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SPT20/ADA5 Encodes a Novel Protein Functionally Related to the TATA-Binding Protein and Important for Transcription in *Saccharomyces cerevisiae*

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Mutations selected as suppressors of Ty and solo δ insertion mutations in *Saccharomyces cerevisiae* have identified a number of genes important for transcription initiation. One of these genes, *SPT15*, encodes the TATA-binding protein, and three others, *SPT3*, *SPT7*, and *SPT8*, encode proteins functionally related to the TATA-binding protein. To identify additional related functions, we have selected for new *spt* mutations. This work has identified one new gene, *SPT20*. Null mutations in *SPT20* cause poor growth and a set of severe transcriptional defects very similar to those caused by null mutations in *SPT3*, *SPT7*, and *SPT8* and also very similar to those caused by certain missense mutations in *SPT15*. Consistent with it having an important function in transcription in vivo, *SPT20* was also recently identified as *ADA5* and has been shown to be important for transcriptional activation (G. A. Marcus, J. Horiuchi, N. Silverman, and L. Guarente, Mol. Cell. Biol. 16:3197–3205, 1996).

Eukaryotic transcription initiation is regulated by a variety of both positive and negative *trans*-acting factors. Many of these factors have been identified by biochemical studies that have reconstituted regulated transcription initiation in vitro. These regulatory factors are believed to act via interactions with more general transcription factors, including RNA polymerase II, the TATA-binding protein (TBP), TBP-associated factors (TAFs), and other proteins that form the preinitiation complex in vitro. Most of these general factors are conserved throughout eukaryotes (8, 14, 16, 26). Other factors that are essential or important for transcriptional control have been identified by genetic studies, primarily in the yeast *Saccharomyces cerevisiae*. Many of these factors are encoded by the *SNF/SWI* (9, 41), *SRB* (30), *ADA* (5, 27, 36), *TSF* (12), *SUA* (6, 43), *SIN* (23), and *SPT* (62) genes. In vitro activities have been established for some but not all of these functions (5, 15, 20, 28, 29, 60).

The *SPT* genes, which encode both positive and negative regulators of transcription, were isolated as suppressors of Ty and solo δ insertion mutations (62). These Ty and δ insertion mutations, generally in the promoter regions of genes, abolish or alter transcription of the adjacent gene, causing an auxotrophy (7, 62). Mutations in *SPT* genes alter transcription in vivo to restore functional transcription. *spt* mutations also cause a variety of other transcriptional effects in vivo. Genetic and molecular studies place many *SPT* genes into two groups. The first group, the histone group, encodes histones and at least four other products whose functions are believed to control transcription by causing changes in chromatin structure. The second group, the TBP group, includes *SPT15*, the gene encoding TBP, and three other genes, *SPT3*, *SPT7*, and *SPT8*, whose products are believed to be related to TBP function.

Two types of studies indicate that Spt3p, Spt7p, and Spt8p help TBP to function at certain TATA regions. First, null mutations in *SPT3*, *SPT7*, and *SPT8* and certain missense mutations in *SPT15* all cause common mutant phenotypes, including defective expression from certain promoters (Ty, *MFa1*, and *MFα1*) but not others (*PYK1*, *TUB2*, and *TPI1*) (18–20, 25, 65, 67). Second, previous studies have provided evidence that TBP and Spt3p interact (17) and that Spt8p helps to promote the TBP-Spt3p interaction (18). The Spt3p protein, therefore, likely functions in a complex with TBP, and Spt8p may control formation of this complex. One possible mechanism for Spt3p, Spt7p, and Spt8p is that they directly assist TBP binding to DNA or that they stabilize a TBP-TATA interaction in a manner similar to that for the mammalian activator Zta/Zebra (32).

In this work we wanted to determine if there are additional Spt proteins that work in conjunction with Spt3p, Spt7p, and Spt8p to affect TBP function. The existence of such proteins seemed likely, since past mutant hunts identified mainly *spt3* mutants and very few mutations in some of the other *SPT* genes (65). Therefore, we performed a mutant hunt to identify new members of the TBP group of *SPT* genes under conditions in which *spt3* and *spt15* mutations would not be detected. One of the mutations identifies a new gene, *SPT20*. Our studies demonstrate that *spt20* mutants have severe transcription defects and are phenotypically similar to *spt3*, *spt7*, *spt8*, and *spt15* mutants, suggesting that Spt20p functions in transcription initiation in conjunction with Spt3p, Spt7p, Spt8p, and TBP. *SPT20* has also been recently identified by a different mutant hunt as a member of the *ADA* group of genes, *ADA5* (35). This combination of studies suggests that *SPT20* plays an important role in transcription in vivo.

MATERIALS AND METHODS

Yeast strains and media. Table 1 lists all *S. cerevisiae* strains used in this study. All yeast strains are congenic to FY2, an S288C derivative (66).

To create strains that contain duplications of *SPT3* and *SPT15*, the integrating plasmid pSR13 was linearized at the unique *Stu*I site in *URA3* and used to transform strain FY1085. One of the Ura$^+$ transformants was used to generate strains FY1082 and FY1083 from crosses. The gene duplications encoded by pSR13 are indicated by brackets in Table 1. The *his4-917*δ and *his4-912*δ Ty insertion mutations have been described previously (62).

The *spt20* null strains were constructed by transforming diploid strain FY632 with the 2.3-kb *Cla*I-*Xba*I fragment from pSR56, which contains the *spt20Δ100*::*URA3* allele. One of the resulting Ura$^+$ transformants was sporulated and dis-

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TABLE 1. *S. cerevisiae* strains

Strain	Genotype
L914	*MAT*α *leu2Δ1 lys2-173R2 ura3-52 his4-917δ spt8-113*
FY3	*MAT***a** *ura3-52*
FY21	*MAT***a** *ura3-52*
FY51	*MAT***a** *his4-917δ trp1Δ63 ura3-52 spt3-203::TRP1 leu2Δ1*
FY66	*MAT*α *leu2Δ1 his4-917δ*
FY67	*MAT***a** *trp1Δ63*
FY70	*MAT*α *leu2Δ1*
FY154	*MAT***a** *his4-917δ lys2-173R2 trp1Δ1 ura3-52*
FY463	*MAT*α *his4-917δ lys2-173R2 trp1Δ63 leu2Δ1 ura3-52 spt8-302::LEU2*
FY630	*MAT*α *his4-917δ lys2-173R2 trp1Δ63 leu2Δ1 ura3-52*
FY631	*MAT*α *his4-917δ lys2-173R2 trp1Δ63 leu2Δ1 ura3-52*
FY632	*MAT***a**/*MAT*α *his4-917δ/his4-917δ trp1Δ63/trp1Δ63 leu2Δ1/leu2Δ1 ura3-52/ura3-52 lys2-173R2/lys2-173R2*
FY963	*MAT***a** *his4-917δ ura3-52 leu2Δ1 spt7Δ402::LEU2*
FY1082	*MAT*α *his4-917δ lys2-173R2 can1 cyh2 leu2Δ1* [*ura3-52 SPT3 SPT15 URA3*] *trp1Δ63*
FY1083	*MAT***a** *his4-917δ lys2-173R2 can1 cyh2 ade8* [*ura3-52 SPT3 SPT15 URA3*] *trp1Δ63*
FY1084	*MAT***a** *ade8 ura3-52 cyh2*
FY1085	*MAT*α *leu2Δ1 lys2-173R2 his4-917δ trp1Δ63 ura3-52 can1*
FY1086	*MAT*α *leu2Δ1 lys2-173R2 his4-917δ trp1Δ63* [*ura3-52 SPT3 SPT15 URA3*] *can1*
FY1087	*MAT*α *his4-917δ leu2Δ1 spt3Δ203::TRP1 trp1Δ63 ura3-52 can1*
FY1088	*MAT***a** *his4-917δ lys2-173R2 trp1Δ63 spt3Δ203::TRP1 ade8 can1 cyh2*
FY1089	*MAT*α *spt7-217 his4-917δ leu2Δ1 lys2173R2 trp1Δ63*
FY1090	*MAT***a** *his4-917δ spt8-302::LEU2 leu2Δ1 lys2-173R2 ura3-52 ade8 cyh2 trp1Δ63*
FY1091	*MAT*α *his4-917δ leu2Δ1 spt15-21 ura3-52 trp1Δ63 lys2-173R2*
FY1092	*MAT***a** *ade8 his4-917δ spt15-21 lys2-173R2 trp1Δ63 ura3-52 cyh2*
FY1093	*MAT***a** *ade8 his4-917δ lys2-173R2 leu2Δ1 spt7Δ402::LEU2 ura3-52 trp1Δ63*
FY1095	*MAT***a** *his4-917δ lys2-173R2 spt20Δ100::URA3 trp1Δ63 leu2Δ1 ura3-52*
FY1096	*MAT*α *leu2Δ1 spt20Δ100::URA3 ura3-52*
FY1097	*MAT***a** *trp1Δ63 spt20Δ100::URA3 ura3-52*
FY1098	*MAT***a** *his3Δ200 spt20Δ100::URA3 leu2Δ1 ura3-52*
FY1099	*MAT*α *leu2Δ1 his4-917δ spt20-61 ura3-52 can1*
FY1102	*MAT***a** *his4-912δ lys2-128δ trp1Δ63 ura3-52 leu2Δ1 SPT5::URA3*
FY1106	*MAT*α *his4-917δ lys2-173R2 spt20Δ100::URA3 trp1Δ63 leu2Δ1 ura3-52*
FY1111	*MAT***a** *his4-917δ lys2-173R2 spt20-61 ura3-52 can1 ade8 cyh2*

sected to generate haploid *spt20Δ100::URA3* progeny. In all strain designations, the *spt20Δ100::URA3* allele is denoted *spt20Δ100.*

Rich (yeast extract-peptone-dextrose [YPD]), minimal (synthetic dextrose), synthetic complete, and sporulation media were prepared as described previously (51). Standard protocols for transformation and tetrad analysis were used in strain constructions (51). To measure sporulation frequency, cultures were incubated for 1 day at 25°C and for 3 days at 30°C in liquid sporulation medium with constant agitation. Sporulated cultures were visualized by light microscopy and quantitated with a hemacytometer. The sporulation frequency is defined as the number of tetrads observed divided by the sum of tetrads and unsporulated cells. Values are the averages for three diploids for each set of parents.

Plasmids. All plasmids were constructed by standard procedures (3). The pRS series of yeast-*Escherichia coli* shuttle vectors (13, 55) was used for all subcloning experiments.

The plasmid pSR13, used to construct the gene duplications in FY1082 and FY1083, contains the 2.5-kb *Eco*RI-*Bgl*II *SPT3* fragment from pFW27 (63) and the 2.0-kb *Eco*RI *SPT15* fragment from pFW218 (19) subcloned into pRS406.

pSR34 and pSR35 are two of the original clone isolates that contain *SPT20*. Several subclones were created for analysis of *SPT20*-complementing activity. Plasmids pSR36 and pSR38 were constructed by subcloning the 8-kb *Hin*dIII fragment of pSR34 into pRS316 and pRS306, respectively. pSR37 contains the 8-kb *Hin*dIII fragment from pSR35 in pRS316. pSR40 contains the 3.4-kb *Cla*I fragment from pSR37 in pRS316. pSR46 is a pSR40 derivative that lacks the 750-bp *Xba*I-*Xba*I fragment. The 270-bp *Nhe*I-*Xho*I fragment was removed from pSR46 to form pSR48.

pSR56 contains the *spt20Δ100::URA3* allele. This plasmid was made by subcloning *URA3* into *SPT20* between the *Bst*XI and *Hpa*I sites of pSR46. Thus, in *spt20Δ100::URA3*, codons 11 to 403 of *SPT20* are replaced with the *URA3* gene.

Plasmid pSR65 encodes HA1 epitope-tagged Spt20p, which was constructed in two steps. First, plasmid pSR38 was digested with *Bst*XI to release a 3.8-kb fragment. The remaining vector sequence was blunt ended with T4 DNA polymerase and religated with the insertion of phosphorylated 8-bp *Eco*RI linkers (New England BioLabs) to create plasmid pSR60. Then, the 2.3-kb *Eco*RI-*Xho*I fragment from pSR60 encoding Spt20p amino acids 15 to 604 was cloned into pJG4-6 (22) to make the *TRP1*, 2μm plasmid pSR65. The HA1 epitope-Spt20p junction of pSR65 was shown to be in frame by DNA sequence analysis. Expression of HA1-Spt20p is under control of the *GAL1* promoter.

The plasmid pSR77, encoding untagged Spt20p, for use as a negative control for pSR65 has the 2.3-kb *Eco*RI-*Xho*I fragment from pSR60 cloned into pJG4-4 (22). The parental vector, pJG4-4, is similar to pJG4-6 but lacks the HA1 epitope sequence. Although both pSR65 and pSR77 can complement an *spt20Δ100* mutation, Western blot (immunoblot) analysis demonstrated that only HA1-*SPT20* is recognized by the 12CA5 antibody to the HA1 epitope (48).

Isolation and characterization of new *spt* mutants. To select for His$^+$ revertants of *his4-917δ*, 76 independent cultures of strains FY1082 and FY1083 were grown to saturation in synthetic complete medium lacking uracil. Cells were washed two times in sterile water, and then 5×10^6 cells were spread onto synthetic complete medium plates lacking histidine and uracil. Four of the plates were then irradiated with 300 ergs of UV light per mm^2. An average of 8 His$^+$ colonies appeared on plates which had not been irradiated, and an average of 19 His$^+$ colonies appeared on plates which had been exposed to UV light. His$^+$ revertants were picked after 7 days at 30°C, purified on YPD, and retested for their His$^+$ phenotype. All His$^+$ revertants were screened for a Lys$^-$ phenotype to test for suppression of *lys2-173R2* (57), another Ty insertion allele suppressed by many *spt3*, *spt7*, *spt8*, and *spt15* mutations (65). (Spt$^+$ strains containing the *lys2-173R2* insertion allele are Lys$^+$, while Spt$^-$ strains with this allele are Lys$^-$.) Seventy-five of the 1,200 original His$^+$ candidates that were retested had a His$^+$ phenotype and also had a Lys$^-$ phenotype. In these initial tests, the Lys$^-$ phenotype ranged from very weak to strong. Subsequent purifications and retests showed several of the 75 mutants to actually be Lys$^+$. These mutants were included in our subsequent analyses.

To test for dominance, the candidates were mated to the *SPT*$^+$ strains FY66 and FY1083, and the resulting diploids were tested for their His$^+$ phenotype. To test whether the recessive mutations were in previously identified *SPT* genes, we performed diploid complementation tests with *spt* mutant strains (FY1087, FY1088, FY1089, FY1090, FY1091, FY1092, FY1093, and L914) and plasmid complementation tests (with plasmids pCC1 [*SPT3*] [1], pFW127 [*SPT7*] [20], pMG1 [*SPT8*] [18], pDE18 [*SPT15*] [19], and pMS4 [*SPT5*] [59]). For *spt5* mutants we used strain FY1102 for linkage analysis. For the *spt5* and *spt20* mutations, 2:2 segregation was demonstrated by tetrad analysis of crosses of mutant strains with strains FY66 and FY1083.

Sequencing analysis. Restriction fragments containing the *SPT20* gene were subcloned from plasmid pSR37 into pRS316, and their nucleotide sequences were determined (52) with the Sequenase version 2.0 kit (U.S. Biochemical Corp.) and universal M13 and synthetic primers. DNA sequence analysis was completed on both strands.

TABLE 2. Mutant hunt summary

Mutant class	No. of isolates
*his4-917*δ rearrangements	39
spt3	0
spt7	27
spt8	6
spt15	0
spt5	2
spt20	1
Total	75

Immunofluorescence. Indirect immunofluorescence and DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) staining were performed as described previously (51). Strain FY1095 was transformed with plasmid pSR65 or pSR77. Transformants were grown to a density of 5×10^6 cells per ml in selective synthetic complete medium containing 2% raffinose. Galactose was added to 2%, and the cells were grown 4 to 5 h longer before preparation. The polylysine stock was purchased from Sigma and was used at a dilution of 1:10. The primary antibody, 12CA5 (kindly provided by Frank McKeon), recognizes the HA1 epitope sequence and was used at a dilution of 1:1,000 overnight. The secondary antibody, CY3, (Jackson Immunoresearch Laboratories) was used at a dilution of 1:300 for 2 h. DAPI was purchased from Sigma. Cells were visualized with a Zeiss Axiphot microscope with a 100× oil immersion objective and photographed with Kodak TMAX-400 black-and-white film.

RNA isolation and Northern (RNA) analysis. RNA isolation and Northern analysis were performed as previously described (3, 59). Cells were grown to a density of 1×10^7 to 2×10^7 cells per ml in either minimal medium (for *MFA1* and *HIS4* Northern blots) or YPD (for Ty Northern blots). For *INO1* Northern blots, cells were grown as described previously (20). The plasmids pFW45 (*HIS4*) (64), pYST138 (*TUB2*) (58), B161 (TY), (67), and pSM39 (*MFA1*) (38) were nick translated with a Boehringer Mannheim kit for Northern probes. The *Eco*RI-*Hin*dIII fragment from pJH310 (*INO1*) (24) was randomly primed with a Boehringer Mannheim kit.

Mating assays. *S. cerevisiae* FY67, FY70, FY1096, and FY1097 were grown to saturation in YPD. All cultures were diluted to 8.5×10^7 cells per ml. Twofold dilutions of each strain were spotted on four YPD plates. Cells were mixed and allowed to mate by replica plating *SPT20*$^+$ and *spt20*Δ tester lawns to the spotted plates. The cells were allowed to mate for 24 h at 30°C and then replica plated to minimal medium to select for diploids.

Nucleotide sequence accession number. The GenBank accession number for *SPT20* is U22063.

RESULTS

Isolation of new *spt* mutants. In our current studies, we wanted to identify new genes that, when mutant, could cause phenotypes similar to those of the TBP group of *spt* mutants. In previous studies, a large percentage of such isolates contained *spt3* mutations, all of which were recessive (65). We reasoned that we might find mutations in new *SPT* genes if we could prevent the isolation of more mutations in *SPT3*. Pilot experiments demonstrated that duplication of *SPT3* eliminated detection of *spt3* mutations and also suggested that we should duplicate *SPT15* (48). Therefore, we constructed strains FY1082 and FY1083, in which *SPT3* and *SPT15* were both duplicated, and we selected for mutations that could suppress the His$^-$ phenotype caused by the insertion mutation *his4-917*δ.

Among the His$^+$ revertants, 75 candidates were chosen for further study. These mutants fell into several classes based on complementation, linkage, and phenotypic analyses (Table 2). For the first class, the His$^+$ phenotype was linked to *HIS4*. Previous work showed that reversion of Ty insertion mutations can be associated with genomic rearrangements (11, 49). On the basis of Southern analysis, the Spt$^-$ phenotype of the *HIS4*-linked mutations was associated with rearrangement of the *his4-917*δ mutation (48). As expected, these rearrangements had no effect on the other insertion mutation in these strains, *lys2-173R2.*

Of the mutations unlinked to *HIS4*, all but one were in previously identified genes. Within the TBP group of *SPT* genes, while there were no isolates in the duplicated genes *SPT3* and *SPT15*, there were several in *SPT7* and *SPT8*. In addition, we identified two mutations in *SPT5*, a gene that is a member of the histone group of *SPT* genes. This was an unexpected result, since previous work had suggested that suppression of *his4-917*δ was exclusive to the TBP group of *spt* mutations (62, 64, 65, 67). Therefore, we investigated suppression of *his4-917*δ by these and other *spt5* mutations and observed that suppression of *his4-917*δ by *spt5* mutations is allele specific (48). Neither of the *spt5* mutations tested suppressed *lys2-173R2.*

In addition to mutations in previously identified genes, we isolated one mutation in a new gene that we have designated *SPT20*. Analysis of its Spt$^-$ phenotype demonstrated that *spt20-61* caused suppression of both *his4-917*δ and *lys2-173R2*. In addition, *spt20-61* strains were inositol auxotrophs, a phenotype also found in *spt7* and certain *spt15* mutants (2, 20). These phenotypes suggested that *SPT20* might be functionally related to the TBP group of *SPT* genes.

Cloning, mapping, and sequencing of *SPT20*. To clone *SPT20*, the strain containing the *spt20-61* mutation, FY1111, was transformed with a YCp50-based yeast plasmid library (50) and Ino$^+$ transformants were selected. Three of the nine Ino$^+$ transformants were also Ura$^+$. Restriction analysis of plasmid DNAs from these three candidates showed that they contained overlapping restriction fragments. Subcloning demonstrated that an 8.0-kb *Hin*dIII fragment was able to complement *spt20-61.*

To confirm that this fragment contained the *SPT20* gene, we examined the ability of the clone to direct integration to the *SPT20* locus. First we subcloned the 8.0-kb *Hin*dIII fragment into the integrating plasmid pRS306 to construct pSR38. pSR38 was used to transform the *SPT20*$^+$ strain FY154, and one of the resulting Ura$^+$ transformants was crossed to the *spt20-61* strain FY1099. Following sporulation and dissection of this diploid, tetrad analysis demonstrated that every Ura$^+$ spore was also Spt$^+$ in each of the 18 four-spore tetrads we examined. This result demonstrated that the insert in plasmid pSR38 directed integration to the *SPT20* locus and therefore contains *SPT20.*

To determine if *SPT20* was a newly identified gene, we determined its map position. First, we hybridized the pSR38 insert to a set of overlapping ordered yeast genomic clones (47) and found that *SPT20* mapped to two clones, 5468 and 5954. These clones contain a 6.0-kb overlapping *Hin*dIII-*Eco*RI fragment near the left telomere of chromosome XV. To confirm that *SPT20* mapped to this region, we performed linkage analysis between *SPT20* and a marker in this region, *ARG8*. Tetrad analysis of 13 four-spore tetrads showed that *SPT20* is 3.8 centimorgans away from *ARG8* (14 parental ditype, 0 nonparental ditype, 2 tetratype).

To localize *SPT20* further, we constructed several subclones and sequenced the gene. The smallest subclone that retained *SPT20*$^+$ function, pSR48, contained a 2.3-kb *Xba*I-*Nhe*I fragment. This fragment was sequenced and found to contain a single open reading frame that remained open at the *Nhe*I site. Further sequencing of plasmid pSR37 3′ of the *Nhe*I site revealed a termination codon that completed a 1,812-bp open reading frame. After our sequence determination, this region was also sequenced as part of the *S. cerevisiae* genome project (10) (GenBank accession number Z48239). The size of this open reading frame is consistent with the estimated size (2.7 kb) of *SPT20* mRNA (48). Furthermore, we found that other subclones containing deletions within this open reading frame do not complement *spt20-61*. Finally, we determined that the

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  1  MSANSPTGND PHVFGIPVNA TPSNMGSPGS PVNVPPPMNP AVANVNHPVM
 51  RTNSNSNANE GTRTLTREQI QQLQQRQRLL LQQRLLEQQR KQQALQNYEA
101  QFYQMLMTLN KRPKRLYNFV EDADSILKKY EQYLHSFEFH IYENNYKICA
151  PANSRLQQQQ KQPELTSDGL ILTKNNETLK EFLEYVARGR IPDAIMEVLR
201  DCNIQFYEGN LILQVYDHTN TVDVTPKENK PNLNSSSSPS NNNSTQDNSK
251  IQQPSEPNSG VANTGANTAN KKASFKRPRV YRTLLKPNDL TTYYDMMSYA
301  DNARFSDSIY QQFESEILTL TKRNLSLSVP LNPYEHRDML EETAFSEPHW
351  DSEKKSFIHE HRAESTREGT KGVVGHIEER DEFPQHSSNY EQLMLIMNER
401  TTTITNSTFA VSLTKNAMEI ASSSSNGVRG ASSSTSNSAS NTRNNSLANG
451  NQVALAAAAA AAAVGSTMGN DNNQFSRLKF IEQWRINKEK RKQQALSANI
501  NPTPFNARIS MTAPLTPQQQ LLQRQQQALE QQQNGGAMKN ANKRSGNNAT
551  SNNNNNNNNL DKPKVKRPRK NAKKSESGTP APKKKRMTKK KQSASSTPSS
601  TTMS*
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FIG. 1. Amino acid sequence of *SPT20*. Glutamine and asparagine residues are shown in boldface.

spt20-61 mutation is a nonsense mutation caused by a C-to-T substitution at codon 78, changing a CGA (arginine) to a UGA stop codon (48). Therefore, we believe this open reading frame encodes the Spt20p protein.

The predicted Spt20p protein is 604 amino acids (Fig. 1) with a molecular mass of 67,796 Da, a net charge of +19, and an isoelectric point of 10.43. No known structural motifs or significant sequence similarities were predicted when the *SPT20* nucleotide and predicted amino acid sequences were used to search the GenBank database. The Spt20p amino acid sequence contains a large number of glutamine and asparagine residues, comprising 19% of the amino acids. Although there is a highly basic region contained within codons 562 to 591 of Spt20p, a subclone with this region deleted (pSR40) retained $SPT20^+$ function.

Construction and analysis of an *spt20* null mutant. To determine the phenotype caused by complete loss of Spt20p function, we constructed an *spt20* null allele, *spt20Δ100*, by replacing codons 11 to 413 of *SPT20* with the *URA3* gene (see Materials and Methods). To create an *spt20* null strain, *spt20Δ100* was integrated by one-step gene replacement into diploid strain FY632, and the resulting *spt20Δ100* heterozygous diploid was sporulated and dissected. Tetrad analysis showed 2:2 segregation of large-small colony size and of Ura^+-Ura^- in the eight four-spore tetrads examined. In every tetrad, small colony size cosegregated with the Ura^+ phenotype. The slow growth of Ura^+ spores demonstrates that *SPT20* is not essential for viability but is required for normal growth. Further analysis demonstrated that *SPT20* is not essential for growth at 15, 25, 30, or 37°C. The doubling time of *spt20Δ100* strains in YPD at 30°C is 1.4 times longer than that of *SPT20* strains. By Northern analysis, *spt20Δ100* strains contained no detectable *SPT20* transcripts (48).

The *spt20Δ100* strains were checked for other phenotypes that had been observed in *spt20-61* strains. First, *spt20Δ100* strains are Spt^-, indicating that this phenotype is caused by loss of function of *SPT20* (Fig. 2). In addition, *spt20Δ100* strains are inositol auxotrophs and grow poorly on medium with galactose as the carbon source (Fig. 2).

***spt20Δ100* phenotypes are associated with transcriptional defects.** To determine whether the phenotypes caused by *spt20Δ100* were at the transcriptional level, we performed Northern analysis. First, Ty transcripts were examined. Similar to the case with *spt3*, *spt7*, *spt8*, and *spt15* mutants, the level of full-length Ty transcripts is greatly reduced in *spt20Δ100* strains, and a shorter Ty transcript is present (Fig. 3A). Previous studies with *spt3*, *spt7*, and *spt8* mutants have shown that this shorter transcript is likely the result of an altered transcription initiation start site (65).

Next, *HIS4* transcripts from both HIS^+ and *his4-917δ* alleles were examined. In these experiments, cells were grown under conditions derepressing for *HIS4* transcription. Our results demonstrate that for $HIS4^+$, transcript levels are significantly decreased in *spt20Δ100* mutants (Fig. 3B). Previous studies showed a similar decrease in *spt3Δ* mutants (67). For *his4-917δ*, an *spt20Δ100* mutation causes an effect similar to that previously shown for *spt3*, *spt7*, *spt8*, and *spt15* mutations (18, 20, 67). In an $SPT20^+$ strain, *his4-917δ* produces a longer-than-normal *HIS4* transcript that is probably nonfunctional for translation (67). In *spt20Δ100* mutants, both the longer and normal-length *HIS4* transcripts are observed (48). Thus, *spt20Δ100* reduces the level of wild-type *HIS4* transcripts at $HIS4^+$ but elevates the level of wild-type *HIS4* transcripts at *his4-917δ*.

The inositol auxotrophy observed in *spt20* mutants was particularly interesting because mutations in several other genes important for transcription initiation, including certain *RPB*, *SNF/SWI*, and *SRB* genes (29, 40, 53), cause decreased levels of *INO1* mRNA. In addition, among *spt3*, *spt7*, *spt8*, and *spt15* mutants, only *spt7Δ* and certain *spt15* alleles cause defects in *INO1* transcription. To determine whether the Ino^- phenotype observed in *spt20Δ100* mutants correlated with a transcriptional defect, we analyzed *INO1* mRNA in *spt20Δ100* mutants. Northern analysis demonstrated that *INO1* transcripts are not detectable in *spt20Δ100* mutants, even after long exposures (Fig. 3C). We also observed that the leaky Gal^- phenotype of *spt20Δ100* mutants correlated with decreases in wild-type-length *GAL1* and *GAL10* mRNAs (48).

Since *spt* mutations are known to affect transcription start site selection for Ty elements, we cannot rule out the unlikely possibility that a similar event occurs at the other affected

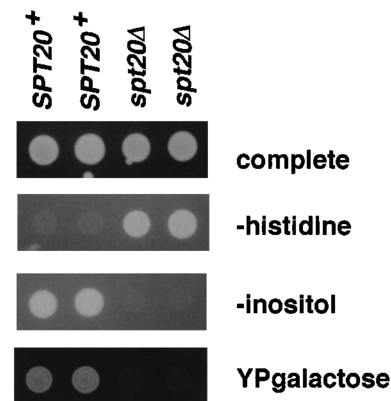

FIG. 2. Phenotypes of *spt20Δ100* mutants. Two *spt20Δ100* strains and two control strains were grown overnight in YPD. Approximately 2.6×10^6 cells were spotted to test for growth on the indicated plates. Both *spt20* null and control strains contain the *his4-917δ* allele. Photographs were taken after 3 days of growth at 30°C. Synthetic complete medium was used for the complete and histidine-lacking (−histidine) plates. Plates lacking inositol consist of minimal medium with the required amino acids. The strains shown are FY630 ($SPT20^+$), FY631 ($SPT20^+$), FY1095 (*spt20Δ*), and FY1106 (*spt20Δ*). This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

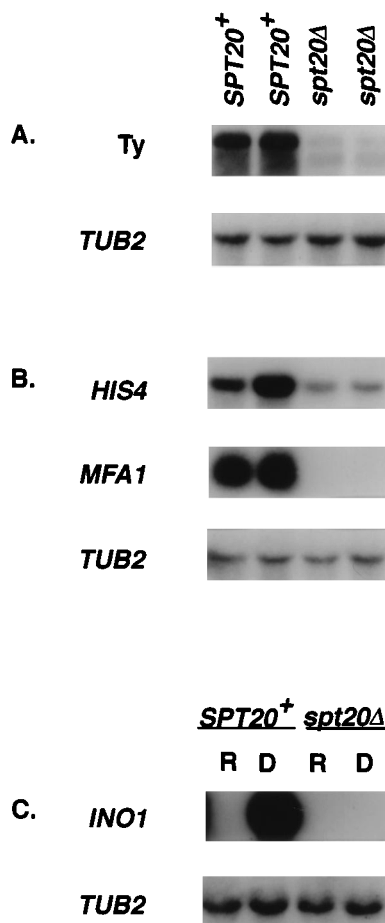

FIG. 3. *spt20Δ100* mutants are defective for transcription of particular genes. Strains were prepared for Northern analysis as described in Materials and Methods. (A and B) Analysis of Ty (A) and *HIS4* and *MFA1* (B) transcription in $SPT20^+$ (FY3 and FY21) and *spt20Δ* (FY1098 and FY1097) strains. (C) Analysis of *INO1* mRNA levels under repressing (200 μM inositol) and derepressing (10 μM inositol) conditions in $SPT20^+$ (FY21) and *spt20Δ* (FY1098) strains. All blots were probed with the *TUB2* gene as an internal control. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

genes and that the altered RNA is not detected because it is too small or extremely unstable. However, we can conclude that in every case examined, an *spt20* mutant phenotype corresponds to a reduction in transcript levels.

***spt20* mutants have defects in mating and sporulation.** Mutations in the TBP group of *SPT* genes cause defects in mating and sporulation (18–20, 25, 65, 67). To investigate whether *spt20* mutations cause similar defects, we first performed mating assays with $SPT20^+$ and *spt20Δ100* strains. Haploid cells with opposite mating types were mated for 24 h on YPD plates and then transferred to minimal medium to select for diploids. When *SPT20* strains were crossed with *spt20Δ100* strains, the strains mated no less efficiently than for *SPT20* strains crossed with *SPT20* strains (Fig. 4). However, for *spt20Δ100* strains crossed with *spt20Δ100* strains, there was a dramatic reduction in the number of diploids formed, and those diploids that formed grew very poorly (Fig. 4). When examined under a phase-contrast microscope, *spt20Δ100/spt20Δ100* diploids exhibit an abnormal morphology. The cells are often very elongated and irregularly shaped, in contrast to *spt20Δ100* haploids, which are only slightly elongated. Previous studies have

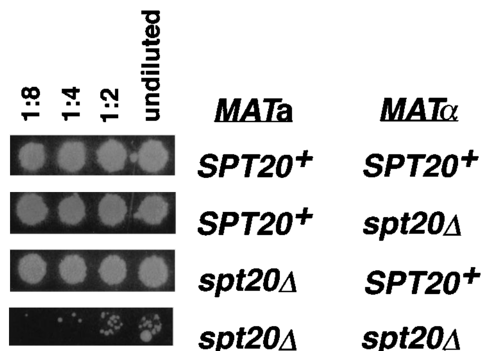

FIG. 4. *spt20Δ100* mutants are defective for mating. $SPT20^+$ and *spt20Δ* strains were prepared as described in Materials and Methods and allowed to mate for 24 h before being replica plated to select for diploids. Undiluted cultures were at a concentration of 8.5×10^7 cells/ml. Photographs were taken after 3 days of growth. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

shown that the mating defect observed in *spt* mutants correlates with the decreased transcription of mating pheromone genes. Therefore, we examined *MFA1* transcripts in *spt20Δ100* mutants and found that, like *spt3* and *spt7* mutants (20, 25), *spt20Δ100* mutants show decreased levels of *MFA1* transcripts (Fig. 3B).

We also tested the ability of *spt20Δ100/spt20Δ100* diploids to sporulate. Consistent with mutations in other members of the TBP group of *SPT* genes, these diploids were sporulation defective (Table 3).

Double mutant and high-copy suppression analyses. To examine the genetic relationship between *SPT20* and the other members of the TBP group of *SPT* genes, we have conducted double mutant and high-copy suppression analyses. In these analyses we monitored growth and the Spt and Ino phenotypes (48). First, we constructed double mutants carrying *spt20Δ* and null mutations in *SPT3*, *SPT7*, and *SPT8*. Each double mutant had the same phenotypes with respect to growth, Spt^-, and Ino^- as did the strongest single mutation present in the strain. Second, we transformed a high-copy-number *SPT20* plasmid into *spt3*, *spt7*, and *spt8* mutant strains and found that there was no suppression of the mutant phenotypes. Finally, we transformed high-copy-number plasmids that contain *SPT3*, *SPT7*, *SPT8*, and *SPT15* into an *spt20Δ* strain and likewise found no suppression. These results suggest that these Spt functions each contribute to the same step in transcription initiation in a nonredundant fashion.

TABLE 3. Sporulation frequency[a]

Diploid	Parent	*MAT* allele	*SPT20* allele	% Sporulation
1	FY67	**a**	*SPT20*	8
	FY70	α	*SPT20*	
2	FY67	**a**	*SPT20*	6
	FY1096	α	*spt20Δ*	
3	FY1097	**a**	*spt20Δ*	6
	FY70	α	*SPT20*	
4	FY1097	**a**	*spt20Δ*	<0.7
	FY1096	α	*spt20Δ*	

[a] All diploids have the same genotype except as indicated. Sporulation was measured as described in Materials and Methods.

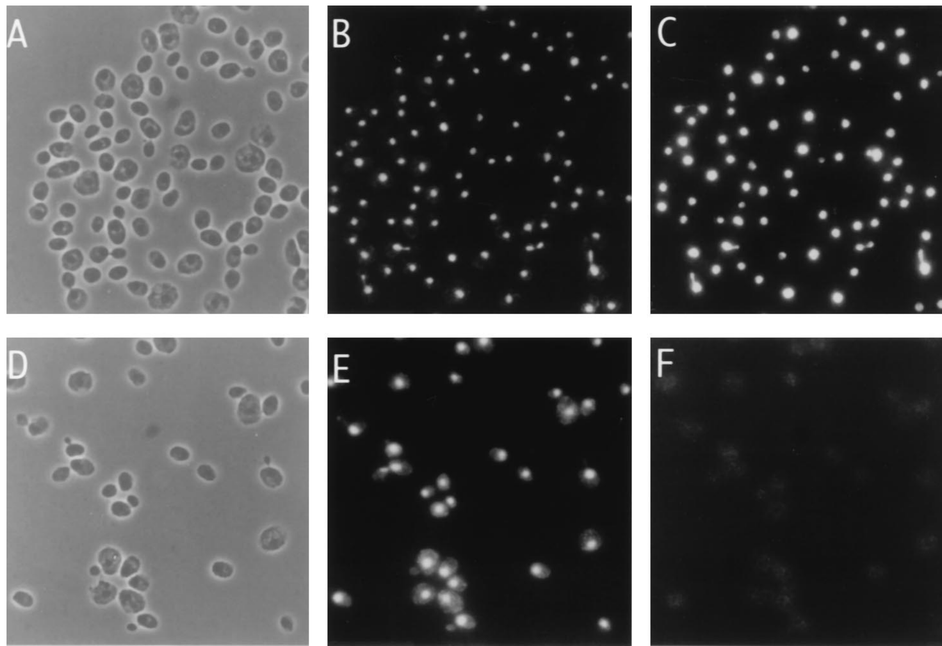

FIG. 5. Nuclear localization of *SPT20* by indirect immunofluorescence. An *spt20Δ100* haploid (FY1097) was transformed with HA1 epitope-tagged Spt20p (A to C) or untagged Spt20p (D to F). Transformants were prepared for immunofluorescence as described in Materials and Methods. The primary antibody was monoclonal antibody 12CA5, which recognizes the HA1 epitope, and the secondary antibody was anti-mouse immunoglobulin G conjugated to CY3. (A and D) Phase contrast; (B and E); DAPI fluorescence to visualize DNA; (C and F) CY3 fluorescence. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

Identification and subcellular localization of Spt20p. To determine the subcellular location of Spt20p, we performed indirect immunofluorescence microscopy with haploid *spt20Δ100* strains expressing either epitope-tagged or untagged Spt20p proteins. In cells expressing HA1 epitope-tagged Spt20p, the fluorescent staining colocalized with the DAPI staining of nuclear DNA, indicating that HA1-Spt20p is a nuclear protein (Fig. 5B and C). This fluorescent staining was not detected either in cells expressing the untagged Spt20p construct (Fig. 5F) or in the absence of a primary antibody (48). In these experiments, Spt20p was expressed at a substantially higher level than normal, possibly leading to mislocalization. We think that this is unlikely, since these overexpression strains had wild-type growth, Spt, and Ino phenotypes.

***SPT20* is the same gene as *ADA5*.** During the course of this work, we discovered, on the basis of identical DNA sequences, that *SPT20* is the same gene as *ADA5* (35). In support of this conclusion, *spt20-61* fails to complement the *ada5-1* mutation (48). *ADA5* was identified by a mutation that confers resistance to the toxicity caused by overexpression of the artificial transcriptional activator, Gal4-VP16 (36). *ADA* genes are candidates to serve as intermediaries between transcriptional activators and general transcription factors (4, 5, 56). Results presented in the accompanying paper (35) show that *ada5* mutations decrease transcription at several promoters in vivo. In addition to *ADA5*, three other *ADA* genes (*ADA2*, *ADA3*, and *GCN5*) have been studied with respect to effects on transcriptional activation (5, 27, 36). To test if these other genes are functionally related to the TBP group of *SPT* genes, we determined if *ada2*, *ada3*, or *gcn5* mutations could suppress an insertion mutation and thereby cause an Spt^- phenotype. We constructed double mutants carrying each *ada* mutation and either *his4-917δ* or *his4-912δ*. Our results showed that in each case, the *ada* mutation did not suppress either *his4-917δ* or *his4-912δ* (48). Therefore, *ada2*, *ada3*, and *gcn5* mutations are phenotypically distinct from *spt20/ada5* mutations.

DISCUSSION

By duplicating two known *SPT* genes and looking for new suppressors of the *his4-917δ* insertion mutation, we have identified a new gene, *SPT20*. We have demonstrated that a null mutation in *SPT20* causes strong transcriptional defects and other phenotypes similar to those caused by null mutations in *SPT3*, *SPT7*, and *SPT8*. These phenotypes also resemble those of certain missense mutations in the gene encoding TBP, *SPT15*. These five genes do not appear to regulate each other's transcription (48, 65), and in the cases in which it has been tested, they do not regulate each other's translation (20). Therefore, the similar mutant phenotypes suggest that Spt20p, along with Spt3p, Spt7p, and Spt8p, are required for normal TBP function at certain promoters.

How do the TBP group of Spt proteins affect TBP function? The model that this group of Spt functions is required for TBP to function at specific promoters is based on three sets of results. First, null mutations in *SPT3*, *SPT7*, *SPT8*, and *SPT20* cause defects at certain promoters. Second, particular alleles of *SPT15* cause defects at the same set of promoters. Third, both genetic and biochemical evidence have shown that Spt3p interacts with TBP, which provides evidence for a functional interaction of at least one of the members with the transcription apparatus. Thus, these Spt proteins may act as specificity factors, necessary for TBP to bind to and/or function at particular TATA boxes.

Previous studies have thus far been unable to define the roles of these Spt functions in affecting TBP activity. There is no evidence that these proteins are part of the conserved complex of TAFs that has been identified in *S. cerevisiae* and other organisms (20, 21, 45, 46). In addition, there is no evidence that these Spt proteins are part of the holoenzyme-mediator complex (28, 30). However, Spt3p does show some sequence similarity to a human TAF, $TAF_{II}18$ (37). $TAF_{II}18$ is believed to be in a distinct complex with TBP that is separate

TABLE 4. Comparison of TBP class *spt* mutant phenotypes

Relevant genotype	Phenotype[a]			
	Spt[b]	Growth[c]	Gal[d]	Ino[e]
SPT$^+$	+	+	+	+
*spt20*Δ	−	−/+	−/+	−
*spt7*Δ	−	−/+	−/+	−
*spt3*Δ	−	+/−	+/−	+
*spt8*Δ	−	+	+	+
spt15-21[f]	−	+/−	+/−	−
spt15-341	+/−	−/+	−	−

[a] Phenotypes were scored after 2 days of growth at 30°C. Symbols (except for Spt): +, strong growth; +/−, growth; −/+, poor growth; −, no growth.
[b] The Spt phenotype is based on the ability of the mutation to suppress the Ty insertion mutation, *his4-917*δ. −, strong suppression; +/−, weak suppression; +, no suppression.
[c] Growth was measured as the ability of the strain to grow on YPD.
[d] Ability of the strain to grow on galactose as a carbon source.
[e] Ability of the strain to grow on medium lacking inositol.
[f] *spt15* null mutants are not viable.

from most of the other TAFs. By analogy, the Spt3p, Spt7p, Spt8p, and Spt20p proteins could be in a similar complex with yeast TBP. Although coimmunoprecipitation experiments have failed to detect a physical interaction between TBP and Spt7p, Spt8p, or Spt20p (18, 20, 48), additional studies will be required before we can conclude whether or not Spt7p, Spt8p, or Spt20p is part of a physical complex with Spt3p and TBP.

While *spt3*, *spt7*, *spt8*, and *spt20* mutants share many mutant phenotypes, they do not have identical mutant phenotypes (Table 4). *spt20* mutants are phenotypically most similar to *spt7* mutants; both *spt7* and *spt20* null mutants grow poorly on rich media, and both are Gal$^-$ and Ino$^-$. The phenotypic resemblance of *spt20* and *spt7* mutants suggests that Spt7p and Spt20p may function in an identical role and that this role is distinct from that of Spt3p and Spt8p.

Spt20p function. Similar to other TBP group *spt* mutants, *spt20* null mutants are defective for transcription of many genes, and therefore, the *SPT20* gene product formally functions as a transcriptional activator. Consistent with this, a LexA-SPT20 fusion protein which fully complements an *spt20Δ100* mutation can activate transcription in vivo (48). However, such an activity for a LexA fusion protein is not strong evidence for a normal role of Spt20p in transcriptional activation (33, 42). The predicted Spt20p amino acid sequence contains several homopolymeric amino acid stretches, most notably, glutamine and asparagine, that are often present in eukaryotic transcriptional activators (61). However, there is no evidence suggesting that glutamine-rich regions are required for transcriptional activation in *S. cerevisiae* (31, 39, 44, 54). A highly basic region in the C-terminal half of Spt20p could encode a DNA-binding domain. However, deletion studies and complementation analysis demonstrated that this region is not required for Spt20p function. Thus, while Spt20p may function as a transcriptional activator, further genetic and biochemical experiments are required to address this issue.

Are the *SPT* and *ADA* genes functionally related? *SPT20*, also known as *ADA5*, is the only gene that shares common mutant phenotypes with both the *SPT* and *ADA* groups of genes. We have shown that other *ada* mutations do not confer Spt$^-$ phenotypes, and *spt3* and *spt8* mutations do not confer Ada$^-$ phenotypes (34). Since both groups of genes participate in transcription initiation, it is possible that Spt20p/Ada5p serves as an intermediary between Spt and Ada functions. For example, both Spt3p and Ada2p have been shown to interact with TBP (4, 17). Although there is no evidence that they are in the same complex, Spt20p/Ada5p could theoretically act as a bridge to connect these or other Spt and Ada proteins.

In summary, our results and those in the accompanying paper (35) demonstrate that Spt20p/Ada5p is very important for transcription in vivo. The fact that it has not yet been identified as one of the factors that is required for transcription in vitro suggests that significant aspects of transcription remain to be defined biochemically. Both biochemical and genetic experiments should help us to understand the role of Spt20p/Ada5p in transcription initiation and the functional relationships between *SPT* and *ADA* genes during transcription initiation.

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