* and *GAL10* promoters. Strong and rapid induction of *GAL1* and *GAL10* was observed in *BUR*$^+$ strains grown in galactose medium, as expected. The level of induction was much lower for both *GAL1* and *GAL10* (Fig. 7) in the *bur6-1* and *mot1-301* strains throughout the time course, consistent with their Gal$^-$ phenotype. Expression of the *SPT15* gene was unaffected by either mutation and therefore serves as an internal loading control for this experiment. The Gal$^-$ defect is not likely to be due to reduced expression of the Gal4 activator, since overexpression of Gal4p from a 2μm plasmid does not suppress the *bur6-1* or *mot1-301* Gal$^-$ defect. These results indicate that Bur6p and Mot1p are required, either directly or indirectly, for induction of *GAL1* and *GAL10* mRNAs.

To investigate the *cis*-acting sequences responsible for the Gal$^-$ defect, a series of promoter fusions was employed. *BUR6*$^+$ and *bur6-1* strains were first transformed with a plasmid that expresses the intact *GAL1-lacZ* promoter (pRY131) and with pRY131 deletion derivatives that remove most (p1Δ7) or all (p1Δ1) of the Gal4p binding sites. *lacZ* expression from pRY131 was greatly reduced in a *bur6-1* strain, faithfully reproducing effects observed with the genomic *GAL1* gene (Fig. 8). The p1Δ7 deletion that removes two of the four Gal4p binding sites also exhibits approximately fivefold lower expression in a *bur6-1* strain. In contrast, a deletion that removes all of the Gal4p binding sites (p1Δ1) but leaves the *GAL1* core promoter intact is unaffected by a *bur6-1* mutation.

To investigate whether the Gal$^-$ defect is a property of the

FIG. 7. *bur6-1* and *mot1-301* cause reduced levels of *GAL1* and *GAL10* mRNA induction. Yeast strains GY315 (*BUR6*$^+$), GY215 (*bur6-1*), and GY236 (*mot1-301*) were grown in YP plus raffinose (noninducing and nonrepressing) medium, harvested, and resuspended in YP plus galactose (inducing) liquid medium. RNAs were isolated from these strains at 0, 1, 2, and 4 h after galactose induction. Three micrograms of total RNA was separated in a 1% formaldehyde agarose gel, transferred to a GeneScreen filter, and hybridized with a *GAL1*- and *GAL10*-specific probe. The filters were subsequently stripped and reprobed with an *SPT15*-specific probe as a loading control. The positions of the *GAL1*, *GAL10*, and *SPT15* mRNAs are indicated.

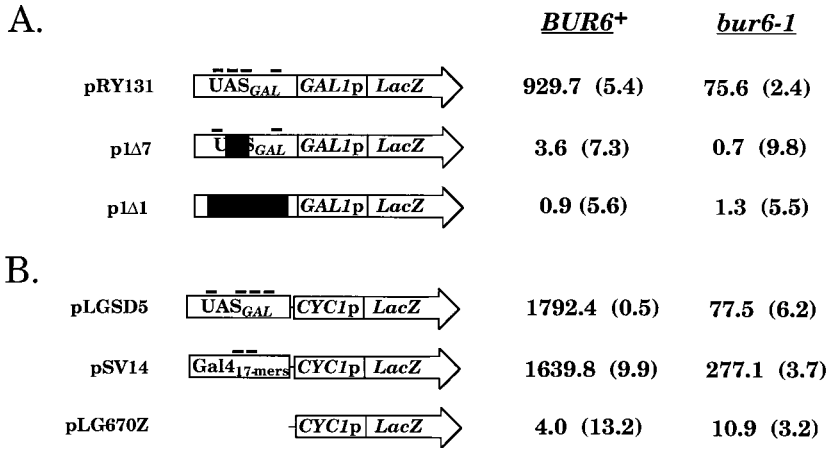

FIG. 8. A *bur6* mutation reduces expression from UAS_{GAL}-*lacZ* and *HO-lacZ* reporter plasmids. (A) Plasmid pRY131 (*GAL1-lacZ*) and two deletion derivatives (p1Δ7 and p1Δ1) were transformed into GY215 (*bur6-1*) and GY315 (BUR^+), and expression of β-galactosidase was assayed after 1.5 h of induction by galactose. The locations of the four Gal4p binding sites within the UAS are depicted above the UAS. Regions deleted in p1Δ7 and p1Δ1 are depicted as filled boxes. The figure is not drawn to scale. (B) Plasmids that contain the UAS-less *CYC1-lacZ* reporter (pLG670Z), *CYC1-lacZ* under the control of UAS_{GAL1} (pLGSD5), or two Gal4p 17-mer binding sites (pSV14) were transformed into GY215 (*bur6-1*) and GY315 (BUR^+), and expression of β-galactosidase was assayed after 1.5 h of galactose induction. Assays were performed in triplicate, and the units of β-galactosidase expression are shown on the right, with percents error in parentheses.

UAS, plasmids that contain either the intact *GAL10* UAS (pLGSD5) or duplicated Gal4p binding sites (pSV14) fused to the UAS-less *CYC1p-lacZ* reporter were transformed into $BUR6^+$ and *bur6-1* strains and β-galactosidase levels were quantitated 1.5 h after induction by galactose. Both pLGSD5 and pSV14 directed high levels of β-galactosidase in a BUR^+ strain upon galactose induction but were defective for induction in a *bur6-1* strain (Fig. 8). In contrast, β-galactosidase expression from the UAS-less *CYC1p-lacZ* plasmid was slightly increased in the *bur6-1* strain, as might be expected. Combined, these results indicate that *BUR6* affects other genes besides *SUC2*, affects some intact wild-type promoters, and has both positive and negative roles on transcription in vivo.

DISCUSSION

The Bur selection was designed to identify components or regulators of the basal transcription machinery. The data presented here, combined with our previous results, indicate that this approach has been successful. The *bur* mutations were previously grouped into two phenotypic classes, with the implication that these phenotypic classes are due to mutations that suppress *suc2*Δ*uas* by biochemically distinct mechanisms. The previously reported cloning of *BUR1*, *BUR4*, and *BUR5* (47), combined with the results presented here, supports this hypothesis.

The *bur* mutations appear to comprise two phenotypic classes: one class of *BUR* genes includes *BUR1*, *BUR2*, *BUR4*, and *BUR5*, while the other class consists of *BUR3* and *BUR6*. Because *BUR5* is identical to *HHT1*, which encodes histone H3, mutations in the first class were proposed to suppress *suc2*Δ*uas* by interfering with nucleosome repression of the GTFs (47). The identification of *BUR4* as *SPT21* further supports this classification, as *SPT21* has a role in both positively and negatively modulating transcription of a large number of differentially regulated genes, possibly by affecting the levels of histone mRNA (14, 52). Finally, the majority of mutations identified by the Bur selection were in genes that encode histones H2A and H2B and in other *SPT* genes that are proposed to have roles in chromatin repression (5, 62). The specific roles of *BUR2*, which encodes a novel protein (46), and *BUR1* (also called *SGV1*), which encodes a putative protein kinase (26), remain unknown but are currently under investigation.

The second class of *BUR* genes, consisting of *BUR3* and *BUR6*, are the subject of this report. These genes were of particular interest because of their proposed roles as chromatin-independent repressors of basal transcription. The identification of *BUR3* as *MOT1* lends strong support to this model, since *MOT1* encodes an ATP-dependent inhibitor of TBP (3). The Mot1 protein specifically displaces TBP from the TATA box in the presence of hydrolyzable forms of ATP but has no effect on two other site-specific DNA-binding proteins tested. This biochemical characterization of Mot1p is consistent with the phenotype of partial loss-of-function *mot1* mutations, which increase transcription from ΔUAS promoters in vivo (13). The identification of *BUR3* as *MOT1* was particularly intriguing in light of the phenotypic similarities between the *bur3* and *bur6* mutations, as these results suggested that *BUR6* functions in a manner similar to that of *MOT1* in vivo.

The molecular cloning of *BUR6* revealed a region of significant amino acid similarity to the histone fold family of proteins. This similarity at first suggested that Bur6p might function via nucleosome-mediated repression of the general factors; however, certain results favor a nucleosome-independent model. First, the *bur6-1* mutation causes a subset of phenotypes that are more similar to those caused by the *bur3-1* (*mot1*) mutation than to those conferred by the histone class of *bur* mutations. Second, the similarity between Bur6p and histone H2A is limited strictly to the histone fold domain; there is no similarity to histones outside the histone fold domain, and whereas the core histones are highly positively charged in both the amino- and carboxy-terminal tails, Bur6p is negatively charged outside of the histone fold domain. Finally, the sequence of Bur6p is most similar to a recently identified human protein known as DRAP1 (38) or NC2α (19), which functions as a repressor of TBP in vitro. Deletion analysis (Fig. 5) indicates that the region of *BUR6* that is required in vivo correlates well with the extent of the DRAP1/NC2α-homologous region, while nonessential amino-terminal and carboxy-terminal regions are not conserved in distantly related organisms. Further support of the proposal that *BUR6* functions as a DRAP1/NC2α homolog is provided by the yeast genome sequencing project: a yeast Dr1/NC2β homolog has been identified on chromosome IV, and *BUR6* is the only yeast gene similar to the Dr1 binding partner DRAP1/NC2α. Finally, Bur6p copurifies

with yeast Dr1 over several column chromatography steps and binds directly to yeast Dr1 (17, 18, 31). Bur6p therefore also displays the biochemical characteristics expected of a DRAP1/NC2α homolog.

Although *BUR6* was initially identified by a selection for mutations that affect the *suc2*Δ*uas* promoter, its effects are clearly not limited to *SUC2* or to basal transcription. The *bur6-1* mutation causes increased transcription from three ΔUAS promoters tested (*SUC2*, *HO*, and *CYC1*), although the effects are rather modest when compared to full levels of induction. Similar partial increase in expression was observed in a series of studies examining the effects of histone H4 depletion in yeast (15, 21) and in strains containing mutations in *MOT1* (13), *SIN4* (7, 28), *SPT5* (56), *SPT6* (43, 57), *SPT16*/*CDC68* (36), and *GAL11* (7). The relatively modest effects of the *bur6-1* mutation may also be due to the fact that only a single *bur6* allele was isolated and that a *bur6* null allele causes extremely slow growth, so only partial loss-of-function alleles were likely to be isolated. Finally, other repressors, such as Mot1p (13), the Spt proteins (62), the Not proteins (11), and nucleosomes, are still likely to be repressing basal transcription in the *bur6* mutant cells, thereby preventing complete derepression.

In addition to increasing transcription from promoters that have had their UAS deleted, the *bur6-1* and *mot1-301* mutations also reduce activated levels of transcription from the wild-type *GAL1* and *GAL10* promoters. The reduced levels of *GAL1* and *GAL10* mRNAs are not simply due to reduced activity of the Gal4p activator, since transformation of a *bur6-1* strain with a plasmid that overexpresses Gal4p does not revert the Gal$^-$ phenotype (46). The activation defect is not likely to be restricted to galactose-induced genes, since an *HO-lacZ* reporter was also defective for expression in a *bur6-1* strain. *BUR6* is not required for activation by all UAS elements, however, as expression of the wild-type *SPT15* and *CYC1* genes was unaffected in *bur6-1* strains. Results presented here indicate that the *GAL10* activation defect is due to an inability of either the intact *GAL10* UAS or duplicated Gal4p binding sites to function as a UAS, since either of these elements fused to the *CYC1* promoter induces strong galactose-dependent expression in a *BUR*$^+$ strain but not in a *bur6-1* background. The reduction of *GAL1* and *GAL10* transcript levels in *bur6-1* and *mot1-301* strains was surprising, since all previously published in vitro characterization of Mot1 and DRAP1/NC2α indicated that these proteins function as repressors, which was consistent with the existing genetic characterization of *mot1* mutations. However, the results presented here suggest that Bur6p and Mot1p may have dual functions in vivo, both as activators and as repressors. Recent results from other labs are also consistent with dual activator and repressor roles for Mot1p in vivo (10, 35). Proteins that have such dual activating and repressing roles are not uncommon. For example, mutations in the histone genes, *SPT21*, *RPD3*, *MCM1*, and *SIN3* all cause increased transcription at some promoters but decreased transcription at others (16, 29, 30, 41, 59–61). How might such dual effects be explained? One model would be that Bur6p and Mot1p may each be associated with two different complexes, one of which activates and the other of which represses transcription. Such a direct dual role has been demonstrated for the yeast Mcm1 protein and a number of mammalian transcription factors. Alternatively, it is possible that one function is direct while the other is indirect. For example, Bur6p may have direct effects only as a repressor but may phenotypically appear to be an activator of *GAL* genes if it represses a *GAL*-specific repressor. Future experiments will be required to address the specific roles of *BUR6* in repression and/or activation.

The existence of systems for global repression of transcription that is dependent upon proteins such as Bur6p, Mot1p, and histones leads to the interesting question of how the cell overcomes this repression machinery to allow transcriptional activation under the appropriate conditions. It appears that the SNF-SWI complex plays an important role in overcoming nucleosomal repression, but it remains to be seen whether SNF-SWI or other activities that are thought to function through chromatin remodeling also play a role in Mot1p- and Bur6p-dependent repression. The inability of *bur6* and *mot1* mutations to suppress *snf5*Δ suggests that SNF-SWI does not overcome repression by Mot1p or Bur6p. Recent studies suggest that TFIIA may play a role in stabilizing TBP from the displacement activity of Mot1p, but it remains to be seen whether TFIIA performs the same role in vivo. It seems likely that at least part of the function of activator proteins is to overcome the effects of repressors such as Bur6p, either directly or through coactivator intermediates. Detecting these proposed activator-repressor interactions and testing their possible functional significance in vivo should be possible in an organism, such as yeast, that allows sophisticated genetic analysis.

The combination of biochemical and genetic analysis of proteins, such as *MOT1* and *BUR6*, that modulate the activities of the GTFs will contribute to a more detailed understanding of the intricacies of transcriptional control in all eukaryotic cells, including the interplay between proteins that activate and repress transcription.

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