The *NOT*, *SPT3*, and *MOT1* Genes Functionally Interact To Regulate Transcription at Core Promoters

MARTINE A. COLLART*

Department of Medical Biochemistry, University of Geneva
Medical School, 1211 Geneva 4, Switzerland

Previous studies demonstrated that mutations in the *Saccharomyces cerevisiae NOT* genes increase transcription from TATA-less promoters. In this report, I show that in contrast, mutations in the yeast *MOT1* gene decrease transcription from TATA-less promoters. I also demonstrate specific genetic interactions between the Not complex, Mot1p, and another global regulator of transcription in *S. cerevisiae*, Spt3p. Five distinct genetic interactions have been established. First, a null allele of *SPT3*, or a mutation in *SPT15* that disrupts the interaction between Spt3p and TATA-binding protein (TBP), allele specifically suppressed the *not1-2* mutation. Second, in contrast to *not* mutations, mutations in *MOT1* decreased *HIS3* and *HIS4* TATA-less transcription. Third, *not* mutations suppressed toxicity due to overexpression of TBP in *mot1-1* mutants. Finally, overexpression of *SPT3* caused a weak Not⁻ mutant phenotype in *mot1-1* mutants. Collectively, these results suggest a novel type of transcriptional regulation whereby the distribution of limiting TBP (TFIID) on weak and strong TBP-binding core promoters is regulated: Mot1p releases stably bound TBP to allow its redistribution to low-affinity sites, and the Not proteins negatively regulate the activity of factors such as Spt3p that favor distribution of TBP to these low-affinity sites.

Accurate transcription initiation of protein-coding genes depends on the specific recognition of core promoter elements and the assembly of a precisely positioned RNA polymerase II-containing complex over this core promoter region. A large class of eukaryotic core promoters contains a TATA box. In this case, the recognition event is carried out by TFIID (3, 35), which in higher eukaryotes is a multiprotein complex containing the TATA-binding subunit TBP and several additional factors known as TBP-associated factors (TAFs) (for a review, see reference 15). For the promoters that lack a TATA box, the elements involved in the specific recognition event have not clearly been established. TBP has been shown to be required for initiation at such promoters (5, 9, 17, 21, 27, 28, 32, 37, 41–43) but may or may not be involved in the recognition event. Recent studies suggest that several types of TATA-less core promoters may exist (discussed in references 21 and 31), and several mechanisms for TBP recruitment to these different class II promoters have been proposed. These include binding of TBP to low-affinity sites (37, 42), the tethering of TBP by upstream-bound activators interacting with TAFs (28), interaction of TBP with sequence-specific initiator-bound proteins (30, 36), and finally tethering of TBP by initiator-bound factors interacting with TAFs (21). Most of these models are derived from studies performed on mammalian in vitro transcription systems reconstituted with crude nuclear extracts, partially purified components, or purified recombinant proteins. In such systems, TATA-less transcription is extremely inefficient relative to transcription of promoters containing canonical TATA sequences, a fact that suggests that critical factors may be missing in these systems and thus not necessarily reflect an in vivo representation.

We have previously presented a genetic approach to study transcriptional regulation using the *HIS3* gene of the yeast

Saccharomyces cerevisiae, a particularly useful model system to determine differences between core promoters (7). The *HIS3* gene is under the control of two proximal elements, T_C and T_R, that are functionally distinguishable (6, 14, 20, 24, 33). Both elements support basal transcription dependent on TBP, but only T_R can respond to transcriptional activators. Whereas T_R behaves as a canonical TATA box and is referred to as the TATA promoter, T_C lacks a conventional TATA element, is referred to as TATA-less, and is unable to support transcription in vitro. We characterized the *NOT* genes (*NOT1* [*CDC39*], *NOT2* [*CDC36*], *NOT3*, and *NOT4* [*SIG1* {18} or *MOT2* {4, 16}]) that encode global negative regulators of transcription (8). These factors repress T_C- but not T_R-dependent transcription and can thus distinguish between weak and strong TBP-binding core promoters. We have previously suggested that the Not proteins inhibit the basic RNA polymerase II machinery possibly by affecting TFIID function (8).

A number of mutations have identified global positive and negative regulators of transcription (for a review, see reference 34). These mutations affect many genes that are not obviously related but may contain similar structural or functional units in vivo. Mutations in some of these regulators behave phenotypically similarly to mutations in histones (39) and may thus affect the chromatin state of promoter regions. Other global factors might recognize core promoter type and thus be functionally related to the Not proteins. I hence studied two groups of regulators that have known interactions with TBP, in an effort to define the mechanism of Not regulation.

The *SPT3*, *SPT8*, and *SPT15* genes were originally identified by mutations that suppress Ty and δ element insertion mutations in the 5′ noncoding regions of *HIS4* and *LYS2* (for a review, see reference 38). *SPT15* encodes TPB (13), and the other two Spt proteins are thought to play a role in conjunction with TBP to initiate transcription at a subset of core promoters. Spt3p interacts with TBP (11), and Spt8p is required for this interaction to be functional (12). Little is known about the roles of Spt3p and Spt8p in transcription initiation or what defines a gene regulated by these Spt proteins.

* Mailing address: Department of Medical Biochemistry, University of Geneva Medical School, 1 rue Michel Servet, 1211 Geneva 4, Switzerland. Phone: 41-22-702 55 16. Fax: 41-22-702 55 02.

TABLE 1. Strains used

Strain	Genotype	Reference or source
KY803	**a** *ura3-52 trp-Δ1 leu2::PET56 gal2 gcn4-Δ1*	15a
KY804	Isogenic to KY803 except α	8
MY603	Isogenic to KY804 except *mot1-1*	This work
MY779	Isogenic to KY803 except *mot1-1 not1-2*	This work
MY794	Isogenic to KY803 except *mot1-1 not3-2*	This work
MY923	Isogenic to KY803 except *mot1-1033*	This work
MY925	Diploid isogenic to KY803 except *not1-2/NOT1 not3-3/NOT3*	This work
MY972	Isogenic to KY803 except *spt8::LEU2*	This work
MY973	Isogenic to KY804 except *spt8::LEU2 not1-2*	This work
MY978	Isogenic to KY804 except *not1::LEU2* pRS316-*NOT1*	This work
MY1049	Isogenic to KY803 except *spt3::TRP1*	This work
MY1050	Isogenic to KY803 except *spt3::TRP1 not1-2*	This work
MY1054	Isogenic to KY803 except *spt3::TRP1 not1-2 not3-3*	This work
MY1075	Isogenic to KY803 except *spt8::LEU2 spt3::TRP1 not1-2*	This work
MY1166	Isogenic to KY804 except *mot1-1 spt3::TRP1*	This work
MY1173	Isogenic to KY804 except *spt3::TRP1 not4-1*	This work
JD215b	**a** *ura3-52 his4-519 leu2-3,112 trp1-1 can1-101 mot1-1*	10
GL1033	**a** *ura3-52 leu2-3,112 trp1-289 ade5 gal2 can1 mot1-1033*	23
FY631	**a** *ura3-52 leu2Δ1 his4-917δ lys2-173R2 trp1Δ63*	11
FY567	**a** *ura3-52 leu2Δ1 his4-917δlys2-173R2 ade8 spt15-21*	11
FY294	**a** *ura3-52 leu2Δ1 his4-917δ lys2-173R2 trp1Δ63 spt3Δ202*	11
MY1402	Isogenic to FY631 except *not1-2*	This work
MY1404	Isogenic to FY567 except *not1-2*	This work
MY1405	Isogenic to FY294 except *not1-2*	This work

The *MOT1* gene encodes a global negative regulator of RNA polymerase II transcription that was originally identified in a genetic selection for increased basal transcription (10). It encodes an essential protein that specifically dissociates TBP-DNA complexes in an ATP-dependent manner, and it can inhibit transcription in vitro (1, 2). Although a biochemical activity has been clearly defined for Mot1p in vitro, its role in the general regulation of transcription initiation in vivo is not known.

In this paper, I present evidence that the *NOT* and *MOT1* genes participate in a dynamic regulation of RNA polymerase II transcription in vivo. My results suggest that the Not proteins regulate the activity of Spt3p, one of the positive transcription factors that can help recruit and/or stabilize TBP (TFIID) to core promoters with low affinity for TBP. They also suggest that Mot1p removes TBP from high-affinity sites to make it available for core promoters with weaker affinity for TBP and is essential because TBP (TFIID) is limiting in vivo. I thus propose a novel type of transcriptional regulation that involves regulation of the distribution of limiting transcription factors on weak and strong TBP-binding core promoters.

MATERIALS AND METHODS

Yeast strains and culture media. The yeast strains used in this work are listed in Table 1 and were generated by standard genetic techniques. MY604 and MY603, carrying the *mot1-1* allele, were obtained by crossing JD215b (10) to KY804, sporulating diploids, and analyzing tetrads. His⁺, aminotriazole (AT)-sensitive, and temperature-sensitive spores were crossed back twice. All temperature-sensitive spores isolated from the third backcross were found to have similar phenotypes, and Mat**a** and Matα spores were saved as strains MY604 and MY603, respectively. MY923 was obtained similarly, starting with strain GL1033 (23). MY610 was obtained by crossing MY603 to MY3 and selecting for Trp⁺

temperature-sensitive spores. MY1445 and MY1451 were obtained by crossing MY610 carrying the *mot1-1* and *his3::TRP1* alleles to MY80 carrying the *his3-205* allele (7) and selecting for His⁺ temperature-sensitive and His⁺ temperature-resistant spores, respectively. MY1457 and MY1454 were obtained similarly by crossing MY79 carrying the *his3-Δ93* allele to MY610.

The rich medium used in this work was YPD; rich medium selective for a *TRP1* or *URA3* plasmid was minimal medium containing 7% Casamino Acids, uracil or tryptophan, and adenine. Minimal medium referred to in this work consists of 0.67% yeast nitrogen base without amino acids (Difco) and 2% glucose supplemented with amino acids, adenine, or uracil as required.

DNA manipulations. pMAC85, a pBSSK derivative containing the *not1::LEU2* allele, was cloned in two steps. pRS316 was digested by *Hin*dIII-*Sac*I, and the *Xho*I-*Hin*dIII fragment of pBS7 containing the *LEU2* gene (inserted between *Xho*I and *Sal*I in pBSSK) as well as the *Xho*I-*Sac*I fragment of pRS316-Sc3863 (carrying the 5′ half of *NOT1*) (7) were inserted (pMAC84). pMAC84 was digested by *Eco*RI-*Hin*dIII, and the *Eco*RI-*Hin*dIII fragment of pRS316-Sc3863 was inserted. For integration of the null allele to the *NOT1* locus, pMAC85 was digested by *Bam*HI-*Sac*I. FB652 (40), carrying the *spt3::TRP1* allele, was linearized by *Ssp*I for integration to the *SPT3* locus; FB749 (12) carrying the *spt8::LEU2* allele was digested by *Xba*I-*Bam*HI for correct integration to the *SPT8* locus. These integration events were verified by Southern analysis of genomic DNA digested by *Hin*dIII for *SPT3* or *Eco*RI for *SPT8*. YEplac195-*SPT3* (pMAC95) was obtained by cloning the *Eco*RI-*Xho*I fragment of FB153 into the *Sal*I-*Eco*RI sites of YEplac195. pMAC132 is a pET15 (Novagen) derivative used for bacterial expression of Not1p. It was made by cloning the *Eco*RI-*Bam*HI fragment containing the ATG of *NOT1* from plasmid Lex202-*NOT1* (8) into pBSSK. From the resulting clone, an *Xho*I-*Bgl*II fragment was cut out and cloned into pET15b (Novagen) digested with *Xho*I and *Bam*HI.

RNA analysis. Total cellular RNA (30 μg) from cells grown under appropriate conditions (7, 9) was hybridized to completion with an excess (2 ng) of the appropriate oligonucleotides, and the products were digested with S1 nuclease and electrophoretically separated as described previously (7). All hybridization reactions contained multiple probes to ensure that the determinations were controlled internally.

AT resistance assays. All strains analyzed in this work have a chromosomal deletion of *GCN4* (*gcn4-Δ1*) and are *leu2::PET56*. To test mutants for AT resistance, strains are transformed with YCplac111-4363, a *LEU2* centromeric plasmid carrying the *gcn4-C163* derivative. This derivative encodes a mutant Gcn4p lacking a large part of the activation domain. Wild-type Gcn4p activates transcription of the *HIS3* gene such that cells can grow on more than 120 mM AT, a competitive inhibitor of the *HIS3* gene product. In contrast, Gcn4-C163 activates *HIS3* only such that cells can grow on 5 mM AT. Thus, transformants of mutant strains are analyzed on minimal medium lacking histidine and containing various concentrations of AT. Wild-type *gcn4-C163* cells grow on 5 mM AT, whereas *not gcn4-C163* mutants grow on 40 mM AT (7, 8).

Preparation of antiserum. pMAC132 (see above) was transformed into *Escherichia coli* BL21 (Novagen). Transformants were grown in liquid (3 × 1.7 liters) and induced at an optical density at 600 nm of 0.4 for 75 min with 1 mM isopropylthiogalactopyranoside (IPTG). Cells were pelleted and broken by multiple rounds of freeze-thawing and by sonication for 5 min in 50 ml of 25 mM Tris (pH 8.0) and 50 mM NaCl. After centrifugation, the insoluble fraction containing Not1p was resuspended in 20 ml of 6 M urea–0.5 M NaCl–20 mM sodium phosphate–1 mM imidazole and was incubated with 4 ml of Ni-nitrilotriacetic acid (Qiagen). The beads were washed with 100 mM imidazole, and the Not1p was eluted at 300 to 800 mM imidazole in 6 M urea. These elution fractions were dialyzed, lyophilized, and resuspended in phosphate-buffered saline for injection in the rabbits (Elevage Scientifique Des Dombes).

Cell extracts and Western blotting (immunoblotting). Ten-milliliter aliquots of cells grown to half saturation in rich medium were pelleted and washed twice in 25 mM Tris-phosphate (pH 6.8)–2 mM phenylmethylsulfonyl fluoride, and the cell pellet was frozen in liquid nitrogen. The cell pellet was thawed on ice and resuspended in 200 μl of the same buffer, and an equal volume of glass beads was added. Cells were broken by vortexing four times for 50 s each at 4°C. The cell extract was obtained by clarification for 10 min at 15,000 rpm at 4°C in a microcentrifuge. Protein concentration was determined by the Bradford assay (Bio-Rad), and equal amounts of all extracts were loaded on a sodium dodecyl sulfate (SDS)–6% polyacrylamide gel. After transfer to nitrocellulose, Not1p was revealed by probing the blot with the Not1p antiserum used at a 1:3,000 dilution. Secondary goat anti-rabbit antibodies conjugated to alkaline phosphatase (Bio-Rad) were used at a 1:3,000 dilution.

RESULTS

One model for negative regulation by the Not proteins is that they inhibit global positive factors more specifically required for initiation at TATA-less promoters. The Spt3 and Spt8 proteins have been loosely defined as global positive factors of transcription and thus are potential candidates as factors inhibited by the Not proteins. I took a genetic approach to determine whether the genes encoding these factors are func-
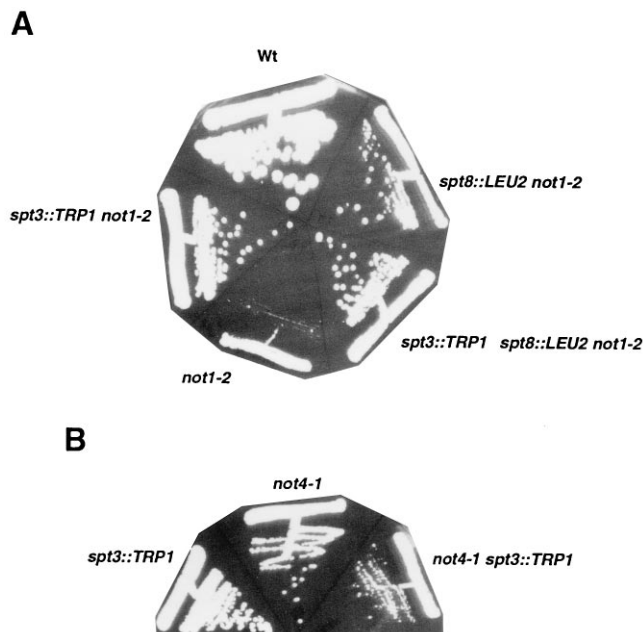
A

B

FIG. 1. (A) Suppression of *not1-2* by *spt3*::*TRP1* and *spt8*::*LEU2*. The indicated strains were streaked on rich medium and grown for 3 days at 37°C. Wt, wild type. (B) Synthetic growth phenotype between *spt3*::*TRP1* and *not4-1*. The indicated strains were streaked on rich medium and grown for 3 days at 30°C.

tionally related. The interest in such an analysis lies in the fact that Spt3p interacts with TBP (11) and Spt8p is required for this interaction to be functional (12). Thus, if genetic interactions can be established between these factors and the Not proteins, one could address if and how the Not proteins affect TFIID function.

spt3 and *spt8* mutations were introduced into strains carrying various mutations in the *NOT* genes. Phenotypes of single and double mutants were compared to determine whether *spt3* and/or *spt8* mutations suppress Not⁻ mutant phenotypes, or alternatively whether specific *not* mutations suppress mutant phenotypes associated with *spt* mutations. Strains carrying mutations in *NOT3* and *NOT1* were initially chosen for this analysis. *not3* mutations do not lead to defective growth phenotypes and are therefore useful to assess suppression of growth defects associated with other mutations. *NOT1*, on the other hand, is the only essential *NOT* gene isolated so far and must therefore be a critical component for Not function.

spt3 and spt8 null mutations suppress the not1-2 allele specifically. The MY925 diploid strain is heterozygous for the *not1-2* and *not3-3* mutations and was transformed with DNA fragments carrying the *spt3*::*TRP1* or *spt8*::*LEU2* allele. Single, double, and triple mutants were obtained by tetrad analysis of the transformants carrying the correct integration events as verified by Southern analysis (data not shown). Phenotypic analysis of the spores determined that null mutations of *SPT3* or *SPT8* are associated with very slow growth in minimal medium. This phenotype was not suppressed by either *not1-2*, *not3-3*, or the two mutations combined. Both *spt* null alleles, however, suppressed the temperature-sensitive growth phenotype associated with *not1-2* and did so the same extent, whether alone or combined (Fig. 1A). Neither *spt* mutation could suppress *not1-2* if the cell was additionally carrying the *not3-3* mutation (not shown).

To determine whether *spt3* or *spt8* null mutations can bypass the cell's essential requirement for *NOT1*, null mutant strains

were crossed to MY978, containing the *not1*::*LEU2* allele and carrying a wild-type copy of *NOT1* on a *URA3* centromeric plasmid. Tetrads were dissected, and four-spore tetrads were tested for growth on 5-fluoroorotic acid. Tetrads showed a 2:2 segregation of viability on 5-fluoroorotic acid regardless of the segregation of the *spt* null alleles. Thus, null mutations in *spt3* or *spt8* cannot suppress a null mutation in *NOT1*.

To further determine the specificity of *not1-2* suppression by the null alleles of *SPT3* and *SPT8*, I crossed the null mutant strains to strains carrying the temperature-sensitive *not1-1*, *not2-1*, *not2-2*, or *not4-1* mutation, sporulated diploids, and analyzed tetrads. I found that null alleles of *SPT3* and *SPT8* could not suppress temperature sensitivity associated with the other *not* mutants tested but on the contrary gave rise to a slight synthetic phenotype, as shown for *not4-1* in Fig. 1B. Thus, the *spt3* and *spt8* null mutations suppressed gene and allele specifically the temperature-sensitive growth phenotype of *not1-2.*

spt3 suppression of not1-2 is not due to an alteration in not1-2 expression. To exclude the possibility that suppression of temperature sensitivity associated with *not1-2* could be due to an increase in expression of the *not1-2* allele by mutations in *SPT3* and *SPT8*, I transformed *not1-2* mutant cells with a high-copy-number plasmid carrying the *not1-2* mutant allele. Overexpression of *not1-2* could not complement the temperature sensitivity of *not1-2* mutant cells (not shown). It thus seems unlikely that the *spt3* and *spt8* null mutations exert their effect through increased expression of *not1-2.*

To further exclude that the Not1-2 mutant protein might be unstable and *spt3*::*TRP1* reverse this instability, I analyzed total cellular extracts from wild-type, *not1-2*, *spt3*::*TRP1*, and *not1-2 spt3*::*TRP1* cells for the presence of the wild-type or mutant Not1 protein by Western blot analysis with an antibody raised against the N-terminal 241 amino acids of Not1p (see Materials and Methods). Figure 2 shows that Not1p was detectable in extracts from wild-type cells as two forms migrating with an apparent molecular mass of approximately 200 kDa (lane 1). The Not1 proteins detectable in extracts from *spt3* cells were indistinguishable (compare lanes 1 and 3). In extracts from *not 1-2* mutants, however, much less 200-kDa Not1p was detectable, and instead two forms of lower apparent molecular mass (either proteolytic degradation or premature
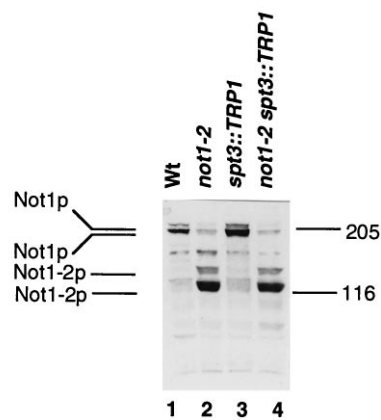

FIG. 2. *spt3*::*TRP1* does not alter the expression of the Not1-2 protein. Twenty-microgram aliquots of total cell extracts of the indicated strains were separated on an SDS–6% polyacrylamide gel, transferred to nitrocellulose, and probed with an antibody raised against the N-terminal 241 amino acids of Not1p. The proteins identified as Not1p or Not1-2p proteolytic degradation products are indicated on the left, and positions of molecular weight standards (in kilodaltons) are indicated on the right. Wt, wild type.
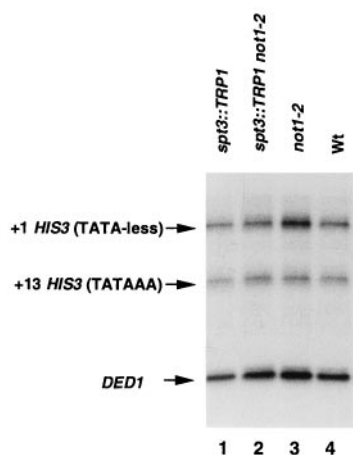
FIG. 3. *SPT3* is required for *not1-2*-induced but not wild-type (Wt) constitutive levels of the *HIS3* TATA-less transcript. *gcn4* deletion strains containing the indicated *not* or *spt* alleles were grown in rich medium at 30°C and analyzed for *HIS3* +1 (TATA-less), *HIS3* +13 (TATA-dependent), and *DED1* RNAs by S1 treatment of RNA-DNA duplexes.

termination products) were seen around 116 kDa (lane 2). This result suggests that the Not1-2 protein is shorter or less stable than wild-type Not1p. However, the levels and forms of Not1-2p detectable in *not1-2* extracts were the same for *SPT3* and *spt3*::*TRP1* strains (compare lanes 2 and 4). The same results were obtained when extracts were incubated for 30 min at 37°C or when extracts were prepared from cells incubated for 90 min at 37°C (not shown). Thus, it seems highly unlikely that *spt3*::*TRP1* suppresses temperature sensitivity associated with *not1-2* by increasing the stability or levels of an unstable Not1-2 protein.

Suppression of *not1-2* by *spt3* and *spt8* null mutations is partial. All *not* mutants were originally isolated because they are more AT resistant than wild-type cells when carrying the defective *gcn4-C163* derivative to complement a chromosomal deletion of *GCN4* (see Materials and Methods). To determine whether increased AT resistance associated with *not1-2* is also suppressed by null mutations in *SPT3* and *SPT8*, I first determined that the *spt3* and *spt8* null mutants display wild-type levels of AT resistance (not shown). I then compared levels of AT resistance of wild-type cells and *spt3*::*TRP1 not1-2*, *spt8*::*LEU2 not1-2*, and *not1-2* mutant cells. All *not1-2* mutants, whether wild type or mutant for *SPT3* and/or *SPT8*, grew similarly on high levels of AT (not shown). Double mutants formed colonies with a slight delay (1 day) relative to *not1-2* single mutants. This, however, may have been due to the slow growth of *spt3* and *spt8* null mutants on minimal medium. Thus, the *spt3* and *spt8* null mutations suppressed temperature sensitivity associated with *not1-2* but not AT resistance. We have previously reported that complementation of temperature sensitivity associated with *not1-2* requires less Not1 function than complementation of the AT resistance phenotype (7). My present results then suggest that suppression of *not1-2* by null alleles of *SPT3* and *SPT8* was partial.

***SPT3* is required for *not1-2*-induced transcriptional derepression.** Another phenotype characteristic of the *not1-2* mutation is increased constitutive transcription from the *HIS3* TATA-less promoter relative to *HIS3* transcription from the canonical-TATA promoter. To determine whether this phenotype is suppressed by null alleles of the *SPT* genes, RNA from wild-type, *not1-2*, *spt3*::*TRP1*, and *not1-2 spt3*::*TRP1* strains was analyzed by S1 mapping for the levels of constitutive *HIS3*

mRNA (Fig. 3). As expected, the *not1-2* mutants had increased *HIS3* TATA-less mRNA relative to the wild-type strain (compare lanes 3 and 4). The *spt3* null mutation reduced this increase (compare lanes 2 and 3) while not significantly altering wild-type levels of the *HIS3* TATA-less transcript (compare lanes 1 and 4). The same observation was made with *spt8*::*LEU2* (not shown). Thus, *SPT3* was required for derepression of *HIS3* TATA-less transcription in *not1-2* mutant cells but was dispensable for wild-type levels of constitutive *HIS3* TATA-less transcription.

A mutation in *SPT15* that prevents TBP's normal interaction with Spt3p suppresses *not1-2* allele specifically. Previous experiments have shown that Spt3p is a TBP-associated protein, and it has been suggested that Spt3p is required for TFIID to function at particular promoters in vivo. To determine whether suppression of *not1-2* by a null mutation in *SPT3* was related to the interaction of Spt3p with TBP, I analyzed the *spt15-21* mutant that is defective in the interaction between TBP and Spt3p (11). The *spt15-21* mutant grows slowly at all temperatures relative to wild-type cells, but it is not temperature sensitive. I could thus determine whether the *spt15-21* mutation suppresses temperature sensitivity associated with *not1-2*. I introduced the *not1-2* mutation into a strain carrying the *spt15-21* mutation (FY567), as well as into isogenic *spt3*::*TRP1* (FY294) and wild-type (FY631) strains. Figure 4 shows that *spt15-21* suppressed temperature sensitivity associated with *not1-2*. *spt15-21 not1-2* double mutants grew less well at high temperatures than *spt3*::*TRP1 not1-2* double mutants, but this correlated with poorer growth of *spt15-21* mutants relative to *spt3*::*TRP1* mutants (Fig. 4). Thus, a null allele of *SPT3* or mutations in *SPT15* that disrupt the interaction between Spt3p and TBP suppressed temperature sensitivity associated with *not1-2*.

To determine whether *spt15-21* suppressed *not1-2* allele specifically, I crossed FY567 to MY8 and MY873 carrying the *not1-2* and *not1-1* alleles, respectively. Tetrad analysis confirmed that *spt15-21* suppressed temperature sensitivity associated with *not1-2* and showed that *spt15-21* did not suppress temperature sensitivity associated with *not1-1* (not shown).

Mutations in *MOT1* drastically decrease *HIS3* and *HIS4* TATA-less transcription. The results presented so far suggest specific interactions between the *SPT3* and *NOT* genes. *MOT1*, like the *NOT* genes, encodes a global negative regulator of transcription and has been suggested to interact with *SPT3*, since a search for mutations that are synthetically lethal with a null allele of *SPT3* led to the isolation of a mutation in *MOT1*
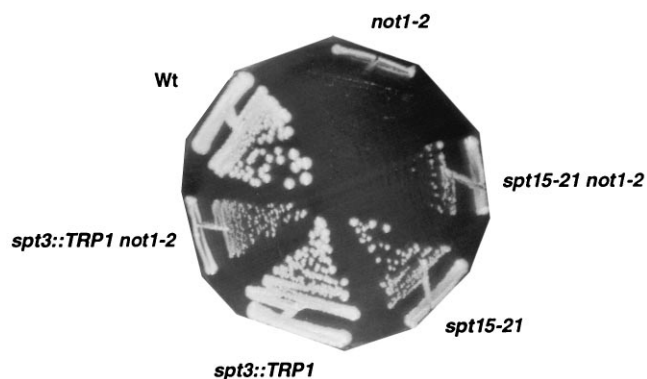

FIG. 4. Mutations in *SPT15* that disrupt the TBP-Spt3p interaction suppress *not1-2*. The indicated strains were streaked on rich medium and grown at 37°C for 3 days. Wt, wild type.
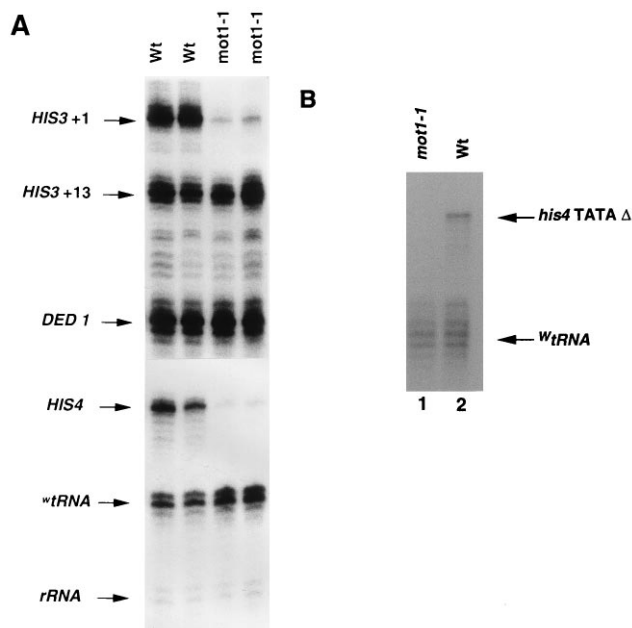
FIG. 5. Transcriptional analysis of *mot1-1* mutants. (A) Isogenic *gcn4* deletion strains containing *MOT1* or the *mot1-1* mutation as indicated were grown in rich medium at the permissive temperature (30°C) and analyzed for *HIS3* (+1 and +13), *DED1*, *HIS4*, ^{W}tRNA, and *rRNA* RNAs by S1 treatment of RNA-DNA duplexes. (B) Isogenic *his4-TATAΔ gcn4-Δ1* strains carrying *MOT1* or *mot1-1* as indicated were grown in rich medium at the permissive temperature and analyzed for ^{W}tRNA and *his4 TATAΔ* RNA levels at permissive temperature (30°C). Wt, wild type.

(19). I thus wanted to determine whether there is a relationship between *MOT1* and the *NOT* genes. *MOT1* is essential, and so I introduced the original temperature-sensitive *mot1-1* mutation (10) into the genetic background of my strains by multiple backcrosses. To first determine which genes *MOT1* may regulate, total cellular RNA was prepared from wild-type or *mot1-1* mutant cells and was analyzed for *HIS3*, *DED1*, and *HIS4* transcription by RNA polymerase II, tryptophan *tRNA* (^{W}tRNA) transcription by RNA polymerase III, and *rRNA* transcription by RNA polymerase I. Figure 5A shows that the only clear difference in transcription detected in *mot1-1* mutants relative to wild-type cells was a strong decrease in the levels of the *HIS3* TATA-less and *HIS4* transcripts. In contrast to what has previously been published (10), the *DED1* transcript was

not increased twofold in *mot1-1* mutants. This experiment also showed that the *mot1-1* mutation does not generally affect transcription by RNA polymerase I or RNA polymerase III.

Both the *HIS3* and *HIS4* promoters that were down-regulated in *mot1-1* mutants are TATA-less. Indeed, although there is a canonical TATA sequence upstream of the transcriptional start site in the *HIS4* gene, basal *HIS4* expression (in the absence of *GCN4* as analyzed in Fig. 5A) is not dependent on the TATA element in the promoter (22). To confirm that transcription independent of the *HIS4* TATA element was decreased in *mot1-1* mutants, I crossed strain L4389 (22) carrying a *his4 TATAΔ* allele to MY604 carrying the *mot1-1* mutation, and double mutants were obtained by tetrad analysis. Figure 5B shows that in a strain carrying a *HIS4* TATA deletion allele, *HIS4* TATA-less transcription was decreased by *mot1-1*. In contrast, Gcn4p-activated transcription of *HIS4* is TATA dependent (22) and was not decreased by *mot1-1* (not shown). Thus, *MOT1* is required for *HIS3* and *HIS4* TATA-less transcription specifically.

To confirm that decreased *HIS3* and *HIS4* TATA-less transcription resulted from a loss of *MOT1* function, I introduced a different recessive *mot1* mutation, *mot1-1033* (23), into the genetic background of my strains. Analysis of total cellular RNA from the *mot1-1033* mutant showed the same, albeit less dramatic, decrease in *HIS3* and *HIS4* TATA-less transcription (not shown). Thus, loss of *MOT1* function led to a decrease in transcription from the *HIS3* and *HIS4* TATA-less promoters. These results define Mot1p as a positive factor for *HIS3* and *HIS4* TATA-less transcription, in contrast to its definition so far as a negative regulator of transcription.

Mot1p is dispensable for *HIS3* TATA-less transcription if the *HIS3* TATA promoter is mutated. The results presented above suggest that *mot1-1* differentially affects TATA-less and TATA-dependent transcription. To confirm this, I analyzed basal and activated *HIS3* mRNA levels in *MOT1* and *mot1-1* strains that were mutated either at the TATA-less promoter (*his3Δ-93*) (20) or at the TATA promoter (*his3-205*) (20) and were transformed with either a vector alone, a plasmid carrying *gcn4-C163*, or a plasmid carrying *GCN4*. As shown on Fig. 6, activation of *HIS3* +13 transcription by Gcn4p in *mot1-1* mutants was identical to activation in wild-type cells (compare lanes 1 to 3 to lanes 4 to 6) and required a functional TATA promoter (compare lanes 4 to 6 to lanes 13 to 15) but not a functional TATA-less promoter (compare lanes 4 to 6 to lanes 10 to 12). Interestingly, when the *HIS3* TATA promoter was mutated, the *HIS3* TATA-less transcript levels were not decreased in *mot1-1* mutants (compare the decrease in *HIS3* +1
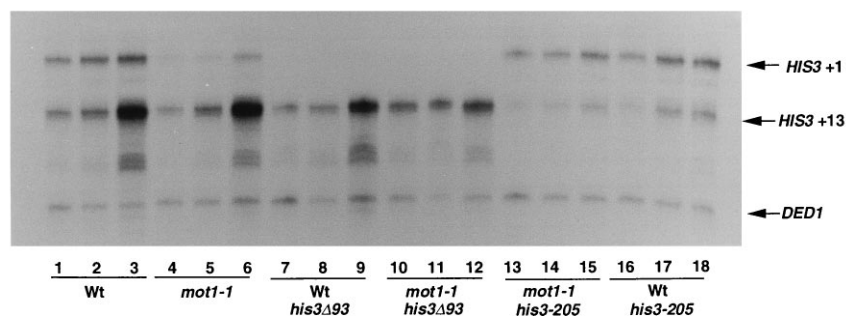

FIG. 6. Mot1p is not required for *HIS3* TATA-less transcription if the *HIS3* TATA promoter is mutated. Isogenic *gcn4* deletion strains carrying the indicated alleles (*his3Δ-93* deletes T_C [−83 to −46], and *his3-205* has a point mutation [TGTAAA] in T_R) (20) were transformed with the pRS316 vector (lanes 1, 4, 7, 10, 13, and 16), a plasmid carrying the *gcn4-C163* derivative (lanes 2, 5, 8, 11, 14, and 17), or a plasmid carrying *GCN4* (3, 6, 9, 12, 15, and 18). Transformants were grown in 10 ml of minimal medium containing Casamino Acids, tryptophan, and adenine to an optical density at 600 nm of 0.8. Total cellular RNA was extracted and analyzed for *HIS3* (+1 and +13) and *DED1* mRNAs by S1 treatment of RNA-DNA duplexes. Wt, wild type.
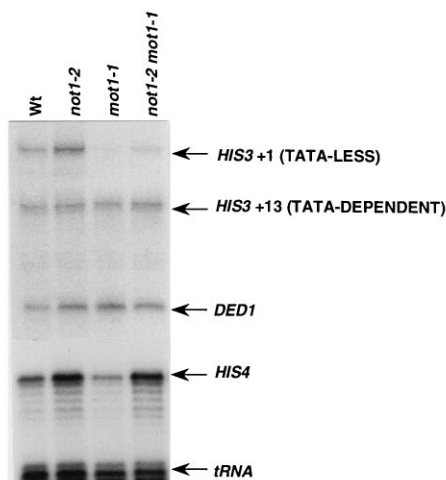
FIG. 7. Alterations in *HIS3* and *HIS4* TATA-less transcript levels by mutations in the *MOT1* and *NOT* genes do not seem dependent. Total cellular RNA was extracted from *gcn4* deletion strains containing the indicated *not* or *mot* mutations and was analyzed for *HIS3* (+1 and +13), *DED1*, *HIS4*, and ^{W}tRNA RNAs by S1 treatment of RNA-DNA duplexes. Wt, wild type.

transcript levels in lanes 4 to 6 relative to lanes 1 to 3 and the absence of decrease in lanes 13 to 15 relative to lanes 16 to 18). *HIS4* TATA-less transcript levels, on the other hand, were strongly decreased in *mot1-1* mutants regardless of whether the *HIS3* TATA promoter was mutated or wild type (not shown). Thus, Mot1p is not required for *HIS3* TATA-less transcription if the *HIS3* TATA promoter is mutated.

Regulation by the *MOT1* and *NOT* genes may not be dependent. Transcription of the *HIS3* and *HIS4* TATA-less promoters is increased in *not* mutants (8) and decreased in *mot1-1* mutants. I thus constructed strains mutant both for *MOT1* and any one of the *NOT* genes to look for mutual suppression. All double mutants were temperature sensitive and displayed increased AT resistance (the *mot1-1* mutants did not display this Not⁻ phenotype [not shown]), demonstrating no mutual suppression. Analysis of total cellular RNA showed that in *mot1-1 not1-2* double mutants, *HIS3* TATA-less transcript levels are somewhat lower than in wild-type cells but higher than in *mot1-1* mutants (Fig. 7). *HIS4* transcript levels, on the other hand, were above wild-type levels in such double mutants (Fig. 7). These results suggest that TATA-less transcription of some genes (such as *HIS3*) cannot be increased by *not* mutations above wild-type levels in strains that are also mutant for *MOT1*, whereas some promoters (such as *HIS4*) can be activated by *not* mutations even in the absence of *MOT1*. Taken together, these results demonstrate no clear epistasis between the *MOT1* and the *NOT* genes, suggesting that they might oppositely affect the same promoters but possibly by independent mechanisms. Alternatively, the difference in *HIS3* and *HIS4* TATA-less transcription in double mutants might stem from their dependence on different upstream elements, namely, a poly(dA)-poly(dT) sequence for *HIS3* and a *BAS1/BAS2*-dependent element for *HIS4*.

Mutations in *NOT1* suppress toxicity due to overexpression of TBP in *mot1-1* mutant cells. It has been proposed that Mot1p is a repressor of transcription. Strikingly, my results define Mot1p, formally at least, as a positive factor for TATA-less promoters. Since *MOT1* encodes a protein that removes TBP from DNA in vitro, the following model could reconcile these opposite effects: TBP (TFIID) is limiting in vivo; thus, if TBP (TFIID) were not constantly removed from certain pro-

moters to which it stably binds, then transcription from these promoters would be unchanged (or increased), while transcription from promoters that have a lower affinity for TBP (TFIID) would be decreased. Such a model is supported by the observation presented above that *MOT1* is not required for *HIS3* TATA-less transcription if the *HIS3* TATA promoter is mutated and predicts that overexpression of TBP should increase TATA-less transcription in *mot1-1* mutants. The overexpression of TBP, however, has been shown to be toxic for *mot1-1* mutants (2), and rather than increase *HIS3* TATA-less transcript levels, it seemed to increase *HIS3* TATA-dependent transcript levels (not shown). Promoters that bind TBP with high affinity might not be saturated and favored upon TBP overexpression in *mot1-1* mutants, thereby exacerbating the imbalance of TATA-less to TATA-dependent transcription.

The absence of increased TATA-less transcription upon TBP overexpression might be due, at least in part, to a limited availability of factors that are required, in addition to TBP, for TATA-less transcription. If mutations in the *NOT* genes do indeed increase the availability of such positive factors, then TBP overexpression may not be toxic in *mot1-1* mutants that are additionally mutant for a *NOT* gene. To test this possibility, I transformed double *mot1-1 not1-2* mutants with the same plasmid overexpressing TBP. Figure 8 shows that indeed, overexpression of TBP in *mot1-1* mutants was not toxic if the cells were additionally mutant for *NOT1*. This was also true if the cells were additionally mutant for *NOT3* (not shown).

These experiments demonstrate that the *NOT* genes regulate TFIID activity, since mutations in the *NOT* genes could suppress toxicity due to overexpression of TBP. Moreover, since overexpression of TBP is not toxic in wild-type cells, these results suggest that mutations in the *NOT* and *MOT1* genes modulate TBP (TFIID) activity such as to have opposite effects on the same promoters.

Overexpression of *SPT3* in *mot1-1* mutants leads to a weak Not⁻ phenotype. The results presented so far support the idea that the Not proteins limit the availability of positive factors for TATA-less promoters and suggest that Spt3p is one such positive factor. I thus investigated whether *mot1-1* mutants could be made AT resistant by overexpressing *SPT3*, since *mot1-1* mutants are AT sensitive, while *mot1-1 not* mutants are AT
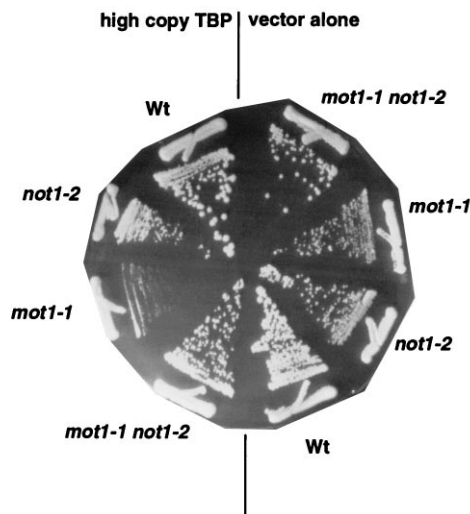

FIG. 8. *not1-2* suppresses toxicity due to overexpression of TBP in a *mot1-1* mutant. Isogenic strains carrying the indicated *not* or *mot* alleles and containing YEplac112-*SPT15* (high-copy-number TBP) or YEplac112 (vector) as indicated were grown for 3 days at 30°C on rich medium selective for the plasmid.
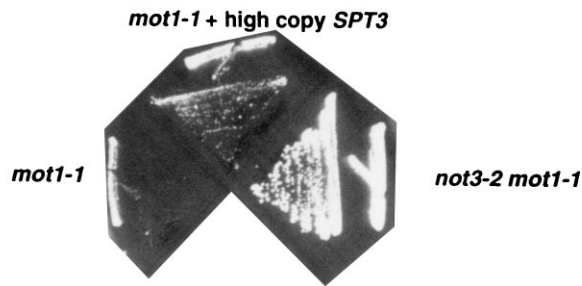
FIG. 9. Overexpression of *SPT3* can partially mimic a Not⁻ phenotype. *gcn4*-deleted strains containing the *gcn4-C163* derivative, carrying the indicated *not* or *mot* mutations, and containing YEplac195-SPT3 (overexpressing *STP3*) or YEplac195 (vector) as indicated were grown for 4 days on minimal medium containing 20 mM AT.

resistant. *mot1-1* mutants were transformed with a plasmid overexpressing *SPT3* and tested for AT resistance. Figure 9 shows that overexpression of *SPT3* allowed partial growth of *mot1-1* mutants on 20 mM AT, an intermediate phenotype between *mot1-1* mutants that did not grow at this concentration of AT and *mot1-1 not* mutants that grew well even on 40 mM AT. Overexpression of Spt3p in wild-type cells did not increase detectably the AT resistance of these cells (not shown), suggesting that the *mot1-1* mutation contributes to this phenotype. Thus, overexpression of *SPT3* can partially mimic a Not⁻ phenotype in a *mot1-1* mutant.

mot1-1 and spt3::TRP1 display a strong synthetic phenotype. If Mot1p is required for the same TATA-less promoters for which Spt3p, negatively regulated by the Not proteins, is a positive factor, cells mutant for both *MOT1* and *SPT3* are expected to be more deficient in TATA-less transcription than either single mutant and therefore may display a synthetic phenotype. Such double mutants would lack both the factor that makes TBP available for low-affinity promoters and one of the positive factors that can help recruit and/or stabilize TBP on these promoters. To determine this, I constructed double *mot1-1 spt3*::TRP1 mutants by crossing single mutants, sporulating the diploid, and dissecting tetrads. Double *spt3*::TRP1 *mot1-1* mutants were viable but grew slowly at 25°C and could not form colonies at 30°C. This dramatic synthetic phenotype supports the idea that Mot1p and Spt3p are required for the same promoters.

DISCUSSION

The transcriptional activity resulting from a functional Spt3p-TBP interaction is inhibited by the Not negative regulator. We have previously suggested that the *NOT* genes define a global negative regulator of transcription that inhibits some component(s) of the basic transcription machinery required more specifically for initiation at TATA-less promoters (8). The work presented in this paper supports this model and indicates that the *NOT* genes inhibit the transcriptional activity resulting from a functional interaction between Spt3p and TBP. Indeed, a null allele of *SPT3* itself, a null allele of *SPT8* required for a functional Spt3p-TBP interaction, or finally mutations in TBP that impede its binding to Spt3p suppressed *not1-2*. This suppression was allele specific, suggesting that it defines genes directly related to Not1p function rather than genes transcriptionally activated by mutations in the *NOT* genes. Moreover, in a *mot1-1* mutant strain, overexpression of Spt3p partially mimicked a Not⁻ phenotype, suggesting that

excess Spt3p function can behave phenotypically similarly to a *not* mutation.

The Not proteins might inhibit Spt3p itself, Spt8p, TBP, or other functionally interacting factors. They might conceivably also inhibit factors that are themselves regulated by Spt3p-TBP. I consider this less likely because overexpression of *SPT3* partially mimicked a *not* mutant phenotype. One would thus have to imagine that the level of Spt3p is strictly regulated and has a direct impact on the regulated factor(s). Whether the inhibition is direct or not, this work suggests that the interaction between Spt3p and TBP may be more specifically involved, directly or through the regulation of other factor(s), in transcription initiation at TATA-less core promoters.

MOT1 is required for TATA-less transcription. *MOT1* has been identified genetically as a global negative regulator of transcription (10) and biochemically as a repressor that decreases the half-life of TBP's binding to DNA (2). There have been suggestions to explain the necessity for such an activity in the cell, such as reorientation of incorrectly positioned TBP, rendering the binding of TFIID rate limiting, or removing TFIID once the elongating polymerase has disengaged from the preinitiation complex. My results define a different role for Mot1p as a positive factor for transcription of core promoters that have a low affinity for TBP (TATA-less). I suggest that in vivo, Mot1p is needed to remove TBP (TFIID) from DNA to which it stably binds because TBP (TFIID) is limiting and must be available for all types of core promoters, in particular promoters with a low inherent affinity for TBP. This model suggests that in *mot1-1* mutants the limited pool of TBP (TFIID) probably remains sequestered at promoters with high affinity for TBP whose expression might then be increased, and this explains why *mot1-1* mutants were initially obtained in a selection for increased basal transcription (10). Meanwhile transcription from other promoters would be severely decreased due to the lack of available TBP, as shown here for the *HIS3* and *HIS4* TATA-less promoters.

TBP is required for transcription by all three RNA polymerases (for a review, see reference 15), yet as shown in this report, transcription by RNA polymerase I and III that is also TATA-less is not affected by mutations in *MOT1*. This can be explained if one considers that the cellular pool of TBP is distributed into different TBP-containing complexes specific for RNA polymerase I (SL1), RNA polymerase II (TFIID), and RNA polymerase III (TFIIIB) and is not readily interchangeable. Thus, it is the pool of TBP in an RNA polymerase II-specific configuration that is limiting in the cell and allowed to be redistributed among polymerase II-specific core promoters by the activity of Mot1p. Indeed, it has recently been shown that yeast TBP, like its human and *Drosophila* counterparts, is stably associated with other factors (25, 26, 29).

The *MOT1-SPT3-NOT* connection. This work provides several pieces of evidence that the promoters regulated by Mot1p, Spt3p, and the Not proteins are the same. First, mutations in *MOT1* decreased and mutations in the *NOT* genes increased *HIS3* and *HIS4* TATA-less transcription. This finding suggests that the *NOT* and *MOT1* genes have opposite effects on the same promoters. That this is true beyond the *HIS3* and *HIS4* TATA-less promoters is suggested by the fact that mutations in the *NOT* genes suppressed toxicity due to overexpression of TBP in *mot1-1* mutants. Indeed, overexpression of TBP is not toxic in wild-type cells, and so this observation demonstrates that the *NOT* genes can modulate TBP function such as to counteract the way TBP function is altered by *mot1-1*. This result is the first demonstration that the *NOT* genes do indeed regulate TBP (TFIID) function. Second, the transcriptional activity resulting from a functional Spt3p-TBP interaction is
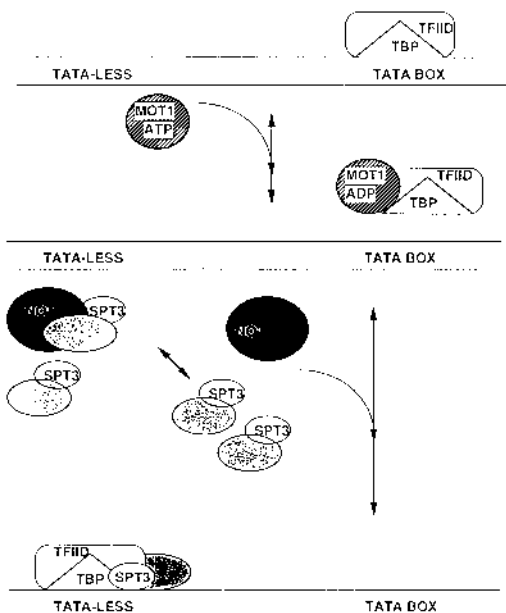
FIG. 10. Model for the roles of Mot1p, the Not proteins, and Spt3p in the regulation of core promoter selection. The factor indicated as Spt3p could be Spt3p itself, a factor associated or functionally interacting with Spt3p, or finally a factor regulated by Spt3p.

inhibited by the Not negative regulator, as discussed above. This finding suggests that the Not proteins and Spt3p have opposite effects on the same promoters. Finally, if the Not proteins and Mot1p or the Not proteins and Spt3p have opposite effects on the same promoters, then Spt3p and Mot1p are positive factors (formally at least) for the same promoters. This view is supported by the observation that *spt3 mot1-1* double mutants display a severe synthetic phenotype and that a mutation in *MOT1* was isolated in a screen for mutations synthetically lethal with a null allele of *SPT3* (19).

Model for regulation of transcription at core promoters. From my results, I propose a model for regulation of core promoter transcription (Fig. 10): Mot1p interacts with promoter-bound TBP (TFIID) and removes it from DNA, whereupon TBP (TFIID) will either bind these TATA-containing promoters again or interact with Spt3p and/or other factors (possibly Spt3p-regulated factors) in a Not-regulated way and thereby bind TATA-less promoters. The terms "TATA containing" and "TATA-less" should be understood as referring to promoter affinity for TBP that can range anywhere from very high to very low. According to this affinity, the requirement of a given promoter for *MOT1* as well as for the factor(s) inhibited by the Not proteins is probably variable. Finally, my experiments with *HIS3* promoter mutants suggest that the close proximity of a strong TBP binding sequence may also increase the requirement of a weak TBP-binding promoter for *MOT1*.

There are several issues which have not been addressed by this work but that are of interest. First, is Mot1p or a similar activity also required to remove TBP from TATA-less promoters, or is the complex formed on promoters with low affinity for TBP inherently unstable? Second, is there a direct interaction between Spt3p and Mot1p that would mediate interactions between Spt3p and free TBP (TFIID)? Finally, a very interesting issue is how the assembly of the general transcription machinery occurs on TATA-less promoters. The identification of the factors that act in concert with Spt3p and Spt8p to drive

transcription to TATA-less promoters will probably be a first step in helping us to address this question in the future.

REFERENCES

1. **Auble, D. T., and S. Hahn.** 1993. An ATP-dependent inhibitor of TBP binding to DNA. Genes Dev. **7:**844–856.
2. **Auble, D. T., K. E. Hansen, C. G. F. Mueller, W. S. Lane, J. Thorner, and S. Hahn.** 1994. Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. Genes Dev. **8:**1920–1934.
3. **Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp.** 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. Cell **56:** 549–561.
4. **Cade, R. M., and B. Errede.** 1994. *MOT2* encodes a negative regulator of gene expression that affects basal expression of pheromone-responsive genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**3139–3149.
5. **Carcamo, J., L. Buckbinder, and D. Reinberg.** 1991. The initiator directs the assembly of a transcription factor IID-dependent transcription complex. Proc. Natl. Acad. Sci. USA **88:**8052–8056.
6. **Chen, W., and K. Struhl.** 1988. Saturation mutagenesis of a yeast *his3* TATA element: genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA **85:**2691–2695.
7. **Collart, M. A., and K. Struhl.** 1993. CDC39, an essential nuclear protein that negatively regulates transcription and differentially affects the constitutive and inducible *his3* promoters. EMBO J. **12:**177–186.
8. **Collart, M. A., and K. Struhl.** 1994. *NOT1* (*CDC39*), *NOT2* (*CDC36*), *NOT3*, and *NOT4* encode a global-negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev. **8:**525–537.
9. **Cormack, B. P., and K. Struhl.** 1992. The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69:**685–696.
10. **Davis, J. L., R. Kunisawa, and J. Thorner.** 1992. A presumptive helicase (*MOT1* gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**1879–1892.
11. **Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney, and F. Winston.** 1992. *SPT3* interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev. **6:**1319–1331.
12. **Eisenmann, D. M., C. Chapon, S. M. Roberts, C. Dollard, and F. Winston.** 1994. The *Saccharomyces cerevisiae SPT8* gene encodes a very acidic protein that is functionally related to Spt3 and TATA-binding protein. Genetics **137:**647–657.
13. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation *in vivo*. Cell **58:**1183–1191.
14. **Harbury, P. A. B., and K. Struhl.** 1989. Functional distinctions between yeast TATA elements. Mol. Cell. Biol. **9:**5298–5304.
15. **Hernandez, N.** 1993. TBP, a universal transcription factor? Genes Dev. **7:**1291–1308.
15a. **Hope, I. A., and K. Struhl.** 1986. Functional dissection of a eukaryotic transcriptional activator protein, GCN4, of yeast. Cell **46:**885–894.
16. **Irie, K., K. Yamaguchi, K. Kawase, and K. Matsumoto.** 1994. The yeast *MOT2* gene encodes a putative zinc finger protein that serves as a global negative regulator affecting expression of several categories of genes, including mating-pheromone-responsive genes. Mol. Cell. Biol. **14:**3150–3157.
17. **Kaufmann, J., and S. T. Smale.** 1994. Direct recognition of initiator elements by a component of the transcription factor IID complex. Genes Dev. **8:**821–829.
18. **Leberer, E., D. Dignard, D. Harcus, M. Whiteway, and D. Y. Thomas.** 1994. Molecular characterization of *SIG1*, a *Saccharomyces cerevisiae* gene involved in negative regulation of G-protein-mediated signal transduction. EMBO J. **13:**3050–3064.
19. **Madison, J., and F. Winston.** Personal communication.
20. **Mahadevan, S., and K. Struhl.** 1990. T$_C$, an unusual promoter element required for constitutive transcription of the yeast *his3* gene. Mol. Cell. Biol. **10:**4447–4455.
21. **Martinez, E., C.-M. Chiang, H. Ge, and R. G. Roeder.** 1994. TATA-binding

protein-associated factors in TFIID function through the initiator to direct basal transcription from a TATA-less class II promoter. EMBO J. **13:**3115–3126.

22. **Pellman, D., M. E. McLaughlin, and G. R. Fink.** 1990. TATA-dependent and TATA-dependent transcription at the *HIS4* gene of yeast. Nature (London) **348:**82–85.

23. **Piatti, S., R. Tazzi, A. Pizzagalli, P. Plevani, and G. Lucchini.** 1992. Control of DNA synthesis genes in budding yeast; involvement of the transcriptional modulator *MOT1* in the expression of the DNA polymerase α gene. Chromosoma **102:**102–113.

24. **Ponticelli, A. S., and K. Struhl.** 1990. Analysis of *Saccharomyces cerevisiae his3* transcription in vitro: biochemical support for multiple mechanisms of transcription. Mol. Cell. Biol. **10:**2832–2839.

25. **Poon, D., A. M. Campbell, Y. Bai, and P. A. Weil.** 1994. Yeast Taf170 is encoded by *MOT1* and exists in a TATA box-binding protein (TBP)-associated factor complex distinct from transcription factor IID. J. Biol. Chem. **269:**23135–23140.

26. **Poon, D., and P. A. Weil.** 1993. Immunopurification of yeast TATA-binding protein and associated factors. J. Biol. Chem. **268:**15325–15328.

27. **Pugh, B. F., and R. Tjian.** 1990. Mechanism of transcriptional activation by SP1: evidence for coactivators. Cell **61:**1187–1197.

28. **Pugh, B. F., and R. Tjian.** 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. Genes Dev. **5:**1935–1945.

29. **Reese, J. C., L. Apone, S. S. Walker, L. A. Griffin, and M. R. Green.** 1994. Yeast TAF$_{II}$s in a multisubunit complex required for activated transcription. Nature (London) **371:**523–527.

30. **Roeder, R. G.** 1991. The complexities of eukaryotic transcription initiation: regulation of preinitiation complex. Trends Biochem. Sci. **16:**402–408.

31. **Smale, S. T.** 1994. Core promoter architecture for eukaryotic protein-coding genes, p. 63–81. *In* R. C. Conaway and J. W. Conaway (ed.), Transcription: mechanisms and regulation. Raven Press, New York.

32. **Smale, S. T., M. C. Schmidt, A. J. Berk, and D. Baltimore.** 1990. Transcriptional activation by SP1 as directed through TATA or initiator: specific requirements for mammalian transcription factor IID. Proc. Natl. Acad. Sci. USA **87:**4509–4513.

33. **Struhl, K.** 1986. Constitutive and inducible *Saccharomyces cerevisiae* promoters: evidence for two distinct molecular mechanisms. Mol. Cell. Biol. **6:**3847–3853.

34. **Struhl, K.** 1993. Yeast transcription factors. Curr. Opin. Cell. Biol. **5:**513–520.

35. **Van Dyke, M. W., R. G. Roeder, and M. Sawadago.** 1988. Physical analysis of transcription preinitiation complex assembly on a class II gene promoter. Science **241:**1335–1338.

36. **Weis, L., and D. Reinberg.** 1992. Transcription by RNA polymerase II: initiator-directed formation of transcription-competent complexes. FASEB J. **6:**3300–3309.

37. **Wiley, S. R., R. J. Kraus, and J. E. Mertz.** 1992. Functional binding of the TATA box binding component of transcription factor TFIID to the −30 region of TATA-less promoters. Proc. Natl. Acad. Sci. USA **89:**5814–5818.

38. **Winston, F.** 1992. Analysis of *SPT* genes: a genetic approach toward analysis of TFIID, histones and other transcription factors of yeast, p. 1271–1293. *In* S. L. McKnight and K. R. Yamamoto (ed.), Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

39. **Winston, F., and M. Carlson.** 1992. Yeast *SNF*/*SWI* transcriptional activators and the *SPT*/*SIN* chromatin connection. Trends Genet. **8:**387–391.

40. **Winston, F., and P. L. Minehart.** 1986. Analysis of the yeast *SPT3* gene and identification of its product, a positive regulator of Ty transcription. Nucleic Acids Res. **14:**6885–6900.

41. **Yoganathan, T., M. Horikoshi, S. Hasegawa, R. G. Roeder, and B. H. Sells.** 1992. Yeast transcription factor IID participates in cell-free transcription of a mammalian ribosomal protein TATA-less promoter. Biochem. J. **285:**721–723.

42. **Zenzie-Gregory, B., A. Khachi, J. P. Garraway, and S. T. Smale.** 1993. Mechanism of initiator-mediated transcription: evidence for a functional interaction between the TATA-binding protein and DNA in the absence of a specific recognition sequence. Mol. Cell. Biol. **13:**3841–3849.

43. **Zhou, Q., P. M. Lieberman, T. G. Boyer, and A. J. Berk.** 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6:**1964–1974.