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# STD1 (MSN3) interacts directly with the TATA-binding protein and modulates transcription of the *SUC2* gene of *Saccharomyces cerevisiae*

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## ABSTRACT

***STD1* (*MSN3*) was isolated independently as a multicopy suppressor of mutations in the TATA-binding protein and in SNF4, suggesting that STD1 might couple the SNF1 kinase signaling pathway to the transcriptional machinery. We report here a direct physical interaction between STD1 and the TATA-binding protein (TBP), observed *in vivo* by the two-hybrid system and *in vitro* by binding studies. STD1 bound both native TBP in yeast cell-free extracts and purified recombinant TBP. This interaction was altered when TBPΔ57 was used, suggesting a role for the non-conserved N-terminal domain of TBP in mediating protein–protein interactions. We also show that perturbation of STD1-TBP stoichiometry alters *SUC2* expression *in vivo* and that this effect is dependent on the N-terminal domain of TBP. The activation of *SUC2* expression by increased copy number of *STD1* occurs at the level of mRNA accumulation and it requires the same TATA element and uses the same transcription start site as does activation of *SUC2* by glucose limitation. Taken together, these results suggest that STD1 modulates *SUC2* transcription through direct interactions with TBP.**

## INTRODUCTION

*STD1* was isolated as a multicopy suppressor of growth defects caused by overexpression of the conserved C-terminal domain of the TATA-binding protein (TBPΔ57) (1). The TATA-binding protein (TBP) is an essential component of the transcriptional machinery of all three nuclear RNA polymerases (2–6). In yeast, TBP contains 240 residues with the C-terminal 180 residues consisting of a DNA binding domain that is highly conserved throughout eukaryotic evolution. The 60 residue N-terminal domain is not conserved and is not required for viability (7–10). Overexpression of wild type TBP in yeast has no known phenotype. In contrast, overexpression of TBPΔ57 from the *GAL1* promoter can result in a dominant negative phenotype of extremely slow growth and defects in both induced and uninduced RNA polymerase II transcription (1,11). This effect is not restricted to growth on galactose media; overexpression of TBPΔ57 (y183c) from the *ADH1* promoter causes poor growth on media with glycerol as a carbon source (8).

Biochemical studies indicate that the N-terminal domain inhibits the DNA binding activity of TBP. The temperature dependence of TBP's DNA binding activity is relaxed by proteolytic removal of the N-terminal domain (12). The N-terminal domain of TBP greatly destabilizes the TBP–DNA complex in electrophoretic mobility shift assays and it increases the activation energy of TBP–DNA complex formation by almost 3 kcal/mol (13). Missense mutations that reduce DNA binding activity of TBP are suppressed *in vitro* by removal of the N-terminal domain (14). These same mutant forms of TBP can bind DNA stably if TFIIA is present, indicating that TFIIA may stabilize the DNA bound form of TBP by blocking the inhibitory effects of the N-terminal domain (14). These data suggest that the N-terminal domain negatively affects the DNA binding activity of TBP and that other proteins may be able to modulate this effect. Since TBPΔ57 causes deleterious effects only when expressed at high levels, TBPΔ57 might be titrating an important component of the transcription machinery.

By searching for multicopy suppressors of TBPΔ57 overexpression, we sought to identify genes encoding proteins that directly interact with or regulate the activity of TBP. *STD1* on a multicopy plasmid suppresses both the growth phenotype and the defects in RNA polymerase II transcription caused by overexpression of TBPΔ57 (1). It does this even though TBPΔ57 accumulates to the same levels and has the same DNA-binding activity as without *STD1* overexpression. High level expression of *STD1* suppresses TBPΔ57 induced defects in transcription at apparently unrelated loci such as *CUP1* and *ACT1*. These findings suggest that STD1 may be an important component of transcription, perhaps interacting directly with the transcription machinery.

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*STD1* (*MSN3*) was also identified as a multicopy suppressor of the raffinose growth defect caused by deletion of the *SNF4* gene (15). Deletion of *SNF4* causes a defect in the SNF1 protein kinase pathway of glucose derepression (16–18). Strains with this defect have pleiotropic phenotypes related to carbon utilization (19–22). Among other things, these strains fail to derepress transcription of *SUC2*, the gene for invertase, and as a result, cannot grow on media containing raffinose as the carbon source.

Several lines of evidence indicate that STD1 acts positively to derepress invertase expression (15). First, increased copy number of *STD1* restores the ability of a *snf4*Δ strain to derepress invertase and grow on raffinose media. Secondly, increased copy number of *STD1* partially relieves glucose repression in wild type cells. Thirdly, deletion of *STD1* and its homologue, *MTH1*, causes a 4-fold reduction in the derepressed level of invertase expression. These activities are all dependent upon an intact *SNF1* gene. Moreover, STD1 can physically associate with SNF1 *in vivo* and *in vitro*. These data suggest that STD1 acts in conjunction with SNF1 to relieve glucose repression of the invertase gene.

STD1 does not appear to be a conventional transcriptional activator. A LexA-STD1 fusion protein fails to activate transcription of a reporter gene containing LexA binding sites (15). This fusion protein is known to be functionally active, since it suppresses the *snf4*Δ phenotype. Sequence analysis of STD1 does not detect any similarity to motifs associated with known families of transcription factors (1,15). Thus the mechanism by which STD1 acts to derepress invertase expression is unclear.

We report here a direct physical interaction between STD1 and TBP. We show that *in vivo*, STD1 interacts with TBP in the two-hybrid system. *In vitro*, purified recombinant STD1 binds both native TBP from yeast whole cell extracts and recombinant TBP purified from *Escherichia coli*. STD1 is also able to bind TBPΔ57, though in a qualitatively different fashion from its binding to TBP. *In vivo*, the expression of *SUC2* is sensitive to perturbations of STD1-TBP stoichiometry and this effect is also dependent on the N-terminal domain of TBP. These results suggest that STD1 activates expression of invertase through its interaction with TBP at *SUC2* and that this interaction is modulated by the N-terminus of TBP.

## MATERIALS AND METHODS

### Strains and genetic methods

*Saccharomyces cerevisiae* strain Y153 (*MATa, leu2-3,112, ura3-52, trp1-901, his3-Δ200, ade2-101, gal4Δ, gal80Δ, URA3::GAL1-lacZ, LYS2::GAL1-HIS3*) was used for the two hybrid analysis (23). Y153 requires GAL4 activity to grow on media lacking histidine. MCY2634 (*MATa, snf4-Δ2, ura3-52, his3-Δ200, leu2-3,112*) was used for testing invertase expression in a *snf4*Δ background (15). MCY2662 (*MATa, ura3-52, trp1-Δ1, his3-Δ200, lys2-801*) was used to prepare yeast extracts to test the ability of GST-STD1 to bind TBP. MCY2649 (*MATα, ura3-52, leu2-3, 112, his3Δ200*) and FY716 (*MATα, ura3-52, leu2Δ1, his4-912δ, lys2-128δ, suc2-104*) were used in invertase assays. Yeast were grown in synthetic complete (SC) media (24), lacking certain supplements where indicated to maintain plasmid selection. Dextrose was used as the carbon source unless otherwise noted.

### Plasmid constructions

Plasmids pGBT9 and pGAD424 were used to construct fusions for two-hybrid analysis (25). pGBT9 expresses the GAL4 DNA binding domain (amino acids 1–147) from the *ADHI* promoter, while pGAD424 expresses the GAL4 activation domain (amino acids 768–881) from the *ADHI* promoter. In both cases the test protein is fused to the C-terminal end of the *GAL4* fragment. The GAL4 binding domain-STD1 fusion (pTT21) was created using PCR with 5′-CCGGAATTCATGTTTGTTTCACCACCTCC and 5′-CGCGAATTCAAATTTACTAGGACATTCCATCAGGCTTCC as primers to amplify a fragment containing the entire *STD1* gene with *Eco*RI sites on each end, in frame with the *GAL4* binding domain fragment in pGBT9. This PCR product was subcloned into the *Eco*RI site of pGBT9 to make pTT21. The GAL4 activation domain-TBP fusion plasmid (pTT31) was similarly constructed except a threonine to lysine mutation was introduced at residue 112 to abolish the DNA binding activity of TBP (10). The mutation was created using the primers 5′-GCGCGAATTCATGGCCGATGAGGAAC with 5′-GCAAAAATTAAAGCTTTAGTTTTTGG and 5′-CCAAAAACTAAAGCTTTAATTTTTGC with the universal primer. The resulting two overlapping PCR products were used with the two outside primers in a new PCR reaction to generate full length TBP with the T112K mutation. This PCR product was digested with *Eco*RI and *Sal*I and inserted into similarly digested pGAD424 to create pTT31. The T112K mutation and the fusion junctions were confirmed by DNA sequence analysis.

Plasmid pGEX-STD1 was constructed using PCR to amplify the full length STD1 using the primers 5′-CGCGGATCCCATATGTTTGTTTCACCACCTCC and 5′-CGCGAATTCAAATTTACTAGGACATTCCA. The resulting PCR product was digested with *Bam*HI and *Eco*RI ligated into similarly digested pGEX-2T (26).

Plasmid pRG84 was constructed by subcloning the 2.4 kb *Eco*RI-*Bam*HI genomic fragment containing the wild type TBP gene, *SPT15*, into similarly digested YEp352 (27). pRG85 is the same as pRG84 with a precise deletion removing amino acids 2–57 in TBP.

Plasmid pGBT9-SNF1 (the GAL4 binding domain-SNF1 fusion) was subcloned from pEE5 (28), pGBT9-LAM (the GAL4 binding domain–human lamin C fusion)was subcloned from pLAM5 (29). pNI12 (28) (the GAL4 activation domain fused to the C-terminus of SNF4), pGST-MSN3 (15) (a glutathione S-transferase-STD1 fusion protein; GST-STD1), pLexA (1–202)+PL (30), pLexA-MSN3 (15) (the LexA binding domain-STD1 fusion) and pDE93-3 (31) (the 2μ vector pRS424 expressing TBP from its native promoter) have all been described previously.

### Two-hybrid assays

For the two-hybrid assays (28,32), yeast strain Y153 was co-transformed with the indicated pGBT9 and pGAD424 derivatives onto synthetic complete media lacking tryptophan and leucine to maintain selection for the plasmids. Transformed cells were grown in selective media to late log phase and adjusted to equal cell number. Ten microliter each of $10^0$, $10^{-1}$, $10^{-2}$ and $10^{-3}$ dilutions were spotted to synthetic complete media with 2% ethanol, 2% galactose and 3% glycerol as a carbon source and lacking tryptophan, leucine and histidine. 3-Aminotriazole (3-AT) was added at 30 mM to increase the requirement for the *HIS3* gene

product (23). These dilutions were also spotted to the same media containing histidine to control for equal viability. Cells were grown for 7 days at 30°C, then photographed.

### Assay for invertase activity in *snf4Δ* strains

Strain MCY2634 was transformed with pLexA(1–202)+PL or pLexA-MSN3 and either YEp352, pRG84 or pRG85. Glucose derepressed cells were prepared (21) and assayed for invertase activity (33).

### Primer extension of *SUC2* mRNA

Total yeast RNA was prepared (34) and analyzed by primer extension (35) using AMV reverse transcriptase. The oligonucleotide primer complementary to *SUC2* mRNA (5′-CCAAAGGTC-TATCGCTAGTTTCGTTTGTCATTGATGCAGATATTTTGG-CTGC) was labeled with [$\gamma$-$^{32}$P]ATP and polynucleotide kinase.

### Assay for binding to GST-STD1

Glutathione S-transferase-STD1 was purified as previously described (15) from *E.coli* strain XL1-blue (Stratagene) transformed with pGST-MSN3 or pGEX-STD1, for the experiments shown in Figures 2 and 3, respectively. To test the ability of GST-STD1 to bind TBP in yeast whole cell extracts, MCY2662 transformed with pDE93-3 (expressing TBP from a multicopy plasmid) was grown to mid-log phase in selective media containing 2% dextrose (repressing). Cells were then shifted to media containing 0.05% dextrose (derepressing) for 3 h. Yeast whole cell extracts were then prepared (16) and 200 µg whole cell extract per assay was incubated with GST or GST-STD1 resin. The resin was washed 10-fold with 1 ml of MTPBS (15), boiled in SDS-PAGE sample buffer and analyzed by 10% SDS-PAGE (36). Western analysis was performed by standard methods (36), using rabbit anti-TBP serum (kindly provided by S. Buratowski, Whitehead Institute) and developed using a chemiluminescent method (Amersham).

For testing the ability of STD1 to bind purified recombinant TBP and TBPΔ57, GST-STD1 bound to glutathione-agarose was incubated in 100 mM NaCl, 1% Triton X100, 20 mM $NaPO_4$ pH 7.3 in the presence of protease inhibitors (2 mM phenylmethylsulfonyl fluoride and 1 µg/ml each aprotinin, leupeptin and pepstatin) for 30 min at 4°C with 2 µg of either purified recombinant TBP or TBPΔ57. This was centrifuged and the supernatant was saved. The resin was washed six times in the same buffer. One half of the resin was then eluted with SDS-PAGE sample buffer. The other half was subjected to sequential salt washes containing 0.15, 0.2, 0.4 and 0.8 M NaCl in 1% Triton X100, 20 mM $NaPO_4$, pH 7.3 and protease inhibitors as indicated above. This was followed by a final wash with a buffer containing 20 mM glutathione, 0.12 M NaCl, 100 mM Tris, pH 8.0. The remaining resin was then eluted with SDS-PAGE sample buffer. The GST samples were treated identically, except that the bound GST was eluted directly with glutathione buffer. These samples were then analyzed by SDS-PAGE in a 12% gel. Western analysis was by standard methods (36). A polyclonal rabbit anti-TBP antibody was used to visualize TBP. TBPΔ57 was visualized by a polyclonal rabbit antibody directed against the C-terminus of TBP (1). The blots were developed using an alkaline phosphatase method (Biorad).

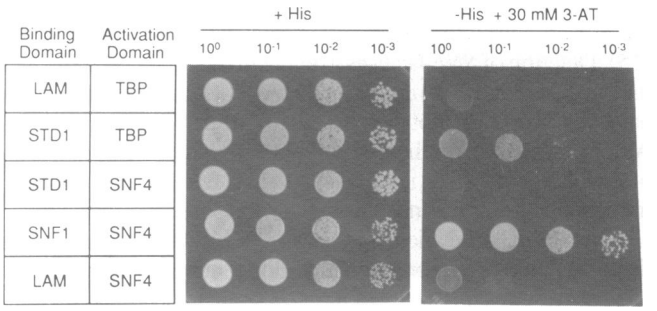


**Figure 1.** STD1 interacts with TBP *in vivo*. Strain Y153 transformed with the indicated plasmids were grown overnight in selective media to late log phase. They were adjusted to equal cell number, then 10 µl of the indicated dilutions were spotted onto synthetic complete media lacking tryptophan and leucine or to media lacking tryptophan, leucine and histidine and containing 30 mM 3-AT. They were grown for 7 days at 30°C, then photographed.

## RESULTS

### STD1 and TBP interact in the two-hybrid system

The ability of STD1 to suppress TBPΔ57-induced defects in a dosage dependent manner suggested that STD1 may interact directly with TBP. We tested this possibility using the two-hybrid system developed by Fields and colleagues (28,32). This assay is based on the fact that GAL4 activation of a reporter gene can be reconstituted in *trans* from separate fusion proteins containing the GAL4 binding ($G_{BD}$) and activation ($G_{AD}$) domains. Reconstitution of GAL4 activity *in vivo* indicates a physical interaction between the two fusion proteins.

Initial experiments determined that a fusion protein composed of wild type yeast TBP and $G_{BD}$ contained significant background activity in the absence of any $G_{AD}$ plasmid. This technical difficulty was overcome by making two adjustments. We used a point mutation in the DNA binding domain of TBP (Thr to Lys at position 112) that eliminates specific binding to TATA boxes (10) and we fused this ($TBP_{T112K}$) to $G_{AD}$. The $G_{AD}$-$TBP_{T112K}$ fusion contained little if any background activity and was used in the two hybrid experiments described below.

Plasmids expressing $G_{BD}$ fused with STD1 ($G_{BD}$-STD1) and $G_{AD}$ fused with $TBP_{T112K}$ ($G_{AD}$-$TBP_{T112K}$) were cotransformed into the yeast strain Y153. Y153 has its *HIS3* gene under the control of the *GAL1* upstream activating sequence and the endogenous *GAL4* gene is deleted. Reconstitution of GAL4 activity from the fusion proteins allows expression of the *HIS3* gene product and the resulting ability to grow on media lacking histidine. 3-Aminotriazole was added to the media to increase the amount of the *HIS3* gene product required, since the background level of transcription that occurs in the absence of any GAL4 activity is sufficient for substantial growth (23). Thus, increasing amounts of 3-AT can be used to titrate the efficiency of GAL4 reconstitution.

Cells containing both $G_{BD}$-STD1 and $G_{AD}$-$TBP_{T112K}$ were able to reconstitute GAL4 activity, demonstrating an interaction between STD1 and TBP. This is shown for serial dilutions of these strains at 30 mM 3-AT in Figure 1, on media containing ethanol, glycerol and galactose as a carbon source. Similar results were obtained with 15 mM 3-AT and on glucose with 15, 30 and 60 mM 3-AT (not shown).

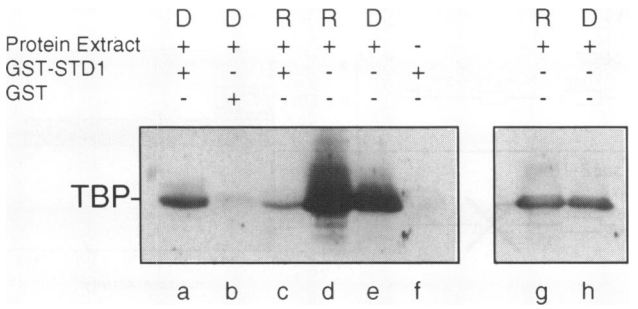


**Figure 2.** Glutathione S-transferase-STD1 binds TBP from yeast whole cell extracts. Strain MCY2662 transformed with pDE93-3 (TBP expressed from a 2 μ vector) was grown to mid-log phase in selective media containing 2% dextrose (repressing, R) and shifted to derepressing (D) media for 3 h. Yeast extracts were prepared and 200 μg protein from each was incubated with GST-STD1 (lanes a and c) or GST (lane b) bound to glutathione-agarose for 1 h at 4°C. After extensive washing, these were boiled in SDS-PAGE sample buffer and subjected to SDS-PAGE on a 10% gel. 25 μg yeast extract was loaded in lanes d and e. Lane f was loaded with GST-STD1 alone. Lanes g and h are the same as d and e, except at a lighter exposure. TBP was detected by Western analysis using rabbit anti-TBP serum and developed by chemiluminescence.

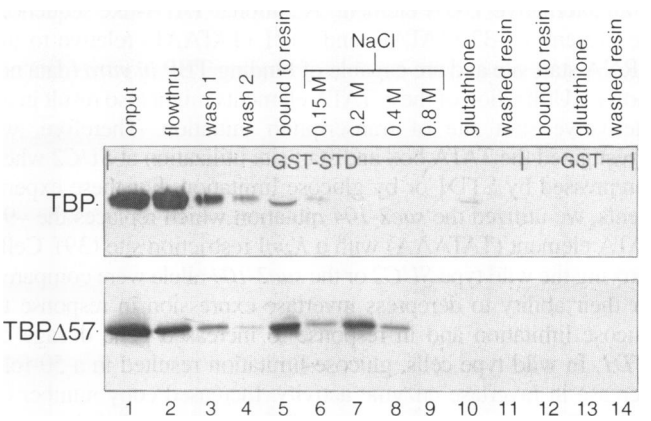


**Figure 3.** Glutathione S-transferase-STD1 directly interacts with TBP and TBPΔ57. Freshly induced GST-STD1 was bound to glutathione-agarose, then incubated in isolation buffer at 0.1 M NaCl for 30 min at 4°C with either purified recombinant TBP or TBPΔ57 (onput). This was centrifuged and the supernatant was saved (flowthru). The resin was washed three times in the same buffer (wash 1), then three more times (wash 2). One half the resin was then eluted with SDS-PAGE sample buffer (bound to resin). The other half was subjected to sequential salt washes in isolation buffer containing 0.15, 0.2, 0.4 and 0.8 M NaCl. Bound proteins were then eluted from the resin with a buffer containing 20 mM glutathione, 0.12 M NaCl, 100 mM Tris, pH 8.0 (glutathione). Any remaining bound proteins were then eluted by boiling with SDS-PAGE sample buffer (washed resin). The GST samples were treated identically, except that one half the resin was eluted with glutathione buffer immediately after wash 2.

The STD-TBP interaction was shown to be specific, in that neither $G_{BD}$-STD1 nor $G_{AD}$-$TBP_{T112K}$ promoted growth when paired with other proteins. For instance, while the $G_{BD}$-LAM (human lamin C)/$G_{AD}$-$TBP_{T112K}$, $G_{BD}$-STD1/SNF4-$G_{AD}$ and $G_{BD}$-LAM/SNF4-$G_{AD}$ protein pairs showed some growth at the $10^0$ dilution, it was not sustained. At the $10^{-1}$ dilution these strains were clearly not growing. The positive control, the $G_{BD}$-SNF1/SNF4-$G_{AD}$ pair, shows a well documented (28,37), relatively strong interaction. The interaction between TBP and STD1 was not dependent on the T112K mutation since similar results were obtained with wild type TBP fused to either the LexA or GAL4 DNA binding domains (data not shown). That the same results were obtained using different constructs and reporter genes shows that the apparent ability of $G_{BD}$-STD1 and $G_{AD}$-$TBP_{T112K}$ to interact was not an artifact of any particular construct. Furthermore, all strains grew on media supplemented with histidine (Fig. 1), indicating that none of the fusion proteins were toxic. Thus, $G_{BD}$-STD1 and $G_{AD}$-$TBP_{T112K}$ were able to reconstitute GAL4 activity through their specific interaction *in vivo*.

## STD1 specifically binds TBP from yeast extracts

The two hybrid data reported above provided evidence that STD1 and TBP interact *in vivo*. We detected this interaction *in vitro* using a GST-STD1 purified from bacteria. Glutathione S-transferase-STD1 or GST bound to glutathione-agarose resin was incubated with 200 μg yeast extract from either glucose repressed or derepressed cells. After extensive washing, the proteins bound to the resin were analyzed by Western blot. GST-STD1 specifically bound TBP (Fig. 2, compare lanes a and e; c and d). Glutathione S-transferase did not (lane b). This biochemical evidence confirms that STD1 specifically interacts with TBP in both glucose repressed and derepressed cells.

## STD1 binds purified recombinant TBP and TBPΔ57

We addressed whether STD1 interacts with TBP directly or through an intermediate by testing the ability of GST-STD1 to bind recombinant TBP purified from *E.coli*. We also tested its ability to bind TBPΔ57, since an increased gene dosage of STD1 can rescue cells from TBPΔ57 toxicity. Glutathione S-transferase-STD1 or GST bound to glutathione-agarose resin was incubated with 2 μg purified recombinant TBP or TBPΔ57. The resin was then washed and eluted with increasing concentrations of NaCl. Figure 3 shows a Western analysis of these fractions using antibodies to TBP and TBPΔ57. Both TBP and TBPΔ57 bound to GST-STD1 (lane 5), but not to GST alone (lane 12). This interaction was not mediated by DNA, since including either ethidium bromide (38) or DNase I in these reactions had no effect relative to controls (data not shown). These results demonstrate a direct physical interaction between STD1 and TBP or TBPΔ57 proteins *in vitro*.

Interestingly, there were striking qualitative and quantitative differences in the binding properties of TBP and TBPΔ57. The same preparation of GST-STD1 resin bound almost half the TBPΔ57, but only a small fraction of the TBP (compare lanes 2 and 5). This difference was not due to different amounts of active TBP or TBPΔ57 in these preparations, since they have similar DNA binding activities as measured by electrophoretic mobility shift assay (data not shown). Furthermore, the binding of STD1 to TBP appeared qualitatively different from that of STD1 to TBPΔ57. Most of the bound TBPΔ57 eluted at 0.2 M NaCl (lane 7), whereas the small amount of TBP that bound either eluted at 0.15 M NaCl or was retained until the glutathione wash. These experiments show that both TBP and TBPΔ57 interact with STD1, but that removal of the non-conserved N-terminal domain of TBP alters its binding properties to STD1.

## STD1 activation of *SUC2* does not alter TATA box or start site selection

Since STD1 protein interacts with the TBP, it is possible that its mechanism of activation of the *SUC2* gene may be to direct TBP

to an alternative TATA element. Additional TATA-like sequences are present at –32 (TATAT) and –121 (TATAAT) relative to the mRNA start site and are capable of binding TBP *in vitro* (data not shown). Utilization of these TATA elements might also result in an alternative start site of transcription initiation. Therefore, we investigated the TATA box and start site utilization at *SUC2* when derepressed by STD1 or by glucose limitation. For these experiments, we utilized the *suc2-104* mutation which replaces the –90 TATA element (TATAAA) with a *Kpn*I restriction site (39). Cells carrying the wild type *SUC2* or the *suc2-104* allele were compared for their ability to derepress invertase expression in response to glucose limitation and in response to increased gene dosage of *STD1*. In wild type cells, glucose limitation resulted in a 50-fold increase in invertase enzyme activity. Increased copy number of the *STD1* gene resulted in a 10-fold increase of invertase expression even though cells were maintained under repressing (high glucose) conditions (Fig. 4A). These results are consistent with earlier studies (15,39). In contrast, the *suc2-104* mutation essentially blocks the ability of cells to derepress *SUC2* expression in response to either glucose limitation or increased copy number of *STD1*. This strain (FY716) has all the *trans*-acting factors needed for regulation of *SUC2* since they efficiently derepress wild type *SUC2* introduced on a plasmid (not shown). These data demonstrate that STD1 activation of *SUC2* depends on the same TATA element that is used in response to glucose limitation.

The initiation site of *SUC2* mRNA was analyzed by primer extension of total RNA using a *SUC2* specific oligonucleotide primer. Using this assay, we compared both the quantity of *SUC2* mRNA and the initiation site selection. Wild type cells show a large increase in *SUC2* mRNA in response to glucose limitation (Fig. 4B, lanes 2 and 5) that closely correlates with the observed increase in invertase activity (Fig. 4A). The mobility of the primer extension product is consistent with the previous mapping of the *SUC2* mRNA start site to 40 bp upstream of the ATG codon (40). Increased copy number of *STD1* increases *SUC2* mRNA accumulation under repressing conditions (lane 3) and the mobility of the primer extension product is identical to that observed for *SUC2* mRNA induced by glucose limitation. No additional primer extension products were observed. Therefore, STD1-activated mRNA has the same 5′ end as mRNA derepressed by glucose limitation. In addition, we examined the level of *SUC2* mRNA in cells which lack both the *STD1* gene and its homologue, *MTH1*. These cells derepress invertase poorly (15) and this defect is apparent at the level of mRNA accumulation (lane 4).

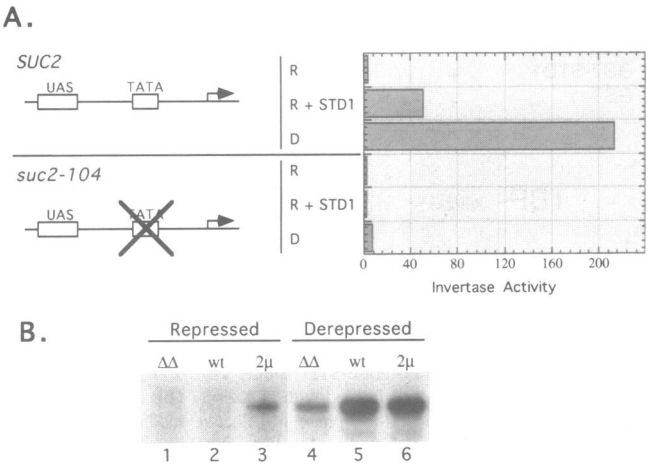


**Figure 4.** *SUC2* promoter elements required for activation by STD1 and glucose limitation. (**A**) TATA box requirement. The requirement for the *SUC2* promoter TATA element was examined by comparing strains MCY2649 (*SUC2*$^+$) and FY716 (*suc2-104*) for the ability to derepress *SUC2* transcription in response to glucose starvation and increased copy number of *STD1*. All cells were transformed with the YEp13 based plasmid (2μ, *LEU2*) and grown in the absence of leucine. Cells transformed with YEp13 are indicated by R and D; cells transformed with p6A8, a plasmid with the *STD1* gene inserted into YEp13 are indicated by R + STD1. Repressed cells (R and R + STD1) were grown in synthetic complete media containing 2% glucose to an OD of 0.5–1.0, and were then harvested and assayed for invertase activity. Derepressed cells (D) were grown to an OD of 0.5–1.0 in the same media and were then harvested, washed in water and resuspended to the same OD in the same media containing 0.05% glucose. After 3 h, cells were harvested and assayed for invertase activity. All errors were <5% for values >2 U/100 mg dry weight. (**B**) Primer extension of *SUC2* mRNA. The cells used in this experiment contained wild type levels of STD1 and MTH1 (wt), increased copy number of *STD1* on a 2μ plasmid (2μ) or contained null alleles of both *std1* and *mth1* (ΔΔ). Total yeast RNA was purified from cells grown under repressing conditions (2% glucose; lanes 1–3) or derepressing conditions (3 h in 0.05% glucose; lanes 4–6). Primer extension products from reactions with 15 μg of RNA were resolved on an 8% polyacrylamide, 7 M urea gel. Control reactions indicated that this analysis was in the linear range for this assay (data not shown). A reaction with twice the RNA (30 μg) from wild type derepressed cells yielded twice the quantity of extension product, whereas a 2-fold increase in primer did not increase the yield of primer extension product.

### TBP can titrate STD1 suppression of the snf4Δ phenotype

Given that STD1 can interact directly with TBP, we sought evidence that this interaction is relevant *in vivo*. Previously, it was shown that STD1 expressed from a multicopy plasmid can partially suppress the defect in invertase expression of a *snf4Δ* strain (15). To test whether this ability of STD1 to suppress the *snf4Δ* defect is mediated by its interaction with TBP, we measured the invertase activity of strains overexpressing both STD1 and TBP.

For this experiment a LexA-STD1 fusion protein under the control of the strong *ADH1* promoter was used. The LexA moiety is not relevant here. As expected from previous studies (15), LexA-STD1 restored significant invertase activity in a *snf4Δ* strain under derepressing conditions and that function was dependent on the STD1 moiety in the fusion protein (Fig. 5, lanes a and d). Increased dosage of the TBP gene on a multicopy plasmid abolished STD1 suppression of *snf4Δ* (lane e). In control experiments, the presence of the parent vector for the TBP plasmid had little effect (lane d), as did increased dosage of TBP in the absence of overexpressed STD1 (lane b). Instead, high levels of TBP seem specifically to interfere with STD1 suppression of the *snf4Δ* defect. In contrast, increased dosage of TBPΔ57 on a multicopy plasmid had little effect on invertase expression in the *snf4Δ* strain, whether LexA-STD1 was present (lane f) or not (lane c). These results are consistent with a direct interaction between TBP and STD1 that is modulated by the N-terminus and further suggest that this interaction is physiologically relevant to the role of STD1 in the regulation of *SUC2* transcription.

## DISCUSSION

The isolation of *STD1* (*MSN3*) as a multicopy suppressor of both a *snf4Δ* mutant and a TBP mutant suggests that this protein might

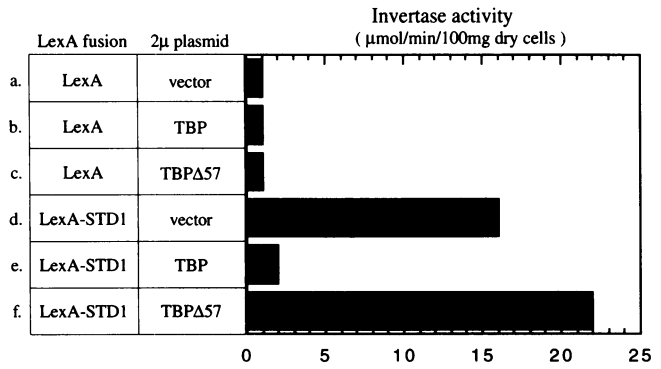


**Figure 5.** High copy expression of TBP abolishes STD1 suppression of *snf4*Δ phenotype. Strain MCY2634 transformed with either pLexA(1–202)+PL (LexA) or pLexA-MSN3 (LexA-STD1) and either YEp352 (vector), pRG84 (TBP) or pRG85 (TBPΔ57) were grown to mid log phase in selective media and shifted to glucose derepressing media (0.05% glucose) for 2 h. Yeast cells were prepared and tested for invertase activity. A unit is defined as micromoles of glucose released per min/100 mg (dry weight) of cells. Values are the average of assays of three transformants for derepressed samples. All errors were <8% for values >2 U/100 mg dry weight.

provide a link between the SNF1/SNF4 protein kinase complex and the transcriptional machinery. Earlier studies demonstrated a physical interaction between STD1 and SNF1, both *in vivo* and *in vitro* (15). Here we demonstrate a direct physical interaction between STD1 and TBP, thus establishing a potential link between SNF1 and the transcriptional machinery.

### Interaction of STD1 and TBP

*In vivo*, STD1 interacted with TBP in the two-hybrid system. This interaction was shown to be specific and independent of particular plasmid constructs or yeast strains. The interaction between STD1 and TBP was also demonstrated *in vitro* using a GST-STD1 fusion protein. When purified GST-STD1 was incubated with extracts from yeast cells, it was shown to bind TBP. This interaction was specific in that GST alone did not bind TBP. The interaction of TBP with GST-STD1 was also detected using purified components, showing that this interaction is direct and not dependent on additional yeast proteins. Together with the two-hybrid results, these data provide conclusive evidence for a direct physical interaction between STD1 and TBP.

Many gene regulatory proteins have been shown to interact with TBP (41–50). In fact, so many TBP-interacting proteins have now been identified that it is reasonable to question which interactions are biologically important. The data presented here indicate that the STD1-TBP interaction is physiologically relevant *in vivo*. Changes in the relative levels of expression of STD1 and TBP or TBPΔ57 cause changes in gene regulation *in vivo* (1,15). For instance, increased expression of TBPΔ57 causes defects in RNA polymerase II transcription *in vivo* that can be reversed by increased expression of STD1. Similarly, increased expression of STD1 causes a partial derepression of *SUC2* in a *snf4*Δ mutant that can be reversed by increased expression of TBP. In both cases, the effects on transcription by overexpressing one of these proteins is reversed by concomitant overexpression of the other. The dosage effects of these phenotypes are consistent with the existence of a STD1-TBP interaction that affects transcriptional regulation *in vivo*.

The mechanism by which the STD1-TBP interaction might affect transcriptional regulation remains speculative. The phenotypes due to TBPΔ57 overexpression and *snf4*Δ mutation correlate with defects in gene expression. Increased copy number of *STD1* can partially suppress both these phenotypes and the data presented here and previously (15) provide a physical link between the components of glucose derepression and general transcription. However, the data do not provide a clear mechanistic model that can adequately explain our *in vitro* binding data. In particular, it is not known whether a STD1-TBP complex is the active species with regard to *SUC2* transcription or whether these proteins affect *SUC2* transcription by titrating one or the other from a different complex. A second issue is the identity of the component of this system which provides gene specificity. The mechanism which directs these components to the *SUC2* gene as opposed to any other TATA-box-containing gene remains unknown.

### Role of the N-terminal domain of TBP

Our data indicate that the interaction of STD1 and TBP is altered by the non-conserved N-terminal domain of TBP. This domain of TBP is not essential *in vivo* and little is known about its functional role. *In vitro*, the N-terminal domain acts as an inhibitor of DNA binding (12,13,51) and this inhibition can be modulated by TFIIA (14). The binding studies using GST-STD1 with purified TBP and TBPΔ57 show that the STD1-TBP interaction is greatly affected by the N-terminal domain of TBP, providing evidence for a role of the N-terminal domain of TBP in modulating protein–protein interactions. The N-terminal domain of TBP also affects the STD1-TBP interaction *in vivo*. Increased expression of STD1 results in increased expression of *SUC2* in a *snf4* mutant. This STD1-mediated derepression is blocked by increased expression of TBP but not TBPΔ57. The finding that the N-terminal domain affects the STD1-TBP interaction may explain earlier studies which reported that cells expressing TBPΔ57 (y183C) grew poorly on some carbon sources (8). Deletion of both *STD1* and its homologue, *MTH1*, also results in poor growth on these media (15). Recently, it was found that the TBPΔ57 but not full-length TBP was able to associate with human TAFs when expressed in a human cell line, suggesting that the N-terminal domain may affect the assembly of the TFIID complex *in vivo* (52). These data suggest that one role of the N-terminal domain may be to modulate the interaction of TBP with the TAFs and other transcriptional regulators.

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