MOLECULAR AND CELLULAR BIOLOGY, Dec. 1999, p. 8461–8468 Vol. 19, No. 12
0270-7306/99/$04.00+0

Conservation of Histone Binding and Transcriptional Repressor Functions in a *Schizosaccharomyces pombe* Tup1p Homolog

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Received 24 May 1999/Returned for modification 30 June 1999/Accepted 13 September 1999

The Ssn6p-Tup1p corepressor complex is important to the regulation of several diverse genes in *Saccharomyces cerevisiae* and serves as a model for corepressor functions. To investigate the evolutionary conservation of these functions, sequences homologous to the *S. cerevisiae TUP1* gene were cloned from *Kluyveromyces lactis* (*TUP1*) and *Schizosaccharomyces pombe* (*tup11*$^+$). Interestingly, while the *K. lactis TUP1* gene complemented an *S. cerevisiae tup1* null mutation, the *S. pombe tup11*$^+$ gene did not, even when expressed under the control of the *S. cerevisiae TUP1* promoter. However, an *S. pombe* Tup11p-LexA fusion protein repressed transcription of a corresponding reporter gene, indicating that this Tup1p homolog has intrinsic repressor activity. Moreover, a chimeric protein containing the amino-terminal Ssn6p-binding domain of *S. cerevisiae* Tup1p and 544 amino acids from the C-terminal region of *S. pombe* Tup11p complemented the *S. cerevisiae tup1* mutation. The failure of native *S. pombe* Tup11p to complement loss of Tup1p functions in *S. cerevisiae* corresponds to an inability to bind to *S. cerevisiae* Ssn6p in vitro. Disruption of *tup11*$^+$ in combination with a disruption of *tup12*$^+$, another *TUP1* homolog gene in *S. pombe*, causes a defect in glucose repression of *fbp1*$^+$, suggesting that *S. pombe* Tup1p homologs function as repressors in *S. pombe*. Furthermore, Tup11p binds specifically to histones H3 and H4 in vitro, indicating that both the repression and histone binding functions of Tup1p-related proteins are conserved across species.

In *Saccharomyces cerevisiae*, the *TUP1* gene encodes a protein required for repression of genes regulated by cell type, glucose, oxygen, DNA damage, and other signals (26, 38). Tup1p forms a complex in vivo with Ssn6p (24, 34). This complex does not bind DNA directly but is recruited to target gene promoters through interaction with a variety of sequence-specific DNA-binding proteins (α2p for mating-type control [16, 28], Mig1p and Nrg1p for glucose repression [23, 31], Rox1p for oxygen repression [1, 39], and Crt1p for DNA damage [12]). Ssn6p may serve as an adapter between Tup1p and these DNA-binding proteins (33). Interestingly, Tup1p-LexA fusion proteins directly mediate repression of appropriate reporter genes, independently of Ssn6p (32). However, Ssn6p-LexA fusions require Tup1p for repression (15). Tup1p, then, appears to directly mediate repression, while Ssn6p does not.

In vitro protein binding experiments and two-hybrid analyses have defined a number of domains in the 713-amino-acid Tup1p protein. The 72 N-terminal amino acids of Tup1p are required for interaction with Ssn6p and self-multimerization (33). The histone binding and repression domain comprises amino acids 73 to 385 (6, 32). WD repeats (amino acids 333 to 706) in the C-terminal region of Tup1p likely form a seven-bladed β-propeller structure (18, 29) that interacts with α2p (16).

Two mechanisms of repression have been proposed for the Ssn6p-Tup1p complex (7, 38). A number of factors necessary for repression, including Sin4p (4, 13), Sin3p/Rpd1p (36), Rpd3p (35), Srb10p/Are1p/Ssn3p, Srb11p/Ssn8p, and Srb8 (3, 17, 37, 38), are associated with subcomplexes within the RNA polymerase II holoenzyme. These findings suggest that Ssn6p-Tup1p may inhibit transcription through interactions with the transcription machinery. In support of this model, a modest amount of repression (two- to fourfold) can be achieved in vitro, in the presence of just the basal transcription machinery (10, 24).

A second model proposes that Tup1p mediates repression through the organization of chromatin. Tup1p interacts directly with the amino-terminal tail domains of histones H3 and H4 in vitro (6), and mutations in these histone domains synergistically reduce repression of multiple classes of Tup1p-regulated genes in vivo (6, 11). Moreover, the H3-H4 binding domain in Tup1p coincides with the repression domain. Ssn6p-Tup1p interactions with components of chromatin may lead to decreased accessibility of promoter regions, thereby effecting repression.

The above-described models for Tup1p repression are not mutually exclusive. Complete repression by Ssn6p-Tup1p may involve interactions with both the basal transcription machinery and the histones. For example, Ssn6p-Tup1p complexes might first halt transcription through altering the activity of the basal apparatus and then maintain the repressed state through organization of chromatin.

To further understand the mechanism of Tup1p repression, we sought functional homologs in other, related and unrelated yeasts. Here, we report a structural and functional analysis of *TUP1* homologs from *Kluyveromyces lactis* and *Schizosaccharomyces pombe*. Our findings suggest that histone binding is a conserved feature of Tup1p repressor functions.

MATERIALS AND METHODS

Yeast strains. *S. cerevisiae* YMH427 (*MATα tup1*::*HIS3 ura3-52 trp1 his3 pho3 pho5 leu2-3,112*::[*LEU2, STE6-PHO5*]) was used for complementation tests of the *tup1* disruption. YMH465 (*MATα leu2-3,112 trp1 pho3 pho5 ura3-52*::[*URA3, lexA4-CYC1-lacZ*]) was used for monitoring the ability of LexA-Tup1p fusions to repress the *CYC1* reporter. TY3 (*MATα ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ1*) was used for preparation of histone proteins. *K. lactis* IFO1267 was used for prepa-

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ration of genomic DNA. The wild-type strain *S. pombe* 972 (h^-) was used for preparation of the genomic DNA and total RNA to be used as the PCR templates, and JY741 (h^- *ura4-D18 leu1-32 ade6-M216*) was used for construction of the *tup11* and *tup12* gene disruptants.

The media used for cultivation and transformation of *S. cerevisiae* and *S. pombe* strains were as described in references 25 and 20, respectively. Determination of mating types was as described previously (21). Acid phosphatase activities (30) of the *STE6-PHO5* reporter gene and β-galactosidase activities (25) of the *lexA4-CYC1-lacZ* reporter gene were measured by standard methods.

Cloning of *K. lactis TUP1* and *S. pombe tup11*$^+$ and *tup12*$^+$ genes. The *K. lactis TUP1* gene was identified by Southern blot hybridization (27) under conditions of low stringency with a PCR product containing the WD repeat region of the *S. cerevisiae TUP1* gene (corresponding to bp +1066 to +1552, relative to ATG) as a probe. A 1.1-kbp *Eco*RI fragment that reproducibly cross-hybridized with the *S. cerevisiae* probe was isolated from the *K. lactis* genomic DNA in pBluescript II KS(+). One of the positive clones, pKL5-2, carried nucleotide sequences similar to those encoding the WD repeats of *S. cerevisiae TUP1* but truncated regions homologous to the N-terminal region. Therefore, a 0.7-kbp *Eco*RI-*Bgl*II fragment from pKL5-2 was used as a probe to identify a 2.0-kbp *Hin*dIII-*Bgl*II fragment containing the N-terminal region of the *K. lactis TUP1* gene. This fragment was cloned into the plasmid pKL4-3. The plasmid pKLTUP1, carrying the entire *K. lactis TUP1* gene, was constructed by ligating the 1.3-kbp *Hin*dIII-*Spe*I fragment from pKL4-3 to the 1.1-kbp *Spe*I-*Eco*RI fragment from pKL5-2. The nucleotide sequence of the insert DNA of pKLTUP1 was determined. The plasmid pYMC105 was constructed by insertion of the 2.3-kbp *Bam*HI-*Sal*I fragment, containing the entire *K. lactis TUP1* gene, into the same site of YCp50, and this single copy vector was used for complementation analysis.

The *S. pombe tup11*$^+$ cDNA was cloned by reverse transcriptase (RT)-PCR with an LA PCR kit (TaKaRa). The oligonucleotides 5′-CTCGGATCCCCATGGCGTCAGTGGAGGATG-3′ (corresponding to the first 19 bp of the coding sequence of a putative Tup1p homolog from the *S. pombe* genome project [accession no. Z50728]) and 5′-CTCGTCGACTCAAGGAGATGCAGGGTCAA-3′ (corresponding to the 20 bp of the end of the coding sequence) were used as primers, and total RNA from *S. pombe* 972 was used as a template. The resultant 1.8-kbp PCR product was digested with *Bam*HI and *Sal*I and ligated into pUC119 to create pYMS264. *S. pombe tup11*$^+$ genomic DNA was also amplified by PCR with the above oligonucleotides as the primers and the chromosomal DNA of the *S. pombe* strain 972 as the template. The 2.1-kbp PCR products were digested with *Bam*HI and *Sal*I and ligated into pUC119 or pBluescript II SK(+), with the resultant plasmids designated pYMS263 or pYMS266, respectively. The 7.5-kbp *Hin*dIII DNA fragment containing the *S. pombe tup11*$^+$ gene was isolated from the genomic DNA of strain 972 by colony hybridization (27) with the 2.1-kbp *Bam*HI-*Sal*I fragment from pYMS263 as a probe. The 2.6-kbp *Hin*cII fragment from the above clone was subcloned into pUC118 in the same direction as the *lacZ* gene to create pYMS285.

pYMS287 was constructed by inserting the 0.3-kbp *Bam*HI-*Bgl*II fragment of pYMS264 into the same gap of pYMS285. A 1.8-kbp *Bam*HI-*Pst*I fragment prepared from pYMS287 was cloned into pBTM116 to create pBTM-tup11, which was used to express the LexA-*S. pombe* Tup11p fusion protein in *S. cerevisiae*.

Plasmid pGEX-tup11N was constructed by cloning a 0.9-kbp *Bam*HI-*Xho*I fragment (corresponding to amino acids 1 to 298 of *S. pombe* Tup11p) amplified by PCR (with pBTM-tup11 as a template) into the same gap of pGEX-6P-1 (Amersham Pharmacia Biotech). This plasmid was used for production of the glutathione S-transferase (GST)-*S. pombe* Tup11p fusion protein in *Escherichia coli*. Plasmid pCITE-tup11 was constructed by cloning a 1.9-kbp *Bam*HI-*Sal*I fragment generated by PCR and containing the full-length coding region of *S. pombe tup11*$^+$ into the *Bam*HI-*Sal*I site of the pCITE-4a vector (Novagen).

The *S. pombe tup12*$^+$ genomic DNA was amplified by PCR with the oligonucleotides 5′-CGGGATCCATGGCGCTCATGAAACAAAC-3′ (corresponding to the first 20 bp of the coding sequence of another *S. pombe* Tup1p homolog [accession no. U92792]) and 5′-GCGTCGACCAGATCCTCATAAGACCAAA-3′ (corresponding to the 20 bp of the end of coding sequence) as primers and the chromosomal DNA of *S. pombe* 972 as a template. The 2.2-kbp PCR product was digested with *Bam*HI and *Sal*I and ligated into pBluescript II KS(+) to create ptup12int.

Construction of *tup11* and *tup12* disruptants. The *tup11*::*ura4*$^+$ disruptant, JY741-Δtup11U, was constructed by transformation of JY741 with the *Bam*HI- and *Hin*dIII-digested plasmid which had the insertion of the *ura4*$^+$ DNA fragment at the *Bgl*II site of pYMS266. The *tup12*::*LEU2* disruptant, JY741-Δtup12L, and the *tup11*::*ura4*$^+$ *tup12*::*LEU2* double disruptant, JY741-Δtup11U, Δtup12L, were constructed by transformation of JY741 and JY741-Δtup11U, respectively, with the *Bam*HI- and *Xho*I-digested plasmid which had the insertion of the *S. cerevisiae LEU2* DNA at the *Bgl*II site of ptup12int.

Construction of the *S. cerevisiae-S. pombe* Tup1p hybrids. The YCp50-based vector pYMC111 carrying the *S. cerevisiae TUP1* promoter was constructed by insertion of a PCR product amplified with the primers 5′-CTCAAGCTTATTTTGCGCACGTTGGATTG-3′ (corresponding to positions −939 to −920 relative to ATG) and 5′-CTCGGATCCCCATATTGGTTTGGATGGAAA-3′ (corresponding to positions +3 to −17) into YCp50 after digestion with *Eco*RI and *Hin*dIII. The *S. cerevisiae TUP1* and the *S. pombe tup11*$^+$ DNA fragments were synthesized by PCR and cloned into the *Hin*dIII-*Sal*I site of pYMC111 for expression in *S. cerevisiae*. Each gene was divided into three regions, roughly corresponding to the amino-terminal Ssn6p-binding domain, the central repression domain, and the C-terminal WD repeats of the *S. cerevisiae* protein. The PPP construct contained only *S. pombe* sequences, and the CCC construct contained only *S. cerevisiae* sequences. PPP was constructed by cloning of the entire coding region (corresponding to amino acid positions 1 to 614) of the *S. pombe tup11*$^+$ gene amplified by PCR into pYMC111. PPC was constructed by replacing the region from positions 298 to 614 of PPP with the region from positions 329 to 713 of *S. cerevisiae TUP1* at the *Sph*I site of the *S. pombe tup11*$^+$ gene. Similarly, CCP was constructed by replacing the region from positions 1 to 297 of PPP with the region from positions 1 to 328 of *S. cerevisiae TUP1* at the *Sph*I site of the *S. pombe tup11*$^+$ gene. CPP was constructed by replacing the region from positions 73 to 328 of CCP with the region from positions 71 to 297 of *S. pombe tup11*$^+$ from the plasmid pBTM-tup11 by using the *Mlu*I and *Sph*I sites. CCC was constructed by ligating the region from positions 1 to 328 of CCP and the region from positions 329 to 713 of PPC. PPC2 was constructed by ligating the region from positions 1 to 351 of *S. pombe tup11*$^+$ and the region from positions 434 to 713 of *S. cerevisiae TUP1* by using the *Pst*I site. Similarly, CCP2 was constructed by ligating the region from positions 1 to 433 of *S. cerevisiae TUP1* and the region from positions 352 to 614 of *S. pombe tup11*$^+$ by using the *Pst*I site.

GST pull-down assays. *S. cerevisiae* Ssn6p and *S. pombe* Tup11p proteins were produced and labeled with ^{35}S-methionine by the TNT Quick Coupled Transcription/Translation system (Promega). Histone proteins were purified from *S. cerevisiae* TY3 as described previously (7, 19). GST-α2p, GST-*S. cerevisiae* Tup1p and GST-*S. pombe* Tup11p fusion proteins were expressed in *E. coli* DH5α and purified with glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) by following the manufacturer's protocol. In vitro-labeled proteins or unlabeled histones were incubated with comparable amounts (as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] analysis) of the different GST fusion proteins bound to beads in binding buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 1 mM glutamate, 1 mM dithiothreitol) at room temperature for 1 h. Then, the beads were collected by centrifugation in a microcentrifuge at 500 × *g* for 2 min. The supernatant was saved, and the beads were washed five times with binding buffer. The washed beads were resuspended directly in 1× SDS-PAGE sample buffer. All samples were separated by SDS-PAGE and autoradiographed for detection of the ^{35}S-labeled proteins or visualized by staining with Coomassie brilliant blue R-250 for observation of histones (27).

Far-Western blot analysis. Separation of histone proteins by SDS-PAGE, electroblotting onto nylon membrane, and binding to ^{35}S-labeled probes were done as previously described (5, 6). Full-length *S. cerevisiae* Tup1p and *S. pombe* Tup11p were synthesized and labeled with ^{35}S-methionine with pCite/Tup1 (6) and pCITE-tup11, respectively, as the templates and purified with microcon 10 spin columns (Amicon) as described previously (6).

Northern blot analysis. Cultivation of *S. cerevisiae* strains was as described previously (25). *S. pombe* strains were grown in YEL with 3% glucose and 2% Casamino Acids to a concentration of 0.5×10^7 to 0.8×10^7 cells/ml. The cells were collected by centrifugation, washed with sterile water, and shaken for 3 h in YEL with 2% Casamino Acids and 8% glucose (repressing conditions) or 0.1% glucose and 3% glycerol (derepressing conditions). Total RNA was prepared and separated on a formaldehyde-agarose gel, transferred to nylon membranes, and hybridized as described previously (25, 27).

RESULTS

Cloning of *TUP1* homologs from *K. lactis* and *S. pombe*. Sequences homologous to *TUP1* were identified in genomic DNA from *K. lactis* by low stringency Southern hybridization with sequences encoding the WD repeat region of *S. cerevisiae TUP1*, and these sequences were subsequently cloned (see Materials and Methods). The nucleotide sequence predicts that the *K. lactis* Tup1p protein consists of 682 amino acids and has 61% overall identity to *S. cerevisiae* Tup1p (75% identity in the WD repeat domain and 45% identity in other regions) (Fig. 1). The predicted Ssn6p-binding domain of *K. lactis* Tup1p is more highly conserved with the corresponding region of *S. cerevisiae* Tup1p (56 of 71 amino acids identical) than that of *C. albicans* Tup1p (39 out of 72 amino acids identical) (2).

A protein database search using the amino acid sequence of *S. cerevisiae* Tup1p revealed that a nucleotide sequence predicted to encode a similar protein had been identified by the *S. pombe* genome project. This sequence, SPAC18B11.10, is located on chromosome I and is termed *tup11*$^+$. Since the *S. pombe tup11*$^+$ sequence also predicted that this gene contains two introns, the *tup11*$^+$ cDNA was cloned by RT-PCR

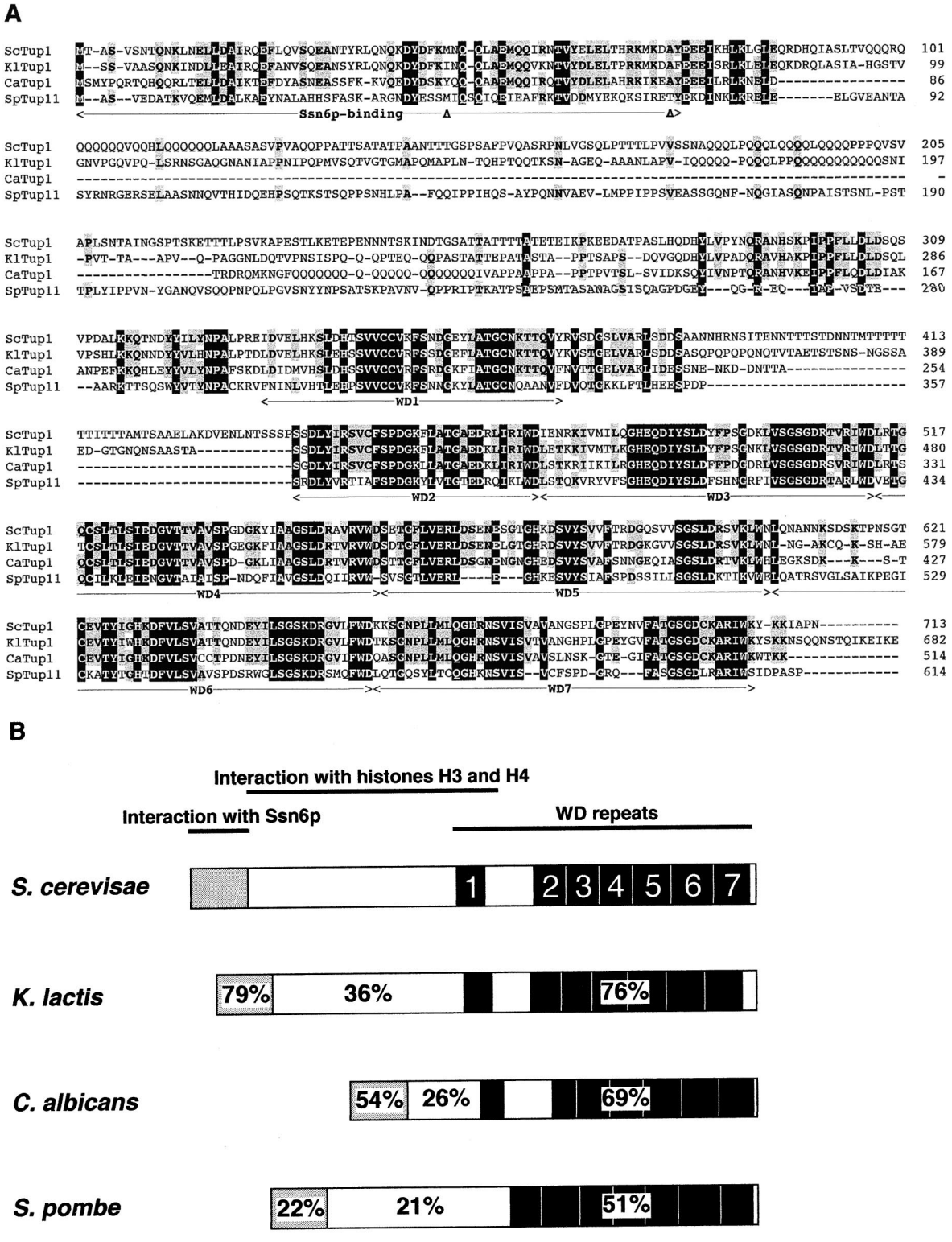

FIG. 1. Comparison of primary structure of Tup1p homologs in yeast. (A) Alignment of Tup1p homologs. Amino acids identical among three or four yeast species are in gray or black, respectively. The predicted Ssn6p-binding region and seven WD repeats are indicated. Triangles represent the positions where introns were inserted in *S. pombe tup11*$^{+}$. ScTup1, *S. cerevisiae* Tup1p; KlTup1, *K. lactis* Tup1p; CaTup1, *C. albicans* Tup1p (2); SpTup11, *S. pombe* Tup11p. (B) Comparison of functional domains within Tup1p homologs. Values indicate the percent identity with *S. cerevisiae* Tup1p. Dotted and closed boxes represent the Ssn6p-binding and WD repeat domains, respectively. The bars indicate the functional domains of *S. cerevisiae* Tup1p.

TABLE 1. Complementation of an *S. cerevisiae tup1* mutation with *TUP1* homologs from other yeasts

Gene[a]	Mating ability	Flocculation	Expression of *STE6-PHO5*[b]
S. cerevisiae TUP1	α	−	0.47 ± 0.07
K. lactis TUP1	α	+[c]	0.88 ± 0.03
S. pombe tup11$^+$	Non[d]	+	3.01 ± 0.31
Vector alone	Non	+	3.71 ± 0.07

[a] Host strain YMH427 (*MAT*α *tup1*::*HIS3 pho3 pho5 leu2-3,112*::[*LEU2, STE6-PHO5*]) was transformed with YCp50-based plasmids containing the indicated *TUP1* homolog.

[b] APase activities (milliunits/milliliter/optical density at 660 nm) were determined in cell suspensions by standard methods. Values are the averages of triplicate determinations ± standard deviations.

[c] When YMH427 was transformed with the high-copy-number plasmid YEp24 containing the *K. lactis TUP1* gene, the flocculent phenotype was suppressed.

[d] Non, nonmating ability.

(see Materials and Methods). Subsequent comparison of the nucleotide sequence of the *tup11*$^+$ cDNA to that of the genomic sequence confirmed the presence of the two introns in the gene. The predicted protein sequence of *S. pombe* Tup11p consists of 614 amino acids and has 50% identity to the WD repeat domain of *S. cerevisiae* Tup1p and 22% identity in other regions (Fig. 1). Interestingly, the *S. pombe* Tup11p has no serine-threonine-rich region between the first and second WD repeats, as is found in Tup1p from the other yeast. Also, no glutamine-rich region, which is located between the Ssn6p-binding region and WD repeats of Tup1p of budding yeast, is found in the *S. pombe* Tup11p. The N-terminal region of *S. pombe* Tup11p (1 to 70 amino acids) corresponding to the first and second exons was similar to but not highly conserved with the Ssn6p-binding region of *S. cerevisiae* Tup1p (16 out of 70 amino acids identical).

***K. lactis TUP1* complemented an *S. cerevisiae tup1* mutation, but *S. pombe tup11*$^+$ did not.** To see whether the above-described *K. lactis* and *S. pombe TUP1* homologs were able to functionally substitute for the *S. cerevisiae TUP1* gene, we expressed each gene in *S. cerevisiae* YMH427 (*MAT*α *tup1*::*URA3 STE6-PHO5*), which is null for *TUP1*. As shown below, the 226-bp promoter region of *K. lactis TUP1* was sufficient to express this gene in *S. cerevisiae*. The *S. pombe tup11*$^+$ cDNA was expressed under the control of the *S. cerevisiae TUP1* promoter.

YMH427 exhibits phenotypes typical of a *tup1* null allele, including defective mating, flocculation, and expression of an *STE6-PHO5* reporter gene. This reporter encodes acid phosphatase (APase) under the control of the **a**-specific *STE6* promoter, which is normally repressed in α cells but expressed in **a** cells. YMH427 cells containing the plasmid harboring the *K. lactis TUP1* gene regained an α-mating phenotype and exhibited reduced expression of the *STE6-PHO5* reporter (Table 1). This reversal of *tup1* phenotype was similar to that achieved upon introduction of a plasmid harboring the native *S. cerevisiae TUP1* gene. The flocculent phenotype of YMH427 was not suppressed when the *K. lactis TUP1* gene was carried on a low-copy-number vector (YCp50), but was suppressed when this gene was carried on a high-copy-number vector (YEp24).

In contrast to the complementation achieved with *K. lactis TUP1*, YMH427 cells expressing the *S. pombe tup11*$^+$ gene maintained the nonmating and flocculent *tup1* null phenotypes. The *STE6-PHO5* reporter gene was not repressed in these cells and was expressed to a level equivalent to that of cells harboring the empty vector (Table 1).

Examination of the expression of endogenous Tup1p-repressed genes by Northern analysis confirmed the above phenotypic observations (Fig. 2). The **a**-cell-specific gene *STE2*, the glucose-repressible gene *SUC2*, and the oxygen-repressed gene *ANB1* were all repressed in YMH427 cells bearing either the *S. cerevisiae* (lane 1) or the *K. lactis* (lane 2) *TUP1* gene. These results are consistent with the previous description of the *K. lactis TUP1* (2), and the *Candida albicans TUP1* gene is also known to complement the *S. cerevisiae tup1* mutation (2). However, cells bearing the *S. pombe tup11*$^+$ gene (lane 3) did not exhibit repression of any of these genes. Furthermore, we expressed a LexA-*S. pombe* Tup11p fusion protein in an *S. cerevisiae tup1* mutant and easily detected expression of this protein using anti-LexA antibodies (data not shown, but see below). However, this fusion protein did not complement the *tup1* mutation. Therefore, we conclude that *K. lactis TUP1* (although somewhat weaker) is functionally exchangeable with *S. cerevisiae TUP1* but *S. pombe tup11*$^+$ is not.

***S. pombe tup11*$^+$ encodes a transcriptional repressor.** The above experiments raise the question of whether the *S. pombe* Tup11p protein serves as a transcriptional repressor in vivo. To examine this question, this protein was fused to the DNA-binding domain of the bacterial LexA protein and expressed in *S. cerevisiae* YMH465, which carries a *CYC1-lacZ* reporter gene containing four copies of the LexA DNA-binding site upstream of the *CYC1* upstream activation sequence. β-Galactosidase activities were determined, and the results (in Miller units) were as follows: for LexA-*S. cerevisiae* Tup1p, 7.3 ± 0.7; for LexA-*S. pombe* Tup11p, 7.5 ± 0.4; and for LexA, 25.7 ± 2.4 (values are the means of 11 independent measurements ± standard deviations). As expected, this reporter was repressed in cells bearing *S. cerevisiae* Tup1p fused to LexA relative to cells bearing LexA alone. Importantly, expression of the reporter in cells bearing the *S. pombe* Tup11p-LexA fusion was also repressed and this repression was equal to that seen in the cells bearing *S. cerevisiae* Tup1p-LexA. These data indicate that *S. pombe* Tup11p can function as a transcriptional repressor in *S. cerevisiae*.

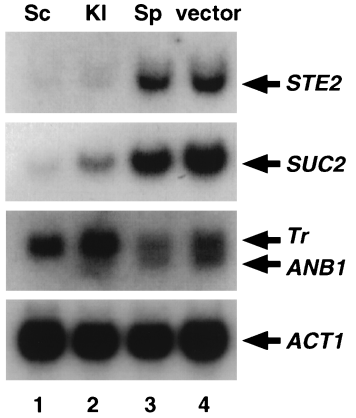

FIG. 2. Regulation of Tup1p-repressed genes in *S. cerevisiae tup1* mutants expressing Tup1p homologs. Total RNA samples were prepared from cells of *tup1*-disrupted strain YMH427 having the plasmid carrying *S. cerevisiae TUP1* (lane 1), *K. lactis TUP1* (lane 2), or *S. pombe tup11*$^+$ (lane 3) or the vector plasmid YCp50 alone (lane 4). Each RNA sample (2 μg per lane) was separated on an agarose gel in the presence of formaldehyde, blotted onto a nylon membrane, and hybridized with ^{32}P-labeled **a**-specific *STE2*, the glucose-repressed *SUC2*, or the oxygen-repressed *ANB1* probe. The same membranes were rehybridized with ^{32}P-labeled *ACT1* as an internal marker. The *ANB1* DNA fragments were also hybridized with the *Tr* transcript which is not regulated by *TUP1*.

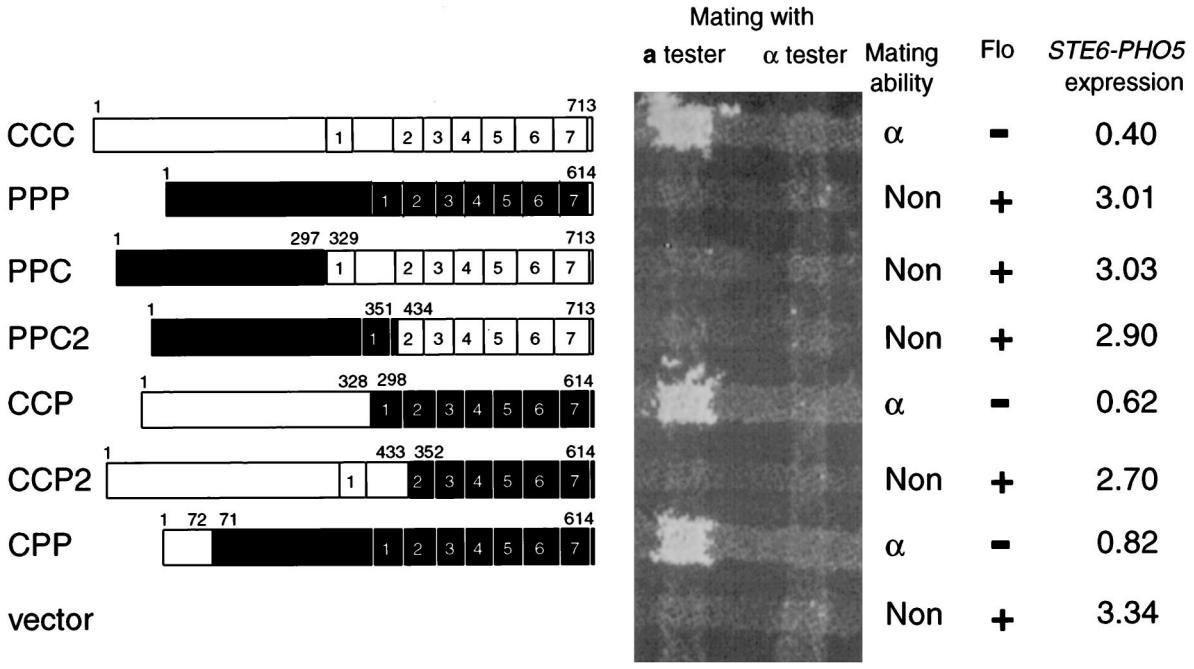

FIG. 3. Identification of functional domains in *S. pombe* Tup11p by creation of chimeric proteins. The *tup1* strain YMH427 was transformed with plasmids harboring the indicated *S. cerevisiae-S. pombe* Tup1p hybrids. The resulting transformants were assayed for mating ability, flocculation, and *STE6-PHO5* expression (APase activity [in milliunits], the average of three measurements with a margin of error of <20%). The open boxes indicate regions derived from *S. cerevisiae* Tup1p, and the closed boxes indicate regions derived from *S. pombe* Tup11p. The amino acid positions of the junctions are indicated. Flo, flocculation; −, nonflocculent; +, flocculent; Non, nonmating ability.

Chimeric Tup11p proteins bearing the Ssn6p-binding region of *S. cerevisiae* Tup1p complement *tup1* null phenotypes. As shown above, the *S. pombe tup11*$^+$ gene was not able to complement the *S. cerevisiae tup1* mutation in spite of its ability to repress transcription in *S. cerevisiae*. To localize the functional differences between the *S. cerevisiae* and *S. pombe* Tup1p proteins, we tested the ability of hybrid Tup1p proteins to functionally substitute for the *S. cerevisiae* Tup1p. Several *S. cerevisiae-S. pombe* chimeric Tup1p proteins were constructed and expressed under the control of the *S. cerevisiae TUP1* promoter in the *tup1* disruptant strain YMH427. For these experiments, each coding region was divided into three sections, roughly corresponding to the amino-terminal Ssn6p-binding domain, the central repression domain, and the C-terminal WD repeats of the *S. cerevisiae* protein. The PPP construct contained only *S. pombe* sequences, and the CCC construct contained only *S. cerevisiae* sequences. Hybrid constructs (PPC, PPC2, CCP, CCP2, and CPP) contained mixtures of *S. pombe* and *S. cerevisiae* sequences as described in Materials and Methods.

YMH427 cells transformed with a plasmid lacking *TUP1* sequences exhibited the expected nonmating and flocculent phenotypes, and these cells expressed the *STE6-PHO5* reporter gene (APase activity, 3.34 mU; Fig. 3). Expression of full-length *S. cerevisiae* Tup1p (Fig. 3) reestablished α-mating and nonflocculent phenotypes and repressed the expression of the *STE6-PHO5* reporter gene (APase activity, 0.40 mU). As shown above, (Table 1) expression of full-length *S. pombe* Tup11p (PPP) did not rescue these phenotypes. Fusion of amino-terminal sequences from *S. pombe* Tup11p to C-terminal sequences of *S. cerevisiae* Tup1p (PPC and PPC2) also failed to complement the *tup1* null phenotypes. However, fusion of the first 328 amino acids of *S. cerevisiae* Tup1p to the WD repeat region of *S. pombe* Tup11p (CCP) fully complemented all three of the *tup1* null phenotypes. These data indicate that the WD repeats of the *S. pombe* protein, which exhibited 50% identity to those of the *S. cerevisiae* protein, can function to target the chimeric protein to Tup1p target genes in *S. cerevisiae*. Similarly, replacement of the first 70 amino acids of the *S. pombe* protein with the first 72 amino acids of the *S. cerevisiae* protein (CPP) complemented the *tup1* null allele. Importantly, we detected comparable amounts of transcripts from the PPP and CPP constructs with *tup11*$^+$ DNA as a probe, indicating that both were expressed well (data not shown). Thus, attachment of the *S. cerevisiae* Ssn6p-binding domain to the bulk of the *S. pombe* protein reconstitutes Tup1p function in *S. cerevisiae*.

Interestingly, fusion of the first 433 amino acids of the *S. cerevisiae* protein, which contain the Ssn6p-binding site, to the last 352 amino acids of the *S. pombe* protein (CCP2) did not restore Tup1p functions. These data indicate that the first WD repeat of the *S. pombe* protein is somehow required for the function of the chimeric proteins in *S. cerevisiae*, perhaps influencing proper folding of the WD propeller domain.

***S. pombe* Tup11p binds to α2p but not to Ssn6p of *S. cerevisiae* in vitro.** Since the WD repeats of *S. cerevisiae* Tup1p were exchangeable with those of *S. pombe* Tup11p, we predicted that the *S. pombe* repeats would interact with DNA-binding proteins that recruit Tup1p to target promoters in *S. cerevisiae*. Therefore, we tested the ability of *S. pombe* Tup11p to bind to α2p in vitro. The α2p protein was fused to GST and expressed in *E. coli. S. pombe* Tup11p was transcribed and translated in vitro in the presence of ^{35}S-methionine. GST-α2p or GST alone was purified from bacterial extracts with glutathione-Sepharose beads, and then equal amounts of the GST fusion proteins were incubated with in vitro-labeled *S. pombe* Tup11p. Bead-bound fractions were analyzed by SDS-PAGE (Fig. 4A). *S. pombe* Tup11p bound to GST-α2p (Fig. 4A, lane

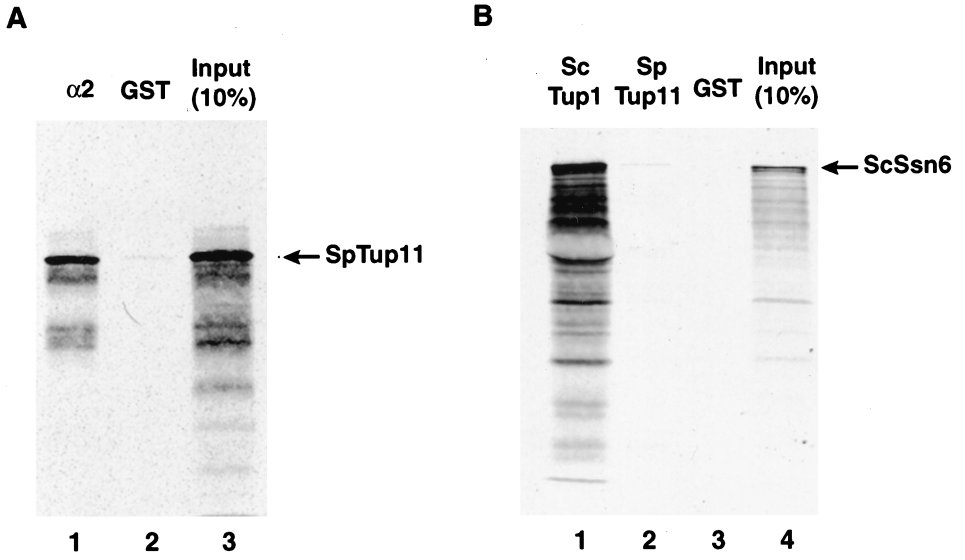

FIG. 4. Interaction of *S. pombe* Tup11p with *S. cerevisiae* α2p and Ssn6p in vitro. (A) Binding of *S. pombe* Tup11p to α2p. In vitro ^{35}S-labeled *S. pombe* Tup11p was incubated with beads bound to GST-α2p (lane 1) or to GST alone (lane 2). After the beads were washed, proteins bound to the beads were analyzed by SDS-PAGE. Shown are autoradiograms detecting the labeled proteins. Input (lane 3) represents 10% of the labeled Tup11p used in the binding reaction. (B) Binding of *S. pombe* Tup11p with *S. cerevisiae* Ssn6p. In vitro ^{35}S-labeled *S. cerevisiae* Ssn6p was incubated with beads bound to GST-*S. cerevisiae* Tup1p (7-253) (lane 1), GST-*S. pombe* Tup11p (1-298) (lane 2), or GST alone (lane 3). Input (lane 4) represents 10% of the labeled Ssn6p used in the binding reaction.

1) but not to GST alone (Fig. 4A, lane 2). These data are consistent with our findings that the chimeric proteins CCP and CPP, whose WD repeats are derived from *S. pombe* Tup11p, function in *S. cerevisiae*.

The failure of native *S. pombe* Tup11p protein to complement loss of *TUP1* in *S. cerevisiae* might be due to an inability to bind to *S. cerevisiae* Ssn6p. Therefore, we examined interactions between these two proteins in vitro. The N-terminal region of either *S. cerevisiae* Tup1p (amino acids 7 to 253) or *S. pombe* Tup11p (amino acids 1 to 298) was fused to GST and expressed in *E. coli*. In vitro-translated *S. cerevisiae* Ssn6p was incubated independently with comparable amounts of each of these GST-Tup1p fusion proteins, which were then isolated with glutathione beads. The bound fractions were analyzed by SDS-PAGE (Fig. 4B). While approximately 20% of the input *S. cerevisiae* Ssn6p bound to GST-*S. cerevisiae* Tup1p (Fig. 4B, lane 1), less than 1% bound to GST-*S. pombe* Tup11p (Fig. 4B, lane 2). No binding to GST alone was observed (Fig. 4B, lane 3). We conclude that *S. pombe* Tup11p does not interact efficiently with *S. cerevisiae* Ssn6p, consistent with its inability to complement Tup1p functions in vivo.

***S. pombe* Tup11p binds specifically to histones H3 and H4.** *S. cerevisiae* Tup1p binds to histones H3 and H4 directly (6). Interestingly, the histone binding domain of *S. cerevisiae* Tup1p was replaced with an analogous region of *S. pombe* Tup11p in the chimeric protein CPP, which effectively substituted for *S. cerevisiae* Tup1p in vivo. This observation raises the possibility that *S. pombe* Tup11p also binds to histones. To test this idea, we isolated histones from *S. cerevisiae* and incubated them with the GST-Tup1p fusion proteins described above. As expected, GST-*S. cerevisiae* Tup1p bound to histones H3 and H4 but not to H2A and H2B (Fig. 5A, lanes 2 and 3). Strikingly, the GST-*S. pombe* Tup11p fusion protein also bound specifically to histones H3 and H4 (Fig. 5A, lanes 4 and 5). In contrast, GST alone did not bind effectively to any of the histones (Fig. 5A, lanes 6 and 7).

Binding of *S. pombe* Tup11p to histones H3 and H4 was confirmed by far-Western analysis. Isolated histones were separated by SDS-PAGE, blotted onto a nylon membrane, and then probed with the full-length *S. cerevisiae* Tup1p and *S. pombe* Tup11p proteins, which were transcribed, translated, and labeled with ^{35}S-methionine in vitro (Fig. 5B). The labeled *S. cerevisiae* Tup1p bound to histones H3 and H4, as shown previously (6). Similarly, the labeled, full-length *S. pombe* Tup11p bound specifically to histones H3 and H4. Together these experiments indicate that histone binding is conserved in *S. pombe* Tup11p and *S. cerevisiae* Tup1p and confirm that this binding does not require the C-terminal WD repeat domains of these proteins.

***S. pombe* Tup1p homologs function as repressors in *S. pombe*.** During the course of this study, another sequence similar to Tup1p was added to the *S. pombe* protein database (accession no. 2555018), which we propose to call *tup12*$^+$. Since these two *TUP1* homologs might provide redundant functions, we created mutants with double disruptions in *tup11*$^+$ and *tup12*$^+$. We examined the expression of a glucose-repressible gene, *fbp1*$^+$, in these strains, since in *S. cerevisiae TUP1* regulates some glucose-repressible functions. Total RNA was prepared from isogenic wild-type (JY741), *tup11* (JY741-Δtup11U), *tup12* (JY741-Δtup12L), or *tup11 tup12* double mutant (JY741-Δtup11U, Δtup12L) cells cultivated in either high (8%) or low (0.1% with 3% glycerol) glucose medium. Northern blot analysis showed that *fbp1*$^+$ was repressed in wild-type cells and the *tup11* and *tup12* single disruptants in the presence of 8% glucose (Fig. 6, lanes 1, 3, and 5). However, *fbp1*$^+$ expression was derepressed in the *tup11 tup12* double disruptants (Fig. 6, lane 7), reaching levels of up to 50% of those observed in the wild-type strain under derepressing conditions (Fig. 6, lane 2). Interestingly, under derepressing conditions the transcription levels of *fbp1*$^+$ also increased two- and threefold in the *tup12* and *tup11 tup12* double disruptants, respectively (Fig. 6, lanes 6 and 8), but not in the *tup11* disruptant (Fig. 6, lane 4). Thus, *tup12*$^+$ may limit *fbp1*$^+$ expression even under derepressing conditions. These data indicate that Tup11p and Tup12p are required for full repression of *fbp1*$^+$ and that these proteins provide redundant functions in *S. pombe*.

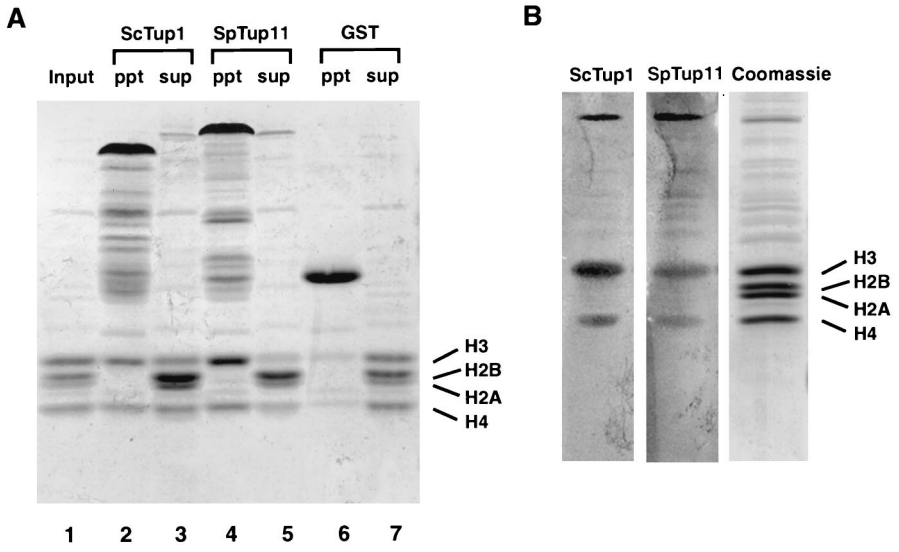

FIG. 5. Interaction of *S. pombe* Tup11p with histones. (A) GST pull-down analysis. Semipurified histones were incubated with beads bound to GST-*S. cerevisiae* Tup1p (amino acids 7 to 253) (lanes 2 and 3), GST-*S. pombe* Tup11p (amino acids 1 to 298) (lanes 4 and 5), or GST alone (lanes 6 and 7). After washing, bound (lanes 2, 4, and 6) and unbound (lanes 3, 5, and 7) fractions were analyzed by SDS-PAGE and visualized by staining with Coomassie brilliant blue R-250. Input (lane 1) represents the total amount of histones used in each binding reaction. (B) Far-Western blot analysis. Samples of yeast histone proteins were separated by SDS-PAGE, electroblotted onto a nylon membrane, and probed with an ^{35}S-labeled *S. cerevisiae* Tup1p (ScTup1) or *S. pombe* Tup11p (SpTup11) probe. A parallel lane was stained with Coomassie brilliant blue R-250. (Coomassie).

DISCUSSION

Tup1p is well characterized as a corepressor in *S. cerevisiae* (7, 38). Here, we report the cloning and characterization of *TUP1* homologs from *K. lactis* and *S. pombe*. We found that the *TUP1* gene from the budding yeast *K. lactis* functionally substituted for *S. cerevisiae TUP1*. Interestingly, the *TUP1* gene from another budding yeast, *C. albicans*, also complemented an *S. cerevisiae tup1* mutation (2), indicating that the mechanism of transcriptional repression by Tup1p is conserved in at least three separate budding yeasts.

Although the *S. pombe tup11*$^+$ gene was not able to complement the *S. cerevisiae tup1* mutation, our data strongly suggest that *S. pombe tup11*$^+$ also encodes a corepressor protein. First, the amino acid sequence of *S. pombe* Tup11p is significantly homologous to Tup1p homologs identified in a number of species, including *Neurospora crassa* Rco-1 (E value of basic local alignment search technique for protein sequences [BLASTP], e^{-124}), *Dictyostelium discoideum* Tup1 (E value of BLASTP, e^{-115}), and *S. cerevisiae* Tup1p (E value of BLASTP, e^{-100}). Second, when *S. pombe* Tup11p was artificially recruited to a promoter region in *S. cerevisiae* via a LexA DNA-binding domain, it repressed transcription of the downstream gene. This same strategy was used by others to define *S. cerevisiae* Tup1p, Ssn6p, and Rpd3p as corepressors (14, 15, 32). Third, a chimeric protein (CPP), which contains the 72 N-terminal amino acids of *S. cerevisiae* Tup1p and the 543 C-terminal amino acids of *S. pombe* Tup11p functionally complemented the *S. cerevisiae tup1* null allele. This result indicates that *S. pombe* Tup11p has corepressor functions but is defective in interacting with *S. cerevisiae* Ssn6p, as confirmed by our biochemical analysis. Fourth, *S. pombe* Tup11p binds to *S. cerevisiae* α2p, suggesting it can be recruited to target promoters by DNA-binding repressors. Fifth, *S. pombe* Tup11p specifically interacts with histones H3 and H4, as does *S. cerevisiae* Tup1p. Many studies indicate that interaction of Tup1p with histones likely plays an important role in the repression mechanism (6, 7). Finally, disruption of *tup11*$^+$ in combination with disruption of *tup12*$^+$, another *TUP1* homolog gene in *S. pombe*, causes a defect in glucose repression of *fbp1*$^+$. Thus, *S. pombe* Tup1p homologs likely function as transcriptional repressors, as does their *S. cerevisiae* counterpart.

Our finding that *S. pombe* Tup11p does not bind to *S. cerevisiae* Ssn6p is consistent with the low conservation of the amino-terminal regions of these proteins (Fig. 1). In contrast, the Ssn6p-binding region of *S. cerevisiae* Tup1p is highly conserved in Tup1ps of other the budding yeasts (*K. lactis* and *C. albicans*), which can complement the *S. cerevisiae tup1* mu-

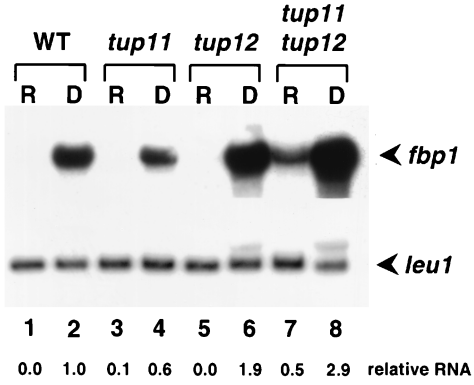

FIG. 6. Transcription of the glucose-repressed *fbp1*$^+$ gene in *tup11* and *tup12* mutants. Total RNA samples were prepared from cells of JY741 (*tup11*$^+$ *tup12*$^+$) (lanes 1 and 2), JY741-Δtup11U (*tup11*::*ura4*$^+$) (lanes 3 and 4), JY741-Δtup12L (*tup12*::*LEU2*) (lanes 5 and 6), JY741-Δtup11U, or Δtup12L (*tup11*::*ura4*$^+$ *tup12*::*LEU2*) (lanes 7 and 8) grown in repressing (R; 8% glucose; lanes 1, 3, 5, and 7) or derepressing (D; 0.1% glucose and 3% glycerol; lanes 2, 4, 6, and 8) conditions. Each RNA sample (10 μg per lane) was separated on an agarose gel in the presence of formaldehyde, blotted onto a nylon membrane, and hybridized with a ^{32}P-labeled PCR product harboring the coding region of *fbp1*$^+$. The same membrane was rehybridized with a ^{32}P-labeled *leu1*$^+$ PCR product as an internal control. Normalized levels of *fbp1*$^+$ RNA relative to *leu1*$^+$ (values averaged from three independent experiments) are shown below the lane numbers. The level of *fbp1*$^+$ in the wild-type strain (WT) under derepressing conditions was set to 1.0 for comparison purposes. Standard deviations were within 10% for all samples except lanes 6 and 8, for which standard deviations were 21 and 27%, respectively.

tations. Interestingly, a database search has revealed a homolog of Ssn6p in *S. pombe* (accession no. 3116127, E value of BLASTP, e^{-129}), and in separate studies, we have shown that *S. pombe* Tup11p interacts with *S. pombe* Ssn6p by two-hybrid and in vitro binding analyses (data to be presented elsewhere). These findings suggests that Tup11p also forms a corepressor complex with Ssn6p to repress transcription in *S. pombe*.

The primary structure of the histone binding region in *S. cerevisiae* Tup1p is not well conserved in either *S. pombe* Tup11p or *K. lactis* Tup1p (Fig. 1). This region is also significantly shorter in *C. albicans* Tup1p, as only 117 amino acids are found between the Ssn6p-binding domain (2) and WD repeats of this protein in contrast to the 260 amino acids in the analogous region of *S. cerevisiae* Tup1p. This diversity of sequence suggests that higher order folding of these regions may be important for forming a histone binding domain.

The mechanism of transcriptional repression by Tup1p-Ssn6p might also be conserved in higher eukaryotes. The Groucho protein in flies and the TLE1 protein in humans have WD repeats and bind to histone proteins (22). TLE1 also binds to a mammalian Ssn6p homolog (9). Groucho binds directly to Hairy-related or Runt domain DNA-binding proteins through the sequence WRPW or WRPY, respectively (8), indicating that it may be recruited to promoters in a manner similar to that of yeast Tup1p. However, the DNA-binding proteins known to interact with *S. cerevisiae* Tup1p do not have WRPW and WRPY motifs, so the details of recruitment are not conserved.

ACKNOWLEDGMENTS

We thank Chikashi Shimoda (Osaka City University, Osaka, Japan) and Kaoru Takegawa (Kagawa University, Kagawa, Japan) for providing the *S. pombe* strains and plasmids and for helpful advice. We also thank members of the Roth lab for helpful discussions.

This work was supported in part by a grant from the NIH (GM51189) to S.Y.R. and by Grants-in-Aid for Scientific Research on Priority Areas (no. 08250210 and no. 09277214) to S.H.

REFERENCES

1. **Balasubramanian, B., C. V. Lowry, and R. S. Zitomer.** 1993. The Rox1 repressor of the *Saccharomyces cerevisiae* hypoxic genes is a specific DNA-binding protein with a high-mobility-group motif. Mol. Cell. Biol. **13:**6071–6078.
2. **Braun, B. R., and A. D. Johnson.** 1997. Control of filament formation in Candida albicans by the transcriptional repressor TUP1. Science **277:**105–109.
3. **Carlson, M.** 1997. Genetics of transcriptional regulation in yeast: connections to the RNA polymerase II CTD. Annu. Rev. Cell Biol. **13:**1–23.
4. **Chen, S., R. W. West, S. L. Johnson, H. Gans, B. Kruger, and J. Ma.** 1993. TSF3, a global regulatory protein that silences transcription of the yeast GAL genes also mediates repression by α2 repressor and is identical to SIN4. Mol. Cell. Biol. **13:**831–840.
5. **Edmondson, D. G., and S. Y. Roth.** 1998. Interactions of transcriptional regulators with histones. Methods **15:**355–364.
6. **Edmondson, D. G., M. M. Smith, and S. Y. Roth.** 1996. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev. **10:**1247–1259.
7. **Edmondson, D. G., W. Zhang, A. Watson, W. Xu, J. R. Bone, Y. Yu, D. Stillman, and S. Y. Roth.** 1998. In vivo functions of histone acetylation/deacetylation in Tup1p repression and Gcn5p activation. Cold Spring Harbor Symp. Quant. Biol. **63:**459–468.
8. **Fisher, A. L., and M. Caudy.** 1998. Groucho proteins: transcriptional corepressors for specific subsets of DNA-binding transcription factors in vertebrates and invertebrates. Genes Dev. **12:**1931–1940.
9. **Grbavec, D., R. Lo, Y. Liu, A. Greenfield, and S. Stifani.** 1999. Groucho/transducin-like enhancer of split (TLE) family members interact with the yeast transcriptional co-repressor SSN6 and mammalian SSN6-related proteins: implications for evolutionary conservation of transcription repression mechanisms. Biochem. J. **337:**13–17.
10. **Herschbach, B. M., M. B. Arnaud, and A. D. Johnson.** 1994. Transcriptional repression directed by the yeast alpha 2 protein in vitro. Nature **370:**309–311.
11. **Huang, L., W. Zhang, and S. Y. Roth.** 1997. Amino termini of histones H3 and H4 are required for a1/α2 repression in yeast. Mol. Cell. Biol. **17:**6555–6562.
12. **Huang, M., Z. Zhou, and S. J. Elledge.** 1998. The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell **94:**595–605.
13. **Jiang, Y. W., and D. J. Stillman.** 1992. Involvement of the SIN4 global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**4503–4514.
14. **Kadosh, D., and K. Struhl.** 1998. Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. Mol. Cell. Biol. **18:**5121–5127.
15. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
16. **Komachi, K., M. J. Redd, and A. D. Johnson.** 1994. The WD repeats of Tup1 interact with the homeo domain protein alpha 2. Genes Dev. **8:**2857–2867.
17. **Kuchin, S., P. Yeghiayan, and M. Carlson.** 1995. Cyclin-dependent protein kinase and cyclin homologs SSN3 and SSN8 contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA **92:**4006–4010.
18. **Lambright, D. G., J. Sondek, A. Bohm, N. P. Skiba, H. E. Hamm, and P. B. Sigler.** 1996. The 2.0 A crystal structure of a heterotrimeric G protein. Nature **379:**311–319.
19. **Macatee, T., Y. W. Jiang, D. J. Stillman, and S. Y. Roth.** 1997. Global alterations in chromatin accessibility associated with loss of SIN4 function. Nucleic Acids Res. **25:**1240–1247.
20. **Moreno, S., A. Klar, and P. Nurse.** 1991. Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods Enzymol. **194:**795–823.
21. **Mukai, Y., Y. Ohno-Yamashita, Y. Oshima, and S. Harashima.** 1997. The role of cysteine residues in the homeodomain protein Mat alpha 2 in mating-type control of Saccharomyces cerevisiae. Mol. Gen. Genet. **255:**166–171.
22. **Palaparti, A., A. Baratz, and S. Stifani.** 1997. The Groucho/transducin-like enhancer of split transcriptional repressors interact with the genetically defined amino terminal silencing domain of histone H3. J. Biol. Chem. **272:**26604–26610.
23. **Park, S. H., S. S. Koh, J. H. Chun, H. J. Hwang, and H. S. Kang.** 1999. Nrg1 is a transcriptional repressor for glucose repression of STA1 gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:**2044–2050.
24. **Redd, M. J., M. B. Arnaud, and A. D. Johnson.** 1997. A complex composed of Tup1 and Ssn6 represses transcription in vitro. J. Biol. Chem. **272:**11193–11197.
25. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Roth, S. Y.** 1995. Chromatin-mediated transcriptional repression in yeast. Curr. Opin. Genet. Dev. **5:**168–173.
27. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Smith, R. L., M. J. Redd, and A. D. Johnson.** 1995. The tetratricopeptide repeats of Ssn6 interact with the homeo domain of alpha2. Genes Dev. **9:**2903–2910.
29. **Sondek, J., A. Bohm, D. G. Lambright, H. E. Hamm, and P. B. Sigler.** 1996. Crystal structure of a G-protein beta gamma dimer at 2.1A resolution. Nature **379:**369–374.
30. **Toh-e, A., Y. Ueda, S.-I. Kakimoto, and Y. Oshima.** 1973. Isolation and characterization of acid phosphatase mutants in *Saccharomyces cerevisiae*. J. Bacteriol. **113:**727–738.
31. **Treitel, M. A., and M. Carlson.** 1995. Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein. Proc. Natl. Acad. Sci. USA **92:**3132–3136.
32. **Tzamarias, D., and K. Struhl.** 1994. Functional dissection of the yeast Cyc8-Tup1 corepressor complex. Nature **369:**758–761.
33. **Tzarmarias, D., and K. Struhl.** 1995. Distinct TPR motifs of CYC8 are involved in recruiting the CYC8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. **9:**821–831.
34. **Varanasi, U. S., M. Klis, P. B. Mikesell, and R. J. Trumbly.** 1996. The Cyc8 (Ssn6)-Tup1 corepressor complex is composed of one Cyc8 and four Tup1 subunits. Mol. Cell. Biol. **16:**6707–6714.
35. **Vidal, M., and R. F. Gaber.** 1991. RPD3 encodes a second factor required to achieve maximum positive and negative transcription states in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**6317–6327.
36. **Vidal, M., R. Strich, R. E. Esposito, and R. F. Gaber.** 1991. RPD1(SIN3/UME4) is required for maximal activation and repression of diverse yeast genes. Mol. Cell. Biol. **11:**6306–6316.
37. **Wahi, M., and A. D. Johnson.** 1995. Identification of genes required for alpha2 repression in Saccharomyces cerevisiae. Genetics **140:**79–90.
38. **Wahi, M., K. Komachi, and A. D. Johnson.** 1998. Gene regulation by the yeast Ssn6-Tup1 corepressor. Cold Spring Harbor Symp. Quant. Biol. **63:**447–457.
39. **Zitomer, R. S., and C. V. Lowry.** 1992. Regulation of gene expression by oxygen in *Saccharomyces cerevisiae*. Microbiol. Rev. **56:**1–11.