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Evidence that Spt3 Functionally Interacts with Mot1, TFIIA, and TATA-Binding Protein To Confer Promoter-Specific Transcriptional Control in *Saccharomyces cerevisiae*

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Spt3 of *Saccharomyces cerevisiae* is a factor required for normal transcription from particular RNA polymerase II-dependent promoters. Previous genetic and biochemical analyses have shown that Spt3 interacts with the yeast TATA-binding protein (TBP). To identify other factors that might interact with Spt3, we have screened for mutations that, in combination with an *spt3* null mutation, lead to inviability. In this way, we have identified a mutation in *MOT1*, which encodes an ATP-dependent inhibitor of TBP binding to TATA boxes. Previous analyses suggested that Mot1 causes repression in vivo. However, our analysis of *mot1* mutants shows that, similar to *spt3* mutants, they have decreased levels of transcription from certain genes, suggesting that Mot1 may function as an activator in vivo. In addition, *mot1* mutants have other phenotypes in common with *spt3*Δ mutants, including suppression of the insertion mutation *his4-912*δ. Motivated by these Spt3-Mot1 genetic interactions, we tested for genetic interactions between Spt3 and the general transcription factor TFIIA. TFIIA has been shown previously to be functionally related to Mot1. We found that overexpression of TFIIA partially suppresses an *spt3*Δ mutation, that *toa1* mutants have Spt$^-$ phenotypes, and that *spt3*Δ *toa1* double mutants are inviable. We believe that, taken together, these data suggest that Spt3, Mot1, and TFIIA cooperate to regulate TBP-DNA interactions, perhaps at the level of TATA box selection in vivo.

For transcription initiation by RNA polymerase II, binding of the TATA-binding protein (TBP) to the TATA element is believed to be one of the central rate-limiting steps in the formation of a functional RNA polymerase II preinitiation complex (6). Studies in vitro have shown that TBP is able to bind with similar affinities to a number of TATA and TATA-like sequences (22). However, evidence in vivo suggests that not all TATA boxes are utilized similarly and that the promoter context influences the function of the TATA box (57a, 59). Furthermore, some promoters, such as the *CYC1* and *GAL1* promoters, have been shown to differ with respect to the occupancy of the TATA box in the inactive state (5, 8, 53). It is unresolved how, in vivo, promoter context and TBP-interacting factors influence different aspects of TBP function, such as TATA box association and dissociation rates.

Several studies have led to the identification of positive regulatory factors that promote stability of the TBP-DNA complex. Among these is the general transcription factor TFIIA, which can stabilize TBP binding to the TATA box in the presence of TBP inhibitors and under suboptimal conditions in vitro (12, 26). Recent evidence from in vivo studies with *Saccharomyces cerevisiae* and from highly purified in vitro systems in *Drosophila melanogaster* suggests that TFIIA can stimulate both activated and basal transcription and may associate with other TBP-associated factors (TAFs) (29, 56, 68). In addition to TFIIA, several studies suggest that TBP-TATA interactions are controlled by certain upstream activators (7, 31, 32, 57, 66). It has also been shown that one function of TAFs in activated transcription is to cooperate with upstream activators to stabilize the TBP-TAF-TFIIA complex (33). Thus, TBP and the TBP-DNA complex are likely to be the targets of multiple factors to control normal transcription in vivo.

In contrast to activities that promote the TBP-TATA box interaction, a number of negative factors that destabilize this interaction have been identified. Several of these TBP inhibitors, such as the HeLa factors Dr1/NC2β, Dr2/TopoI, and NC1, have been purified on the basis of their ability to repress basal transcription in vitro (27, 36, 37, 39, 67). Analysis of Dr1/NC2β suggests that it interacts with another factor, DRAP1/NC2α, to repress transcription by interacting with TBP and excluding the general transcription factors TFIIA and TFIIB, thus precluding the formation of a functional preinitiation complex (21, 30, 40, 67). The in vivo roles of these TBP inhibitors have yet to be determined. However, the existence of these factors suggests that there may be interactions with either DNA or other factors that must be prevented under physiological conditions in order for transcription to occur.

The yeast TBP inhibitor Mot1 has been proposed to block TBP function in a manner distinct from those of Dr1, Dr2, and NC1. Mot1 was identified in vitro in two ways. First, Mot1 was purified on the basis of its ability to remove TBP from TATA boxes in an ATP-dependent fashion (2, 3). In these studies, levels of in vitro basal transcription from *mot1* mutant extracts were higher than levels from wild-type extracts, consistent with a negative role for Mot1. Second, Mot1 was identified as a yeast TAF that exists in a TBP complex distinct from TFIID, the TBP complex that contains the core yeast TAFs (46, 47). Mutations in *MOT1* were identified on the basis of their ability to increase transcription from a number of unrelated RNA polymerase II-dependent promoters (13, 44). Recent work has also suggested that Mot1 is required for repression by Leu3 in vitro and in vivo (60). Since Mot1 activity is counteracted by the general transcription factor TFIIA in vitro, it has been proposed that the balance of TFIIA and Mot1 activities regulate TBP-TATA box interactions (2, 3).

Several other factors identified genetically in yeast are also

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thought to control TBP function in vivo. These factors were identified by mutations that suppress the transcriptional defects caused by insertions of Ty or solo δ elements in the promoters of the *HIS4* and *LYS2* genes (19, 61, 64, 65). These genes were designated *SPT* for suppressor of Ty. Mutations in *SPT15*, which encodes TBP, were originally isolated in this manner (17). Another factor, Spt3, is a nonessential, nuclear protein that has been proposed to regulate TBP-TATA interactions in vivo (15, 64). Mutations in *SPT3* cause mutant phenotypes similar to those caused by particular mutations in *SPT15*, including slow growth, mating defects, sporulation defects, and suppression of similar Ty and solo δ insertion mutations (17, 65). Analysis of transcription in *spt3*Δ mutants has shown that Spt3 is required for transcription of particular RNA polymerase II-dependent genes (24, 65). Moreover, genetic and biochemical analysis of Spt3 has shown that Spt3 interacts with TBP (15). Recently, Spt3 has been shown to be 26% identical to human $TAF_{II}18$ and 21% identical to another yeast factor of unknown function, Fun81 (14, 38). Taken together, the genetic and biochemical data suggest that Spt3 is a TAF required for TBP function at certain promoters.

To identify factors that are functionally related to Spt3, we have screened for mutations that cause lethality in combination with an *spt3* null mutation (*spt3*Δ synthetic lethal mutations). This screen identified a mutation in *MOT1* as an *spt3*Δ synthetic lethal mutation. Further characterization has shown that *mot1* mutants and *spt3*Δ mutants possess some common phenotypes, including decreased levels of certain transcripts. This result suggests that, in contrast to previous models, Mot1 functions as a positive regulatory factor in the transcription of certain genes in vivo. In addition, we have found multiple genetic interactions between TFIIA and *SPT3*, suggesting that these factors may perform functionally similar roles in the regulation of TBP function. These data suggest that Spt3, Mot1, and TFIIA are functionally related and that together they may regulate promoter-specific TBP-DNA interactions.

MATERIALS AND METHODS

Yeast strains and genetic methods. The yeast strains used (Table 1) are derived from an S288C *GAL2*$^+$ derivative (63) and were constructed by standard methods (51). All *spt* mutations used in this study have been described previously (15, 23, 65). The *mot1-1* allele (13) and the *mot1-1033* allele (44) have been described previously, and the *mot1-24* allele was isolated in this study. Integration of the *mot1-1* mutation into an S288C background was done by standard procedures with plasmid pJM75. The *toa1-18GSG* allele has been described previously (29). The *ste12*Δ strains FY1216, FY1217, and FY1218 were constructed by standard methods in an S288C background with the plasmid pLG36. Determination of synthetic lethality was done as described in Table 3, footnote *a*. Yeast strains were transformed by a lithium acetate procedure (18). Standard methods of mating, sporulation, and tetrad analysis were used (51).

Media. Rich (YPD), minimal (SD), synthetic complete (SC) media containing 5-fluoroorotic acid (5-FOA) and sporulation media were prepared as described previously (51). Suppression of insertion mutations was scored on SD medium supplemented with particular nutrients or on SC medium lacking appropriate nutrients. Yeast transformants were selected on the appropriate SC media.

DNA preparation and analysis. *Escherichia coli* HB101 and DH5α were used as hosts for plasmids (52). Plasmids were constructed, maintained, and isolated by standard methods (52). Plasmids were recovered from yeast as described previously (50). Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs (Beverly, Mass.) and Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and used as recommended by the manufacturer.

Plasmids. The pRS series of vectors has been described previously (9, 54). pJM75 contains the *Xho*I-*Not*I fragment of pJM81 subcloned into pRS306. pJM89 contains the *Xho*I-*Not*I *MOT1* fragment from pRS7.1*Bgl*II (13) subcloned into the *Xho*I-*Not*I sites of pRS314. pJM64 is pRS316 with the *Bst*XI polylinker site eliminated. pJM65 contains the *Xho*I-*Not*I *MOT1* fragment from pRS7.1 *Bgl*II (13) subcloned into the *Xho*I-*Not*I sites of pJM64. pJM118 has the *Eco*RI *TOA2*-containing fragment from pSH325 (*TOA2* in pRS316, generously provided by S. Hahn) subcloned into the *Eco*RI site of pRS426. pJM120 has the *Eco*RI-*Not*I *TOA1*-containing fragment from pSH342 (*TOA1* in pRS316, generously provided by S. Hahn) subcloned into the *Eco*RI-*Not*I sites of pRS426. pJM122 has the *Eco*RI *TOA2*-containing fragment from pSH325 subcloned into the *Eco*RI site of pJM120. pLG36 is *ste12*Δ::*LEU2* in pRS306.

Plasmids used for Northern probes were pFW45 (*HIS4*) (62), pB161 (Ty1) (62), pJEH122 (*DED1*) (25), pAB510 (*STE2*) (generously provided by G. Sprague), pHB59 (*TPI1*) (generously provided by H. Baker), pSM39 (*MFa1*) (41), pSM29 (*MFa2*) (41), pYST138 (*TUB2*) (55), pRS7.1*Bgl*II (*MOT1*) (13), and pCC1 (*SPT3*) (10).

DNA sequence analysis. For sequencing portions of both wild-type and mutant *MOT1* genes, the appropriate restriction fragments were first subcloned into pRS316. Sequencing was performed by a protocol provided by the Sequenase version 2.0 kit from U.S. Biochemical Corp. Synthetic primers and M13 universal and reverse primers were used to determine sequences on both strands. The sequence was analyzed by using the BLAST program (1) and compared against known sequences and proteins in the GenBank, EMBL, and PIR databases.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype
FY41	*MAT***a** *ura3-52 leu2Δ1 trp1Δ63*
FY51	*MAT***a** *spt3Δ203:: TRP1 his4-917δ ura3-52 leu2Δ1 trplΔ63*
FY53	*MAT***a** *his4-912δ ura3-52*
FY98	*MAT***a** *ura3-52 leu2Δ1*
FY142	*MAT***a** *his4-912δ lys2-128δ ura3-52 ade8*
FY268	*MAT***a** *his4-912δ lys2-128δ leu2Δ1*
FY294	*MAT***a** *spt3-202 his4-917δ lys2-173R2 ura3-52 leu2Δ1 trp1Δ63*
FY403	*MAT***a** *spt3Δ203*::*TRP1 ura3-52 leu2Δ1 trp1Δ63*
FY567	*MAT***a** *spt15-21 his4-917δ lys2-173R2 ura3-52 leu2Δ1 ade8*
FY631	*MAT***a** *his4-917δ lys2-173R2 ura3-52 leu2Δ1 trp1Δ63*
FY822	*MAT***a** *spt3Δ203*::*TRP1 ste12Δ*::*LEU2 his4-917δ ura3-52 leu2Δ1 trp1Δ63*
FY1210	*MATα mot1-24 his4-917δ ura3-52 trp1Δ63*
FY1211	*MAT***a** *mot1-24 his4-912δ lys2-128δ ura3-52 ade8*
FY1212	*MATα mot1-24 lys2-128δ his4-912δ trp1Δ63 ura3-52 ade8 suc2ΔUAS leu2Δ1*
FY1213	*MAT***a** *mot1-1 his4-912δ lys2-128δ ura3-52 ade8*
FY1214	*MAT***a** *mot1-1 ura3-52 leu2Δ1*
FY1215	*MATα mot1Δ2*::*LEU2 ura3-52 his4-912δ leu2Δ1 trp1Δ63 pRS7.1Bgl*II (*MOT1* pRS316)
FY1216	*MAT***a** *mot1-24 ura3-52 leu2Δ1 ade8*
FY1217	*MAT***a** *ste12Δ*::*LEU2 ura3-52 leu2Δ1*
FY1218	*MAT***a** *ste12Δ*::*LEU2 mot1-24 ura3-52 leu2Δ1 ade8*
FY1219	*MAT***a** *ste12Δ*::*LEU2 mot1-1 ura3-52 leu2Δ1*
JMY30	*MATα mot1-24 his4-917δ ura3-52 trp1Δ63*
JMY177	*MATα spt3Δ203*::*TRP1 mot1-1033 his4-917δ trp1Δ63 ura3-52 leu2Δ1*
JMY179	*MATα mot1-1 spt3*Δ203::*TRP1 his4-917δ trp1Δ63 ura3-52 leu2Δ1*
JMY357	*MATα mot1-1 his4-917δ ura3-52 leu2α1*
JMY483	*MATα spt3-202 toa1-18GSG leu2Δ1 ura3-52 his4-917δ* pCC1 *(SPT3* YCp50)
JMY498	*MAT***a** *toa1-18GSG his4-912δ ura3-52 lys2-128δ*
JMY504	*MAT***a** *toa1-18GSG his4-917δ ura3-52 lys2-128δ*
JMY514	*MAT***a** *mot1-1 toa1-18GSG leu2Δ1 ade8 ura3-52 his4-912δ lys2-128δ*
JMY515	*MATα mot1-24 toa1-18GSG ade8 leu2Δ1 ura3-52 his4-912δ pRS7.1Bgl*II *(MOT1* pRS316)
L603	*MAT***a** *spt3Δ203*::*TRP1 his4-912δ lys2-128δ leu2Δ1 ura3-52*
L641	*MAT***a** *spt3Δ203*::*TRP1 mot1-24 ura3-52 leu2Δ1 trp1Δ63* pCC1 *(SPT3* YCp50)
JD215b	*MAT***a** *mot1-1 leu2-3,112 ura3-52 trp1-1 his4-519 can1-101*
GL1033	*MAT***a** *mot1-1033 ura3-52 leu2-3,112 trp1-289 ade5 gal2 can1*
JKY75	*MAT***a** *toa1-18GSG lys2-801 his3Δ200 ura3-52 trp1Δ63 ade2-101 leu2Δ1*

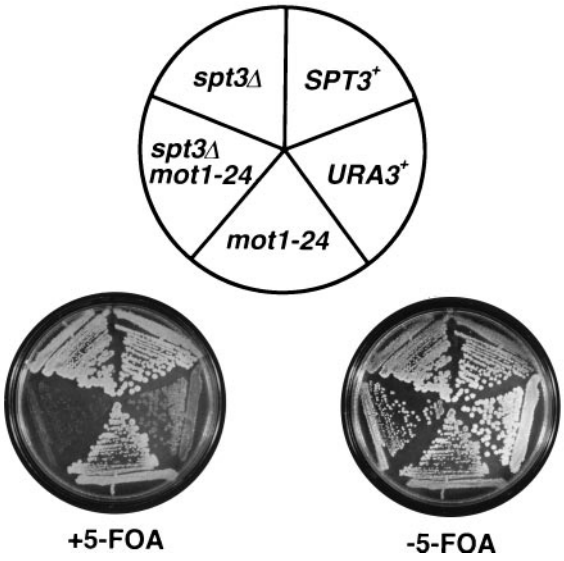

FIG. 1. A *mot1* mutation causes synthetic lethality with an *spt3*Δ mutation. Strains with the indicated genotypes were grown on YPD and then replica plated to SC plates containing (+) or lacking (−) 5-FOA. The strains used are FY41, FY51, L641, FY268, and FY1211. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

RNA preparation and Northern hybridization analysis. Cells for RNA isolation were grown at 30°C in supplemented SD medium to a density of 1×10^7 to 2×10^7 cells/ml. Total RNA was isolated by a hot-phenol method (4). Northern transfer and hybridizations were performed as described previously (58). ^{32}P-labeled probes were generated either with a Boehringer Mannheim Biochemical nick translation kit or by random hexamer labeling (4). Northern blots were quantitated on a Molecular Dynamics PhosphorImager.

Screen for strains carrying *spt3*Δ synthetic lethal mutations. An *spt3*Δ strain, FY51, was transformed with pCC1, an *SPT3 CEN URA3* plasmid. This strain was mutagenized with ethyl methanesulfate by standard methods (4). Mutagenized cells were plated on YPD and incubated at 30°C. Approximately 15,000 colonies were screened for the inability to grow without pCC1 by replica plating to 5-FOA plates. Those colonies that did not grow on 5-FOA but did grow on SC-Ura and SC were chosen for further study. To demonstrate that a single gene in conjunction with an *spt3*Δ mutation was responsible for the synthetic lethality, 5-FOA-sensitive progeny were crossed to FY51 and sporulated, and spores from tetrad dissection were screened for 5-FOA sensitivity. 5-FOA sensitivity was shown to segregate 2:2. 5-FOA-sensitive double mutants were crossed to a wild-type strain, FY41, to isolate the new mutation in an *SPT3* background. These single mutations were verified to cause synthetic lethality with an *spt3*Δ mutation by crossing each candidate to FY51 containing pCC1 and reconstructing the synthetic lethality by loss of the plasmid. Mutations that reconstructed the synthetic lethality were chosen for further study.

Cloning of *mot1* mutations and sequencing of the *mot1-24* mutation. The *mot1-24* mutation was localized by gap repair (43). Plasmid pRS7.1*Bgl*II was gapped with different restriction enzymes. Each gapped plasmid was transformed into FY1210, and the resulting Ura$^+$ transformants were screened for a Ts$^-$ phenotype. An *Nru*I-*Eco*NI gapped plasmid resulted in all Ts$^-$ transformants, indicating that the *mot1-24* mutation was in this region. The plasmid was rescued from yeast, and the *Nru*I-*Eco*NI fragment was subcloned into pJM89 (*MOT1* in pRS314) to create pJM77. pJM77 was shown to carry the mutation by introducing it into FY1215, a *mot1*Δ*2::LEU2* strain, by plasmid shuffle. The plasmid supported viability but caused a Ts$^-$ phenotype. The *Nru*I-*Eco*NI fragment from both a *mot1-24* mutant and a *MOT1* plasmid was sequenced on both strands. We identified a single difference between the *mot1-24* and *MOT1* sequences. The *mot1-24* mutation altered nucleotide 4769 of the published sequence (13), resulting in a predicted arginine-to-lysine change at codon 1507.

The *mot1-1* mutation was cloned based on information generously provided by Jeremy Thorner. pJM65 was digested with *Hpa*I and *Bst*XI and used to transform the *mot1-1* strain JD215b to Ura$^+$. Following transformation to Ura$^+$, the *mot1-1* mutation cloning proceeded as described for the *mot1-24* mutation cloning.

RESULTS

Isolation of mutations that cause lethality with an *spt3*Δ mutation. To identify factors that are functionally related to Spt3, we screened for mutations that in combination with an *spt3*Δ mutation cause inviability (*spt3*Δ synthetic lethal mutations). From this analysis two *spt3*Δ synthetic lethal mutations were identified. One of these mutations is in the previously identified *PTA1* gene (34, 42). The second mutation was shown to be in the *MOT1* gene as described below. Studies arising from the discovery of the *mot1-24 spt3*Δ synthetic lethality comprise the remainder of this report.

***mot1-24* causes synthetic lethality with an *spt3*Δ mutation.** To identify the gene corresponding to this second synthetic lethal mutation, the gene was cloned by complementation of two recessive phenotypes: slow growth at 30°C and Ts$^-$ at 37°C. A single plasmid that fully complemented the slow-growth phenotype at 30°C and partially complemented the Ts$^-$ phenotype at 37°C was identified. Sequencing of a portion of the plasmid insert revealed that it contained the *MOT1* gene. A plasmid containing only *MOT1*, pRS7.1*Bgl*II (13), showed the same complementation as the original library plasmid.

To prove that a mutation in *mot1* had been isolated, we performed several tests. First, complementation analysis showed that *mot1-24* fails to complement two other Ts$^-$ *mot1* mutations, *mot1-1* (13) and *mot1-1033* (44), for growth at 37°C. Second, tetrad analysis of *mot1-1*/*mot1-24* and the *mot1-1033*/*mot1-24* heterozygotes showed Ts$^+$:Ts$^-$ segregation of 0:4 in 40 tetrads for each cross. Therefore, *mot1-24* is very tightly linked to two other *mot1* mutations. Finally, gap rescue and sequence analysis of the *mot1-24* mutation (see Materials and Methods) revealed a single-base-pair change in the *mot1-24* open reading frame, resulting in a predicted arginine-to-lysine change at codon 1507 of *MOT1*. Taken together, these results demonstrate that a mutation in *MOT1*, *mot1-24*, causes lethality in combination with an *spt3*Δ mutation. The synthetic lethal phenotype of a *mot1-24* spt3Δ strain is shown in Fig. 1.

***MOT1* mRNA levels are not significantly altered in an *spt3*Δ mutant.** One possible explanation for the *mot1-24 spt3*Δ synthetic lethality would be that Spt3 controls *MOT1* transcription. By this model, *mot1-24 spt3*Δ lethality would be the result of a greater reduction in the essential function of Mot1 due to the combination of a mutation in *MOT1* and a reduction in its transcription caused by an *spt3*Δ mutation. To test this hypothesis, we examined *MOT1* mRNA levels in *SPT3*$^+$ and *spt3*Δ strains by Northern blotting. As shown in Fig. 2, *MOT1* mRNA levels were not significantly decreased in an *spt3*Δ strain, being within twofold of the levels in an *SPT3*$^+$ strain. This result suggests that the synthetic lethality observed in *spt3*Δ *mot1-24* double mutants is not caused by Spt3 control of *MOT1* mRNA levels.

***mot1* mutants are Spt$^-$.** Since the lethality of a *mot1 spt3* double mutant suggests that Mot1 and Spt3 are functionally

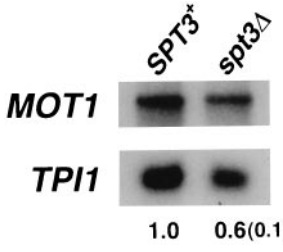

FIG. 2. *MOT1* mRNA levels in an *spt3*Δ mutant. *MOT1* mRNA levels in the *SPT3*$^+$ and *spt3*Δ strains FY41 and FY51, respectively, were examined and normalized to *TPI1* mRNA levels. The fold difference in the *MOT1* mRNA level relative to the wild-type mRNA level is shown below each lane, with the standard error from six experiments indicated in parentheses. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

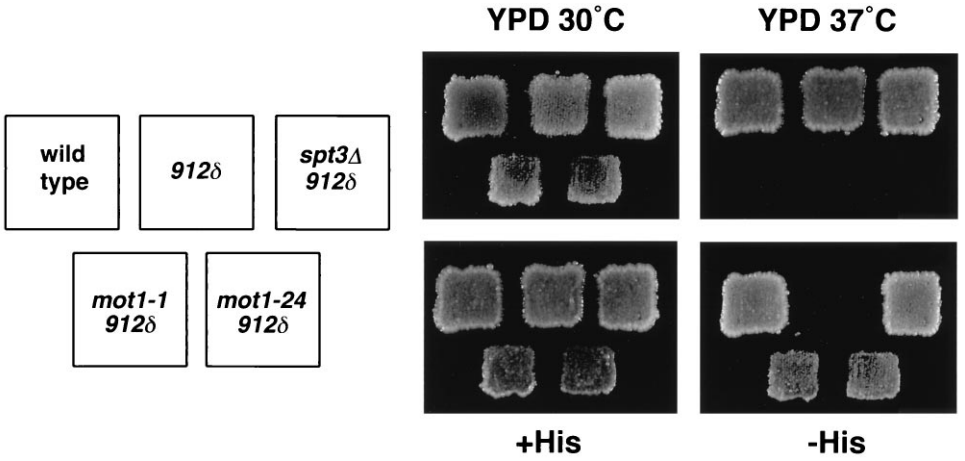

FIG. 3. *mot1* mutants have Spt$^-$ and Ts$^-$ phenotypes. Strains were patched onto a YPD plate and then replica plated to YPD plates at 30 and 37°C and to SD plates containing (+) and lacking (−) histidine. The strains used are L603, FY53, FY98, FY1213, and FY1211. The designation *912*δ indicates the genotype *his4-912*δ. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

related, we tested if *mot1* mutants and *spt3* mutants have any common phenotypes. Previous work has shown that an *spt3*Δ mutation suppresses Ty and solo δ insertion mutations in the *HIS4* and *LYS2* 5′ regions (64). We tested *mot1-1* and *mot1-24* mutants for suppression of the solo δ insertion mutation *his4-912*δ. Our results show that both *mot1* mutations strongly suppress *his4-912*δ (Fig. 3). The fact that two different *mot1* mutants have an Spt$^-$ phenotype suggests that this phenotype is not allele specific. Recently, in a separate study, other *mot1* mutations were shown to suppress a derivative of *his4-912*δ (28). However, *mot1-1* and *mot1-24* mutations fail to suppress a different insertion mutation, *his4-917*δ, that is suppressed by an *spt3*Δ mutation (data not shown). In addition, *spt3*Δ mutants have mating defects that have been correlated with decreased levels of mRNAs for the three major mating factor genes, *MF*α*1*, *MFa1*, and *MFa2* (24). Analysis of mating showed that both *mot1-1* and *mot1-24* mutants do not have detectable mating defects (data not shown). Thus, *mot1* mutant phenotypes overlap with but are not identical to *spt3*Δ phenotypes.

Analysis of *mot1-1 spt3*Δ and *mot1-1033 spt3*Δ double mutants. To determine if the lethality of the *mot1-24 spt3*Δ double mutation is allele specific with respect to *MOT1*, double mutants carrying *spt3*Δ and one of two other *mot1* alleles, *mot1-1* and *mot1-1033* were constructed. At 30°C, both the *mot1-1 spt3*Δ and *mot1-1033 spt3*Δ double mutants are alive but grow more slowly than any of the single mutants (Table 2). The growth of these double mutants correlates with the growth of the single *mot1* mutants at 30°C, where the *mot1* mutants range from fastest to slowest growth as follows: *mot1-1033* > *mot1-1* > *mot1-24* (data not shown). Since *spt3*Δ mutations do not show synthetic lethality or sickness with other *spt* mutations that cause poor growth (15, 62, 65), the *spt3*Δ *mot1* synthetic phenotype suggests that both proteins contribute in a nonredundant fashion toward a common, essential function.

***mot1* mutations cause reduced levels of certain transcripts.** It has been previously shown, both in vivo (13, 44) and in vitro (3), that *mot1* mutations cause increased levels of transcripts from a number of unrelated RNA polymerase II-dependent genes. Since *mot1* mutations cause an Spt$^-$ phenotype, we examined their effects on a set of transcripts that are reduced by an *spt3*Δ mutation. Surprisingly, this analysis showed that both *mot1-1* and *mot1-24* mutations cause greatly decreased levels of both Ty and *HIS4* mRNAs (Fig. 4A) (6- to 10-fold decrease) and also cause a significant decrease in *STE2* mRNA levels (3.2-fold decrease). Quantitation of the Northern blots by PhosphorImager analysis showed that the mRNA levels of all other genes tested (*DED1*, *MFa1*, *MFa2*, *TPI1*, and *TUB2*) were affected less than twofold. In the *spt3*Δ strain we observed greatly decreased levels of Ty, *MFa1*, and *MFa2* transcripts, as previously described for *spt3*Δ mutants. Thus, *spt3* and *mot1* mutations cause decreases in a partially overlapping set of transcripts. We also analyzed mRNA levels in both *mot1-1* and *mot1-24* mutants after 1, 2, and 3 h of growth at 37°C and observed decreases similar to those seen in cells grown at 30°C (data not shown). Thus, in contrast to previous observations, *mot1* mutations cause decreased levels of certain mRNAs.

The *mot1-1* mutation was originally isolated as bypassing the requirement for the activator Ste12 (13). To determine if our *mot1* mutants also had this phenotype, we analyzed the mRNA levels for two different Ste12-dependent genes in both *ste12*Δ and *STE12*$^+$ backgrounds. Based on the previous results, we would expect that in a *mot1* background the *ste12*Δ defect would be partially suppressed. However, our results showed that *ste12*Δ is not suppressed by *mot1* mutations (Fig. 4B); *ste12*Δ causes the same reduction in transcription in both *MOT1*$^+$ and *mot1* strains for two different Ste12-dependent

TABLE 2. Summary of *mot1 spt3*Δ synthetic lethality

Strain	Growth[a]:			Spt[d]
	At 30°C[b]	At 37°C[b]	With *spt3*Δ[c]	
Wild type	+	+	+/−	+
mot1-1033	+	−	+/−	−
mot1-1	+/−	−	−/+	−
mot1-24	−/+	−	−	−

[a] Growth of *mot1* and *mot1 spt3*Δ mutants. +, strong growth; +/−, weak growth; −/+, very weak growth; −, no growth.
[b] Growth on YPD.
[c] *mot1 spt3*Δ double mutant combinations were scored for growth at 30°C on 5-FOA.
[d] Suppression of the *his4-912*δ insertion allele was scored by comparing growth on minimal medium lacking and containing histidine. +, no growth on minimal medium lacking histidine; −, indicates growth on minimal medium lacking histidine.

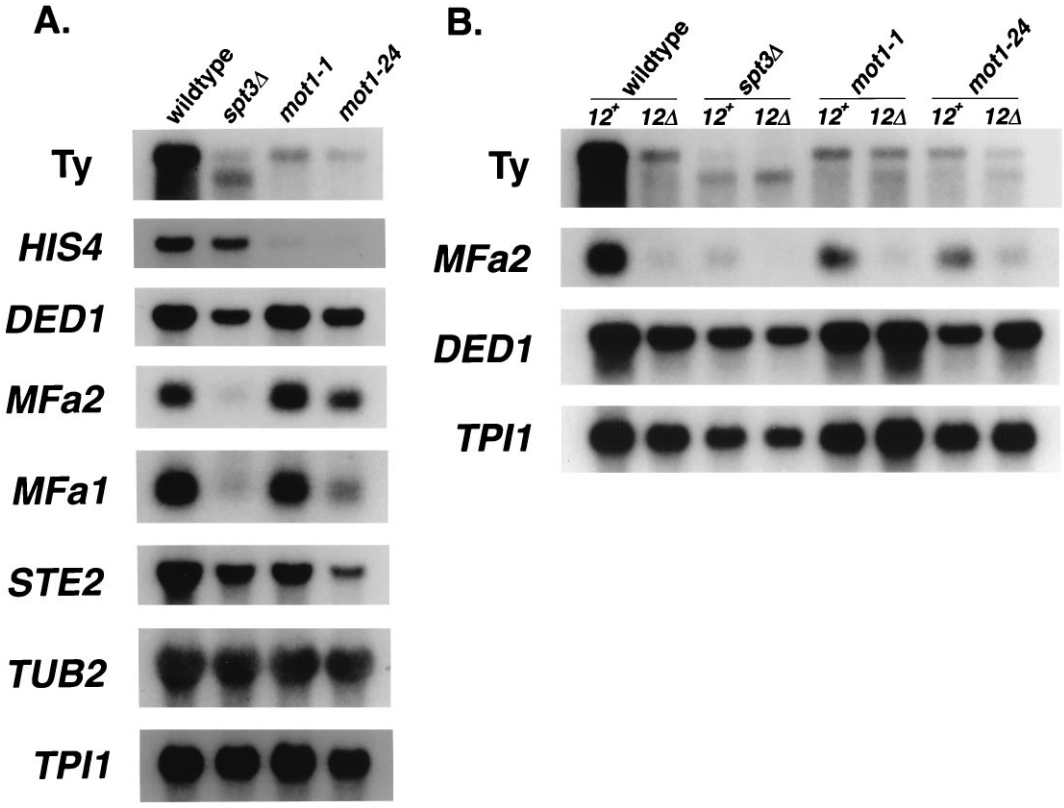

FIG. 4. *mot1* mutations decrease transcription. (A) mRNA levels of the genes indicated at the left were examined in the wild-type strain and in *mot1-1*, *mot1-24*, and *spt3*Δ mutants. The strains used were, from left to right, FY98, FY403, FY1214, and FY1216. Although the *mot1-24* mutation appeared to cause a decrease in *MFa1* message similar to that caused by *spt3*Δ, PhosphorImager analysis showed *MFa1* message levels to be decreased less than twofold compared to the normalization probe. (B) mRNA levels in the wild-type strain and in *mot1-1*, mot1-*24* and *spt3*Δ mutants were analyzed in *STE12*$^+$ and *ste12*Δ backgrounds. The strains used were, from left to right FY98, FY1217, FY403, FY822, FY1214, FY1219, FY1216, and FY1218. Total RNA was prepared from each strain and subjected to Northern analysis. Fifteen micrograms of RNA was run in each lane. The filter was hybridized with the probes listed at the left. After each successive hybridization, the filter was stripped and reprobed. The amount of RNA loaded in each lane varied by less than twofold as determined by normalization to *TPI1* message. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

transcripts, Ty and *MFa2*. Thus, we did not observe the same effects on transcription that Davis et al. (13) observed in the *mot1-1* mutant.

TFIIA overexpression partially suppresses *spt3*Δ. Several results strongly suggest that TFIIA and Mot1 are functionally related (2, 3). In addition, in vitro experiments show that TFIIA can stabilize TBP binding to DNA, and it has been proposed that Spt3 might function to help TBP bind to TATA boxes (15). Therefore, we tested if overexpression of *TOA1* and *TOA2*, the yeast genes that encode the two subunits of TFIIA, could suppress an *spt3*Δ mutation. We also tested for suppression of the *spt15-21* mutation, which has been proposed to cause a defect in a functional interaction between TBP and Spt3 (15). Suppression was examined by assaying the Spt$^-$ phenotype with respect to the insertion mutation *lys2-173R2*. In a wild-type background, *lys2-173R2* strains are Lys$^+$, while in *spt3*Δ or *spt15-21* mutant backgrounds, this insertion causes a Lys$^-$ phenotype (17, 65).

The results demonstrated that overexpression of TFIIA suppresses both *spt3*Δ and *spt15-21* mutations (Fig. 5). Both *spt3*Δ and *spt15-21* strains are Lys$^-$ in the absence of TFIIA overexpression; however, when TFIIA was overexpressed, both the *spt3*Δ and the *spt15-21* strains were Lys$^+$. Overexpression of either *TOA1* or *TOA2* alone fails to suppress *spt3*Δ or *spt15-21*, suggesting that it is the increased level of functional TFIIA that is responsible for the suppression (data not shown). To test if overexpression of any other protein that binds to TBP can generally suppress *spt3*Δ or *spt15-21* mutations, we also overexpressed *SUA7*, which encodes TFIIB (45). As shown in Fig. 5, *SUA7* overexpression does not suppress *spt3*Δ or *spt15-21* mutations. Finally, overexpression of *SPT15* very weakly suppressed an *spt3*Δ mutation (34, 48). Taken together, these results strongly suggest that Spt3 and TFIIA are functionally related.

A *toa1* mutant is Spt$^-$, and the mutation causes synthetic lethality with both an *spt3*Δ mutation and *mot1* mutations. Prompted by the TFIIA dosage suppression of an *spt3*Δ mutation, we tested a *toa1* mutation for Spt$^-$ phenotypes and for synthetic lethality with an *spt3*Δ mutation and with *mot1* mutations. To determine if *toa1* mutants have Spt$^-$ phenotypes, we analyzed *toa1-18GSG* for suppression of the solo δ insertion mutations *his4-912*δ and *his4-917*δ. *toa1-18GSG* was found to suppress *his4-912*δ weakly at 30°C and strongly at 32°C; *his4-917*δ was not suppressed (data not shown).

To test for synthetic lethality between *toa1* and *spt3*Δ mutations, we crossed the *toa1-18GSG* mutant with an *spt3*Δ strain. Tetrad analysis demonstrated that the *toa1-18GSG spt3*Δ double mutant is inviable (Table 3). Toa1 and Toa2 protein levels were unaffected in an *spt3*Δ mutant compared to a wild-type strain (data not shown), suggesting that the *toa1 spt3*Δ synthetic lethality is caused by the combined loss of both activities and not by an effect of *spt3*Δ on *TOA1* or *TOA2* expression. To

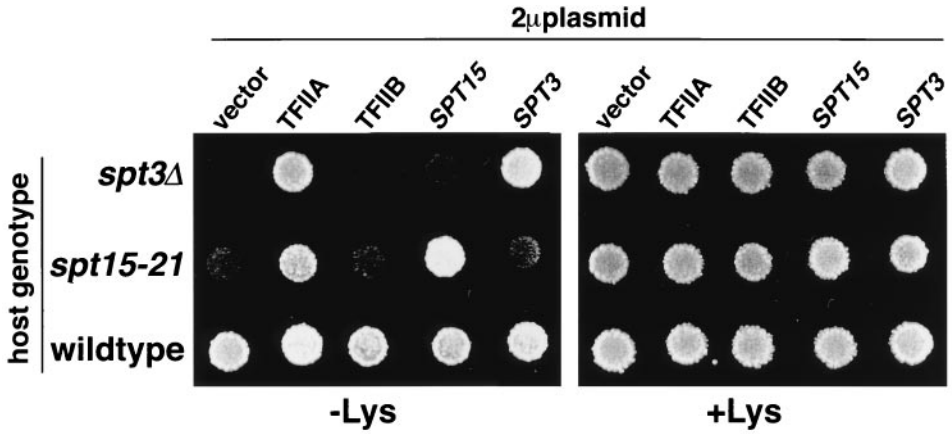

FIG. 5. Overexpression of TFIIA suppresses *spt3*Δ. A wild-type strain (FY631), an *spt3*Δ mutant (FY294), and an *spt15-21* mutant (FY567) containing the insertion mutation *lys2-173R2* were each transformed with the following high-copy-number plasmids: pRS426 (vector), pJM122 (*TOA1* and *TOA2* in pRS426), pSB238, (*SUA7* in pRS426), pDE31-7 (*SPT15* in pCGS42), and pFW32 (*SPT3* in pCGS42). These strains were spotted on SC-Ura and replica plated to both SC-Ura-Lys and SC-Ura. This figure was produced by using Adobe Photoshop and a Fujix Pictography 3000 printer.

complete the analysis of possible synthetic lethal relationships between *SPT3*, *TOA1*, and *MOT1*, we also tested for *toa1 mot1* synthetic lethality. These results (Table 3) demonstrate that *toa1-18GSG mot1-24*mutants are also inviable. Thus, all double mutation combinations among *spt3*, *toa1*, and *mot1* mutations cause synthetic lethality, and each mutation alone causes some Spt$^-$ phenotypes, strongly suggesting that the products of these genes are functionally related and may control similar aspects of TBP function.

DISCUSSION

In these studies we have demonstrated a functional relationship between Spt3, Mot1, and TFIIA. The connection between Spt3 and Mot1 comes from three lines of evidence. First, an *spt3*Δ mutation causes lethality in combination with a *mot1* mutation. Second, *mot1* mutants have an Spt$^-$ phenotype. Third, *mot1* mutations cause a significant decrease in the levels of Ty mRNA. The connection between Spt3 and TFIIA was investigated based on previous studies demonstrating an interaction between Mot1 and TFIIA. Our results show that overproduction of TFIIA suppresses some *spt3*Δ mutant phenotypes, that *toa1* mutants have Spt$^-$ phenotypes, and that *spt3*Δ *toa1* double mutants are inviable. Finally, we have shown that *toa1 mot1* double mutants are also inviable. Taken together, these data (summarized in Fig. 6) suggest that Spt3, Mot1, and TFIIA work together to control promoter-specific TBP-TATA box interactions.

The identification of *mot1* as an *spt3*Δ synthetic lethal mutation has led us to the surprising result that *mot1* mutations cause Spt$^-$ phenotypes and greatly decreased levels of both Ty and *HIS4* transcripts. In contrast, previous analyses showed that *mot1* mutations increased transcription in vivo and in vitro (3, 13, 44). In those studies only one gene, an *MFa2-lacZ* fusion, showed a modest (twofold) decrease (3). We also observed a modest decrease in *MFa2* mRNA levels in our analysis. However, even though we have examined transcription of some of the same genes, our results differ from those of past studies. The discrepancies between the different studies may reflect differences in genetic backgrounds or, in some cases, a

TABLE 3. Combinations of *mot1*, *toa1*, and *spt3*Δ mutations cause lethality

Strain	Viability[a]
Wild type	+
*spt3*Δ	+
toa1-18GSG	+
mot1-24	+
*mot1-24 spt3*Δ	−
*toa1-18GSG spt3*Δ	−
mot1-24 toa1-18GSG	−

[a] Viability or inviability of double mutants was tested by crossing the two single mutants, followed by tetrad analysis. In each cross, one of the two mutants also contained the cognate wild-type gene on a *URA3 CEN* plasmid. These putative double mutants were tested for the ability to lose the plasmid on 5-FOA. The plasmids used were pCC1 (*SPT3 URA3 CEN*) for *mot1-24 spt3*Δ and *toa1-18GSG spt3*Δ double mutants and pRS7.1*Bgl*II, (*MOT1 URA3 CEN*) for both *mot1-24 spt3*Δ and *mot1-24 toal-18GSG* double mutants. In every cross performed we were able to monitor 2:2 segregation of each relevant marker, thus allowing positive identification of the double mutant progeny in every tetrad. The *spt3*Δ mutation was scored by Spt$^-$ and Trp$^+$ phenotypes and in some cases by PCR, the *mot1-24* mutation was scored by a Ts$^-$ phenotype and by complementation, and the *toa1-18GSG* mutation was scored by a Ts$^-$ phenotype and by PCR. For the *toa1-18GSG spt3*Δ cross, 15 tetrads were analyzed. For the other two synthetic lethal combinations, over 40 tetrads were analyzed for each combination. +, viable (5-FOA resistant); −, inviable (5-FOA sensitive).

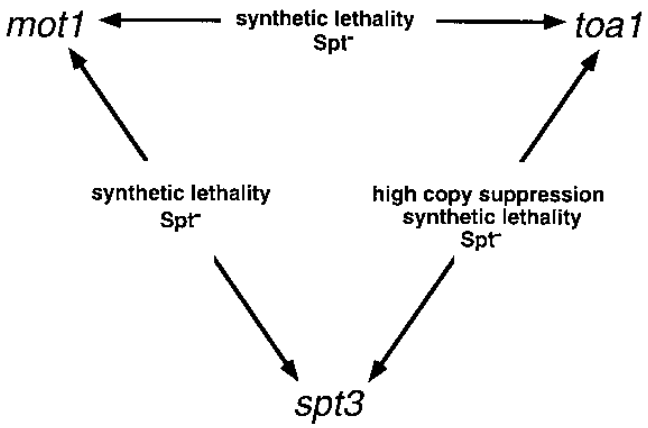

FIG. 6. Summary of the genetic interactions between *mot1*, *toa1*, and *spt3*Δ mutations described in this study. The high-copy suppression specified between *TOA1* and *TOA2* of an *spt3*Δ mutation specifically refers to *TOA1* and *TOA2* high-copy suppression of *spt3*Δ; *SPT3* in high copy does not suppress *toa1* mutants.

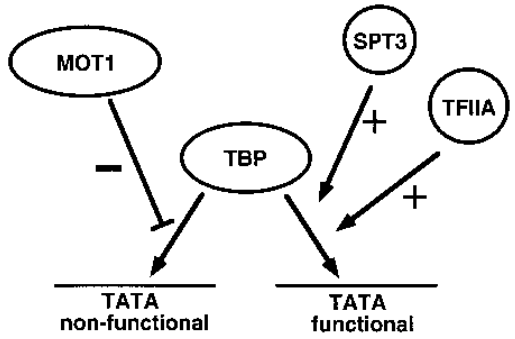

FIG. 7. Possible roles for Mot1, Spt3, and TFIIA at Spt3-dependent promoters. In this model, Mot1 blocks binding of TBP to nonfunctional TATA boxes, and Spt3 and TFIIA promote binding of TBP to functional TATA boxes. The balance of these negative and positive activities may determine TATA box selection in vivo.

variation in results for a relatively small effect. Given the strong dependence of both Ty and *HIS4* transcription on Mot1, our results strongly suggest that Mot1 functions to activate transcription, directly or indirectly, at certain promoters in vivo. Similar effects of *mot1* mutations on transcript levels have also been observed recently in two other independent studies (11, 41a).

In addition to genetic interactions between Mot1 and Spt3, we have demonstrated two types of genetic interactions between Spt3 and TFIIA: suppression of an *spt3*Δ mutation by overexpression of TFIIA and synthetic lethality of *spt3*Δ *toa1* mutants. In addition, a *toa1* mutation causes an Spt$^-$ phenotype. These results suggest that TFIIA and Spt3 might have similar or overlapping functions. Since TFIIA has been shown to stabilize TBP binding to DNA, a similar role seems likely for Spt3. In light of Spt3 being nonessential for growth and required only at certain promoters, its activity could be either to strengthen TFIIA function or to stabilized TBP-DNA interactions in a way independent of but additive with TFIIA. In either case, some as-yet-unrecognized promoter element or aspect of the TATA region must confer Spt3 dependence, either directly or indirectly.

Our results, taken together with past studies of Mot1 and Spt3, lead to a model in which Mot1, TFIIA, and Spt3 all contribute to transcriptional regulation by promoting TBP binding to functional TATA sequences (Fig. 7). In this model, Mot1 acts indirectly, by preventing TBP from binding to nonfunctional TATA sequences, and TFIIA and Spt3 act directly to stabilize TBP on functional TATA sequences. We define a functional TATA box as a TATA sequence located in the context of a functional promoter. A nonfunctional TATA box would be a TATA sequence outside such a context; for example, at an inactive promoter or within the coding region of a gene. This model is consistent with the in vitro activities that have been demonstrated for both Mot1 and TFIIA. An additional consideration of this model is that Spt3 is required only at certain promoters. At Spt3-independent promoters, an Spt3-like activity may not be required, or other factors could work in a functionally similar fashion. One candidate for such a factor with Spt3-like activity is Fun81, the yeast protein that, like Spt3, shows similarity to human $TAF_{II}18$ (14, 38). The *mot1* effects that we have observed are promoter specific, since we detected no effect or very modest effects on transcript levels for six of eight genes examined. However, since *MOT1* is essential, these defects likely represent only a subset of Mot1 activates in vivo. Future genetic analysis of other *mot1* mutants should help clarify the in vivo activity of Mot1 and the specificity of its effects.

This model accounts for our molecular and genetic results in the following manner. First, *mot1* mutations would lead to decreased transcription by allowing TBP to remain bound to nonfunctional TATA sequences, thus lowering the amount of TBP available for preinitiation complexes on functional TATA boxes. Similarly, if either positive factor, Spt3 or TFIIA, is mutant, then TBP would be less stably bound at a functional TATA box, also resulting in reduced transcription levels. In double mutants where two of these functions are defective, the ability of TBP to bind to functional TATA boxes is reduced below a critical threshhold required to maintain viability, resulting in the synthetic lethality that we have observed. In agreement with this model, overexpression of TFIIA exacerbates poor growth of *mot1* mutants (34). In this case we hypothesize that TFIIA is stabilizing TBP bound to nonfunctional TATA sequences. Our model relies on the assumption that the biochemical activity for Mot1 reflects its in vivo activity. Conceivably, other proteins could modify Mot1 activity in vivo such that it acts in a reverse direction, to promote stable TBP-DNA complexes. Such an activity would also be consistent with the decreased transcription we have observed in *mot1* mutants.

Our model suggests possible biochemical interactions and activities for Spt3. We have tested extensively for several of these activities, and the results are summarized here. In many of these experiments we have used a glutathione *S*-transferase–Spt3 (GST-Spt3) fusion that has been purified from yeast and that is fully functional for Spt3 activity in vivo (34). First, we assayed GST-Spt3 for the following TFIIA-like activities: the ability to supershift a TBP-DNA gel shift complex, the stabilization of a TBP-DNA gel shift complex under suboptimal magnesium conditions, and the ability to supershift a TBP-TFIIA-DNA gel shift complex. All of these results have been negative. Second, we have tested Spt3 for the ability to interact with Mot1 and TFIIA by both gel shift and coimmunoprecipitation; again, the results were negative. Finally, we have tested for the ability of GST-Spt3 to affect the activity of Mot1 in vitro. In this assay, Mot1 can supershift a TBP-DNA complex, and upon addition of ATP, the Mot1-TBP-DNA complex is disrupted (2, 3). TFIIA has been shown to stabilize a TBP-DNA complex under these conditions. In the Mot1 assay, we have been unable to show that a GST-Spt3 fusion protein can stabilize a TBP-DNA complex in the presence of Mot1 and ATP. One possible explanation for our inability to demonstrate an activity for Spt3 in vitro is that other proteins or a certain promoter context may be required for the Spt3 activity. Two lines of evidence support this hypothesis. First, Spt3 sediments on glycerol gradients as if it was part of a large complex (48). Second, mutations in other *SPT* genes, including *SPT7* (20), *SPT8* (16), and *SPT20* (35, 49), cause mutant phenotypes similar to those caused by *spt3* and *spt15* mutations, suggesting that the products encoded by these genes might be required for Spt3 activity.

In conclusion, our results suggest that Spt3, Mot1, and TFIIA are functionally related and that together they may control promoter-specific TBP binding. In contrast to previous analyses of Mot1 that suggested it represses transcription, our studies suggest that Mot1 may have a role in activating transcription in vivo. Conceivably, Mot1 could play roles in both activation and repression of transcription. Our studies have revealed a potential overlap in the in vivo function of TFIIA and Spt3, further suggesting that Spt3 may have a biochemical activity that helps specify some aspect of TBP binding to TATA boxes. Ultimately, a better understanding of the inter-

play between Mot1, TFIIA, and Spt3 in the regulation of TBP function at both the biochemical and genetic levels should provide a more complete understanding of transcriptional control in vivo.

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