Identification and Analysis of Homologues of Saccharomyces cerevisiae Spt3 Suggest Conserved Functional Domains

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Spt3 of Saccharomyces cerevisiae is a factor required for normal transcription from particular RNA polymerase II-dependent promoters. As a step towards analysing Spt3 structure-function relationships, we have identified and studied Spt3 homologues from three other yeasts: Kluyveromyces lactis, Clavispora opuntiae and Schizosaccharomyces pombe. Alignment of their predicted amino acid sequences shows an overall identity of 30% between all four homologues and suggests that three conserved domains are present in Spt3. When tested for function in S. cerevisiae, K. lactis SPT3 was shown to fully complement and S. pombe SPT3 to partially complement an spt3Δ mutation. These data demonstrate that Spt3 is functionally conserved among distantly related yeasts. The new sequences have been entered in GenBank: AF005930 (K. lactis SPT3), AF005932 (C. opuntiae SPT3) and AF005931 (S. pombe SPT3).

INTRODUCTION

The transcription machinery necessary to initiate transcription by RNA polymerase II consists of a number of highly conserved components (Zawel and Reinberg, 1993). This machinery includes RNA polymerase itself as well as the general transcription factors required for the assembly and function of an RNA polymerase II preinitiation complex. These general transcription factors were initially identified from mammalian cells based on their requirement for the accurate initiation of transcription in vitro. To date, all of the general transcription factors that have been identified in humans, TFIIA, TFIIB, TBP, TFIIE, TFIIF, TFIIG and TFIH, are conserved in the yeast Saccharomyces cerevisiae (Zawel and Reinberg, 1995). Thus, the factors, and by extension, the mechanism of transcription by RNA polymerase II, is a highly conserved process across species.

In contrast to the biochemical work that identified general transcription factors, other factors, previously unknown to be important for transcription in human or Drosophila cells, have been identified both genetically and biochemically in yeast and then subsequently shown to be important for transcription in other eukaryotes. For example, members of the RNA polymerase II holoenzyme or mediator complex, called Srb5 (Suppressors of RNA polymerase B), were first identified genetically in yeast and were then biochemically characterized as part of a large complex of proteins associated with yeast RNA polymerase II (Kim et al., 1994; Koleske and Young, 1994; Thompson et al., 1993). This complex was also shown to be required both in vitro and in vivo for activated transcription. Subsequently, using homology to Srb7, an RNA polymerase II holoenzyme was shown to exist in human cells as well (Chao et al., 1996; Ossipow et al., 1995; Tassan et al., 1995). Thus, genetic studies in yeast have led to the identification of previously unidentified proteins important for transcription.

There are many other factors in yeast that have been identified genetically as important for...
transcription in vivo that as yet have no known homologue in larger eukaryotes. One such factor that has not been identified in more complex systems is the yeast TBP-associated factor Spt3. Mutations in SPT3 were isolated as suppressors of Ty element insertions or solo δ insertions in the promoters of the HIS4 and LYS2 genes in S. cerevisiae (Winston et al., 1984, 1987). SPT3 encodes a non-essential nuclear protein that is important for transcription of certain RNA polymerase II-dependent genes in vivo (Hirschhorn and Winston, 1988; Winston et al., 1987). In addition, in vivo and in vitro analyses have shown that Spt3 is a TBP-associated factor and may regulate some aspect of TBP function (Eisenmann et al., 1992). Genetic analysis suggests that Spt3 functionally interacts with Mot1 and the general transcription factor TFIIA to specify TBP binding at certain promoters (Madison and Winston, 1997). One other protein has been identified that has significant homology to Spt3, human TAF, 18 (Mengus et al., 1995). Yet, this human TAF shares greater overall size and sequence homology to another yeast protein, Fun81 (Dubois et al., 1987; Mengus et al., 1995). Recent biochemical and genetic analyses have demonstrated that Spt3 is part of a large complex, SAGA, that contains at least eight previously identified transcription factors, including the histone acetyltransferase Gcn5 (Grant et al., 1997; Horiuchi et al., 1997; Roberts and Winston, 1997).

To determine if Spt3 is a conserved transcription factor, we have sought to clone SPT3 homologues. We report here the cloning and analysis of SPT3 homologues from the yeasts Kluyveromyces lactis, Clavispora opuntiae and Schizosaccharomyces pombe. We show that these SPT3 homologues are highly homologous to S. cerevisiae SPT3 and that K. lactis and S. pombe SPT3 can complement all or some of the mutant phenotypes of an spt3Δ mutant. These studies suggest that Spt3 has three conserved domains and that the mechanism of transcription regulation carried out by Spt3 is conserved in other eukaryotes.

MATERIALS AND METHODS

Yeast strains and genetic methods

The two S. cerevisiae yeast strains used in this study were derived from an S288C GAL2+ derivative (Winston et al., 1995) and were constructed by standard methods (Rose et al., 1990). The strains used were: FY294 (MATa spt3-202 his4-917Δ lys2-173R2 ura3-52 leu2Δ1) and FY 631 (MATa his4-917Δ lys2-173R2 ura3-52 leu2Δ1). The S. pombe strain used was FWP1 (h– ura4-294). The yeast species Wickerhamia fluorescens, C. opuntiae, K. lactis, Pichia strasburgensis, Yarrowia lipolytica, Metschnikowia pulcherrima and Hanseniaspora valbians were provided by Todd Milne and David Weaver and were originally obtained from the American Type Culture Collection, Rockville, Maryland. All S. cerevisiae spt mutations used in this study have been described previously (Eisenmann et al., 1992; Happe and Winston, 1992; Winston et al., 1987). Yeast strains were transformed by a lithium acetate procedure (Elble, 1992). Standard methods of mating, sporulation and tetrad analysis were used (Rose et al., 1990).

Media

Rich (YPD), minimal (SD), synthetic complete (SC), 5-FOA, and sporulation media were prepared as described (Rose et al., 1990). Suppression of insertion mutations was scored on SD media supplemented with particular nutrients or on SC media lacking appropriate nutrients. Yeast transformants were selected on the appropriate SC media.

DNA preparation and analysis

Escherichia coli strains HB101 and DH5α were used as hosts for plasmids (Sambrook et al., 1989). Plasmids were constructed, maintained and isolated by standard methods (Sambrook et al., 1989). Plasmids were recovered from yeast as described previously (Robzyk and Kassir, 1992). Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs (Beverly, Massachusetts) and Boehringer Mannheim Biochemicals (Indianapolis, Indiana), and used as recommended by the manufacturer.

DNA sequence analysis

For sequencing portions of SPT3 homologues from K. lactis, C. opuntiae and S. pombe, the appropriate restriction fragments were subcloned into pRS426. Sequencing was performed using a protocol provided by the US Biochemical Corp. Sequenase (TM) Version 2.0 kit. Synthetic primers and m13 universal and reverse primers were used to determine the complete sequence on both strands. The sequence was analysed using
the BLAST program (Altschul et al., 1990) and compared against known sequences and proteins in the GenBank, EMBL and PIR databases. Sequence alignment was also carried out by the Megalign program of the DNAStar sequence analysis program (DNAStar, Inc.).

Plasmids

The pRS series (Christianson et al., 1992; Sikorski and Hieter, 1989) and pRS3MET25 vectors (Mumberg et al., 1994) have been described previously. The remaining plasmids used in this study are described below and listed in Table 2. The plasmid pDE121-6 was generated by PCR-amplifying the SPT3 open reading frame with primers containing BamHI sites, digesting the PCR product, and subcloning into the BamHI site of pRS316.

Cloning K. lactis and C. opuntiae SPT3 homologues

Genomic DNAs from nine yeasts were isolated as described previously (Hoffman and Winston, 1987). Southern blots were performed as described below to identify sequences that cross-hybridized with a full-length SPT3 probe. Unless otherwise noted, all Southern blots were performed with a 32P-dATP-labeled BamHI fragment from pDE121-6, containing only the SPT3 open reading frame. To clone SPT3 sequences from K. lactis and C. opuntiae, genomic yeast DNA was digested with one or two of the following enzymes, HindIII, EcoRI, SacI, and then electrophoresed on 1% TBE gels. Separated DNA fragments were then transferred to Genescreen (New England Nuclear) by Southern blot. Hybridization was carried out using the following solution for prehybridization and hybridization: 10 x Denhardt’s Solution, 3 x SSC, 50 mM HEPES pH 8.0, 50 mg/ml yeast tRNA, 20% deionized formamide. Blots were prehybridized and hybridized at 42°C overnight. Blots were washed in two changes of 2 x SSC, 15 min each at room temperature, followed by washing in two changes of 0.1 x SSC/0.1% SDS, 30 min each at 35°C. Following identification of cross-hybridizing bands, gel slices containing the cross-hybridizing fragments and all other fragments of hybridizing bands, gel slices containing the cross-hybridizing fragments and all other fragments of hybridizing fragments from the gel and rePCR product were excised from the gel, ligated into pRS426, and transformed into DH5α to generate individual size-selected libraries of K. lactis and C. opuntiae genomic DNA. Bacterial colonies from these libraries were plated and colony lifts on Genescreen filters were prepared. Colony lifts were processed as described previously (Buluwela et al., 1989) and then hybridized with a full-length 32P-labeled SPT3 probe as described for the Southern analysis above. DNA was isolated from individual cross-hybridizing colonies and subjected to Southern analysis with a full-length 32P-labeled SPT3 probe to verify the initial cross-hybridization results. Colonies that were positive in secondary screens were chosen for sequencing. Sequencing revealed the presence of an open reading frame from K. lactis and from C. opuntiae with homology to the S. cerevisiae SPT3 gene. The K. lactis SPT3 and C. opuntiae SPT3 open reading frames in pJM1 and pJM3 respectively were sequenced completely on both strands.

Cloning an S. pombe SPT3 homologue

Based on the homology determined by alignment of the K. lactis, C. opuntiae and S. cerevisiae Spt3 predicted amino acid sequences, degenerate PCR primers were designed to isolate an S. pombe SPT3 homologue. A schematic summary of the primer positions in the SPT3 sequence is presented in Figure 1A.

PCR was performed, followed by cross-hybridization with a full-length SPT3 probe as described above for K. lactis and C. opuntiae genomic Southern analysis. Elution of the cross-hybridizing fragments from the gel and rePCR with internal primers yielded a single PCR product of approximately 215 base pairs after three rounds of PCR. The degenerate primer pairs that resulted in the final product are for the first round: JM P2 [G/G I A C G T I A T (A/T/C) G A (G/A) A T]’ M P8 [(A/G) C A I A C (T A G) A T (T/C) T C (A/G) A A I G T]; and for the second and third rounds: JM P3 [C A (T/C) G A (T/C) A A (A/G) G T]’ M P6 [C C A (G/A) T G I A C (G/A) T A (T/C) T C (T/C) T C]. I indicates positions that contain inosine. The conditions for PCR were as follows (using a Perkin Elmer 9600 PCR machine): 94°C, 3 min, 1 cycle; 94°C, 30 s, 35°C, 30 s, the temperature was ramped from 35°C to 72°C over a 2-min period and then remained at 72°C for 1 min, 3 cycles; 94°C, 15 s, 15°C, 15 s, 72°C, 15 s, 30 cycles; 72°C, 5 min. This PCR product was phosphorylated and blunt-end cloned into the Smal site of pRS426 to generate pJM5. Sequencing of pJM5 using the universal and reverse primer (New England Biolabs, Beverly, MA) revealed a partial open reading frame encoding a predicted polypeptide with homology
S. cerevisiae, K. lactis and C. opuntiae Spt3 predicted amino acid sequences. This S. pombe PCR product was labeled and used to screen a genomic Southern containing both S. pombe and S. cerevisiae genomic DNA. Approximately 10,000 colonies of an S. pombe genomic library (Barbet et al., 1992; kindly provided by Tamar Enoch, Harvard University) were screened by colony hybridization and a single cross-hybridizing clone called pJM 14 was obtained that contained a complete S. pombe SPT3 open reading frame. An Hae III fragment containing the S. pombe SPT3 open reading frame was subcloned into the Sma I site of pRS426 to generate pJM15 and the complete S. pombe SPT3 open reading frame was sequenced on both strands.

Expression of SPT3 homologues

To express each SPT3 homologue under the same heterologous promoter, each yeast homologue was PCR-amplified to generate an N-terminal HA-1 epitope-tagged gene with unique restriction sites at the 5' and 3' ends, and was then subcloned under the control of the MET25 promoter. The homologue expression plasmids are listed in Table 1 and their construction is summarized as follows. To generate SPT3 N-terminal HA-1 epitope-tagged PCR fragments, a 5' primer was synthesized that contained a SpeI site for

<table>
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<th>Plasmid</th>
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<tr>
<td>pJM 1</td>
<td>C. opuntiae EcoRI genomic fragment containing C. opuntiae SPT3 in pRS426</td>
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<tr>
<td>pJM 3</td>
<td>K. lactis EcoRI genomic fragment containing K. lactis SPT3 in the EcoRI site of pRS426</td>
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<tr>
<td>pJM 4</td>
<td>K. lactis EcoRI-HindIII fragment in the EcoRI-HindIII site of pRS426</td>
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<tr>
<td>pJM 5</td>
<td>PCR product of S. pombe SPT3 cloned into the Smal site of pRS426</td>
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<tr>
<td>pJM 14</td>
<td>S. pombe SPT3 genomic clone from S. pombe REP genomic library</td>
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<tr>
<td>pJM 15</td>
<td>2.5 kb HaelII S. pombe SPT3 genomic fragment from pJM 14 in Smal site of pRS426</td>
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<tr>
<td>pJM 198</td>
<td>HA-1 tagged S. cerevisiae SPT3 amino acids 1-315 in the SpeI-BamHI sites of p425MET25</td>
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<tr>
<td>pJM 183</td>
<td>HA-1 epitope-tagged S. cerevisiae SPT3 cloned into the SpeI-BamHI site of p425MET25</td>
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<tr>
<td>pJM 184</td>
<td>HA-1 epitope-tagged C. opuntiae SPT3 cloned into the SpeI-BamHI site of p425MET25</td>
</tr>
<tr>
<td>pJM 185</td>
<td>HA-1 epitope-tagged S. pombe SPT3 cloned into the SpeI-XhoI site of p425MET25</td>
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<tr>
<td>pJM 186</td>
<td>HA-1 epitope-tagged K. lactis SPT3 cloned into the SpeI-BamHI site of p425MET25</td>
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Figure 1. Isolation of an S. pombe SPT3 homologue. (A) Diagram of degenerate primers. The thick line represents the SPT3 gene and the thinner lines are degenerate PCR primers used to PCR an S. pombe SPT3 gene. The numbers represent each degenerate primer JMP2, JMP3, JMP6 and JMP8. (B) Genomic Southern blot with the putative S. pombe SPT3 gene. The PCR product isolated from S. pombe SPT3 with the primers described in Figure 1A was labeled with [32P]dATP and used to probe a genomic Southern blot containing S. pombe genomic DNA in lanes 1–3 and S. cerevisiae genomic DNA in lane 4. Genomic DNAs were digested with the restriction enzymes as indicated in each lane, run on a 1% agarose gel, transferred to Genescreen, and then hybridized with the probe. DNA digested with BstEII is in lane 5 as a size marker. Abbreviations for restriction enzymes are: RI, EcoRI; HII, HindIII; BII, BstEII.

Table 1. Plasmids

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identification and analysis of homologues of Saccharomyces cerevisiae Subcloning and that encoded the initiator methionine codon, an HA-1 epitope tag containing the epitope YPYDVPDYA, and the next five amino acids of the desired SPT3 homologue. The 3' primer contained a BamHI site used for subcloning, a translation termination codon, and DNA sequences encoding the last five C-terminal residues of the protein to be expressed. To clone the S. pombe SPT3 homologue, a XhoI site, instead of a BamHI site, was used for the 3' primer of the S. pombe expression construct, due to the presence of an internal BamHI restriction site. PCR was carried out with Pfu Polymerase (Stratagene) using the following conditions (on a Perkin Elmer 9600 PCR machine): 94°C, 3 min, 1 cycle; 94°C, 1 min, 55°C, 1 min, 72°C, 2 min for 30 cycles; 72°C, 5 min, 1 cycle. PCR products were extracted once with phenol:chloroform, ethanol precipitated, cut with the appropriate restriction enzymes and ligated into either the Spel and BamHI sites or the Spel and XhoI sites of the p425M ET25 vector. Five clones for each homologue were tested for complementation of an spt3Δ mutation after transformation into FY294.

Extract preparation and Western analysis of strains expressing SPT3 homologues All homologues were expressed from the MET25 promoter on plasmid p425M ET25 in media lacking methionine and leucine. For Western blots, FY294, an spt3Δ strain, or FY631, an SPT3+ strain, were grown in SD media supplemented with the appropriate amino acids. Cultures (10 ml) were grown overnight at 30°C to saturation and then diluted to 1 × 10⁶ cells/ml and grown to a density of 1–2 × 10⁷ cells/ml. Cultures were harvested, and extracts were prepared by washing once in ice-cold buffer H consisting of 25 mM-Tris pH 7.4, 15 mM-EGTA, 15 mM-MgCl₂, 1 mM-DTT, 0.1% Triton-X-100, 1 mM-NaN₃, 0.2 mM-phenyl methyl sulfonyle fluoride and then quick frozen in an ethanol dry-ice bath. Cells were broken by adding 250 μl cold bufer H to the cell pellet, glass beads to the minicure, and vortexing at 4°C for 5 min. Another 200 μl of buffer H was added, the extract was vortexed briefly, and the supernatant was transferred to a clean tube. Extracts were spun for 10 min at 14,000 rpm in a Eppendorf microcentrifuge. The supernatant was removed to a fresh tube and extracts were spun for a further 10 min at 14,000 rpm at 4°C. Protein concentrations were determined with Bio-Rad Bradford Reagent diluted 1:4 with water. Bovine serum albumin (BSA) was used as a standard.

Extracts prepared from strains expressing SPT3 homologues were run on 12.5% SDS-polyacrylamide gels. Following separation, proteins were transferred to an Immobilon membrane (New England Nuclear) with a semi-dry electrophot blot apparatus (W.E.P. Company, Seattle, Washington) using a transfer buffer consisting of 50 mM-Tris, 28 mM-glycine. Following transfer, even loading and transfer were verified by Ponceau S staining. Subsequently, membranes were blocked with 1% BSA, 1% milk (Carnation) for 45–120 min at room temperature or overnight at 4°C. A mouse monoclonal 12CA5 antisera against the HA-1 epitope (Boehringer Mannheim) was used at a dilution of 1:1000 in 1% BSA, 1% milk. Membranes were probed for 1 h at room temperature with rocking and then washed with agitation in 1 × TBS, 0.1% Tween for 45 min at room temperature with three changes of wash solution. Membranes were then incubated with a secondary goat anti-mouse antibody (Bio-Rad) conjugated to horseradish peroxidase at a concentration of 1:10,000 in 1% BSA, 1% milk with rocking for 1 h at room temperature. Membranes were then washed with agitation for 45–120 min in 1 × TBS, 0.1% Tween with three changes of wash solution. Western blots were developed using the ECL Detection System (Amersham) and exposed to autoradiographic film.

RESULTS Cloning K. lactis and C. opuntiae SPT3 homologues To learn more about the structure and conservation of Spt3, we decided to clone SPT3 homologues from other yeast species. As a first step, Southern blots containing genomic DNA from nine yeast species, digested as described in Materials and Methods, were hybridized with a 32P-labeled full-length SPT3 probe. Hybridization was detected with five yeasts. The degree of hybridization from greatest to least was with K. lactis, C. opuntiae, P. strastburgensis, W. fluorescens and P. heedi (data not shown). Yeasts that did not show cross-hybridization under the conditions used here were Y. lipolytica, M. pulcherrima, H. valbyensis and S. pombe (data not shown).

Based on the Southern hybridization experiments, we chose to clone SPT3 homologues from...
K. lactis and C. opuntiae. Individual size-selected libraries were constructed from both K. lactis and C. opuntiae based on the fragment sizes detected by the Southern blots. These libraries were then screened by colony hybridization using \( \alpha\-^{32}\text{P} \)-labeled SPT3 probe. Single positive clones for K. lactis and C. opuntiae were sequenced and each clone contained an open reading frame with significant homology to S. cerevisiae SPT3. The predicted amino acid sequences of K. lactis Spt3 and C. opuntiae Spt3 are shown in Figure 2A.

Cloning an S. pombe SPT3 homologue

We used a degenerate PCR approach to clone an S. pombe SPT3 homologue. Based on the predicted regions of highest homology between S. cerevisiae, K. lactis and C. opuntiae SPT3, degenerate primers containing inosine at positions of highest degeneracy were designed corresponding to several highly conserved amino acid stretches (summarized in Figure 1A). PCR, Southern hybridization, and reamplification of cross-hybridizing PCR fragments, as described in Materials and Methods, resulted in the isolation of a single PCR fragment (data not shown). To confirm that this band was actually S. pombe DNA and not a contaminating S. cerevisiae fragment, the resulting single band was \( \alpha\-^{32}\text{P} \)-labeled and used to probe S. pombe and S. cerevisiae genomic DNA. As shown in Figure 1B, this PCR fragment cross-hybridized strongly to a single band in S. pombe genomic DNA and cross-hybridized poorly with S. cerevisiae genomic DNA. This PCR product was subcloned and sequenced. Sequence analysis revealed a partial open reading frame with significant homology to all three yeast SPT3 homologues. This PCR product was used to screen a genomic S. pombe DNA library to isolate a full-length S. pombe SPT3 gene that was subsequently sequenced and for which the predicted amino acid sequence is shown in Figure 2A.

Alignment of SPT3 homologues

Alignment of the predicted Spt3 homologue amino acid sequences revealed significant homology to S. cerevisiae Spt3 over the complete length of the sequence. The overall identity of these homologues is 30% while the similarity is 50% as determined by sequence analysis with the BESTFIT sequence comparison program (Genetics Computer Group, Inc.). The similarity among these homologues is summarized in Table 2. These data suggest that phylogenetically S. cerevisiae is related to these homologues in the following order from most to least similar, with the similarity in parentheses: K. lactis (78%), C. opuntiae (61%), S. pombe (53%). Furthermore, the homology appears to be clustered in three regions (Figure 2B). The largest homology region (1 in Figure 2B) is in the N-terminal half of the sequence.
alignment from residues 35 to 111. This region is also homologous to S. cerevisiae Fun81 and human TAF₁₁₁₈, although the homology is not as great (M engus et al., 1995). For example, this Spt3 region shares 30% identity with Fun81 and human TAF₁₁₁₈ (data not shown). In the C-terminal half of the Spt3 homologues, two other homologous regions are found. Region number 2 (residues 196 to 234) has also been identified by a cluster of mutations in a hunt for dominant negative Spt phenotype (D. Rose and F. Winston, unpublished). Region 3 (residues 248–271) contains the residues E240, the position of allele-specific changes that suppress particular mutations in TBP (Eisenmann et al., 1992). E240 is conserved in each homologue. Taken together, the homology regions defined by the alignment of these SPT3 homologues suggest that Spt3 may have several functional domains.

Functional analysis of SPT3 homologues

To determine if these SPT3 homologues were also functional homologues of S. cerevisiae SPT3, we tested each one for complementation in an spt3Δ mutant. Previous analysis has shown that spt3Δ mutations suppress the insertion mutations his4-917α and lys2-173R2. A n SPT3+ strain with his4-917α and lys2-173R2 is His− Lys+, while an spt3Δ strain is His+ Lys− (Winston et al., 1987). Weak spt3 mutations have a His+ Lys+ phenotype (Eisenmann et al., 1992). To measure the function of the SPT3 homologues, we tested for complementation of these Spt− phenotypes. Each HA-1 epitope-tagged homologue was cloned under the control of the MET25 promoter in a high copy plasmid and transformed into SPT3+ and spt3Δ strains. S. cerevisiae SPT3 had been determined previously to be unaffected by an epitope tag at the N-terminus (Eisenmann et al., 1992). Complementation of spt3Δ was assessed by observing growth on SD-His and SD-Lys media as described in Materials and Methods.

The results (Fig. 3) demonstrate that two of the SPT3 homologues can fully or partially complement an spt3Δ mutation. Expression of S. cerevisiae or K. lactis SPT3 fully complemented spt3Δ, as judged by their His− Lys+ phenotypes. Expression of S. pombe SPT3 resulted in partial complementation of spt3Δ as judged by a His+ Lys+ phenotype. Finally, expression of C. opuntiae resulted in no complementation, and a His+ Lys− phenotype similar to an spt3Δ strain. None of the SPT3 homologues affected the Spt+ phenotype of the wild-type strain.

To determine if complementation by each of the homologues correlated with its level of protein expression, Western immunoblot analysis was carried out. As shown in Fig. 4, this analysis revealed different levels of expression of the S. cerevisiae, K. lactis and S. pombe SPT3 proteins. S. cerevisiae and K. lactis Spt3 were expressed the best, with K. lactis Spt3 being expressed at slightly lower levels than S. cerevisiae Spt3. S. pombe SPT3 was expressed at lower levels than either S. cerevisiae and K. lactis. No band for C. opuntiae was observed even at long exposures (data not shown).

The S. pombe and K. lactis Spt3 proteins were observed to be smaller than S. cerevisiae Spt3, consistent with the size difference of the proteins being expressed. Thus, expression levels of Spt3
homologues roughly correlate with the ability to complement spt3Δ, and lower expression may account for the lack of complementation by the S. pombe and C. opuntiae Spt3 homologues.

**DISCUSSION**

In these studies we have identified and analysed three homologues of S. cerevisiae SPT3. Several pieces of data support this conclusion. First, the overall size and sequence homology between S. cerevisiae SPT3 and the SPT3 homologues of S. pombe, C. opuntiae and K. lactis suggest that these genes are true homologues. Second, expression of some of these SPT3 homologues in an spt3Δ strain resulted in partial to complete complementation of the Spt null phenotype. These data demonstrate the existence of functional SPT3 homologues in other eukaryotes.

The sequence alignment and the functional analysis of Spt3 homologues suggest possible functional domains in Spt3. Most significantly, the first Spt3 homology region (Figure 2B) extends over much of the N-terminus and also shares homology with yeast Fun81 and human TAF118. Yeast Fun81 has recently been shown to associate with TBP and is a sequence homologue of TAF118 (Moqtaderi et al., 1996). This homology suggests that region 1 of Spt3 might contain a TBP interaction surface. It is unlikely that Fun81 and Spt3 have overlapping functions because overexpression of Fun81 shows no suppression of any phenotypes of an spt3Δ mutation (J. M. Madison and F. Winston, unpublished data) and unlike SPT3, FUN81 is an essential gene. The second Spt3 homology region contains a region mutated in a number of dominant negative spt3 mutants that have been isolated recently (D. Rose and F. Winston, unpublished results). The third homology region of Spt3 includes the residue E240. This residue is altered by an spt3 mutation that causes allele-specific interactions between Spt3 and TBP (Eisenmann et al., 1992). Thus, this region of Spt3 may also be important in interactions with TBP.

In conclusion, we have identified and studied SPT3 homologues in other yeasts. Functional analysis strongly suggests that SPT3 has been conserved in other eukaryotes. In support of this, a human homologue of SPT3 has recently been identified (J.-M. Yuan, J. Madison, S. Mundlos, F. Winston and B. Olsen, in preparation). The cloning of these SPT3 homologues and their analysis will help guide both biochemical and genetic experiments to unravel the function of Spt3 in the regulation of RNA polymerase II transcription.

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