Yeast Ume6p repressor permits activator binding but restricts TBP binding at the HOP1 promoter

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ABSTRACT

Ume6p plays essential roles in the regulation of early meiotic genes in Saccharomyces cerevisiae. Ume6p exerts repression via recruitment of the Sin3p-Rpd3p histone deacetylase and Isw2p chromatin remodeling complexes. The transcriptional step that is ultimately inhibited by Ume6p is unknown. Here, in vivo footprinting shows that transcriptional activators Hap1p and Abf1p occupy upstream sites in repressed and derepressed promoters. In contrast, chromatin immunoprecipitation shows that TATA box-binding protein (TBP)-promoter binding is reduced upon repression of HOP1. Fusion of TBP to a zinc cluster DNA binding domain relieves repression at a HOP1 promoter modified to include the zinc cluster target site. We suggest that TBP binding is inhibited through chromatin modification by the Sin3p-Rpd3p and Isw2p complexes recruited by Ume6p.

INTRODUCTION

In the yeast Saccharomyces cerevisiae, repression of early meiotic genes depends on histone deacetylation and chromatin remodeling activities. These genes are repressed during mitotic growth by the binding of Ume6p to the URS1 site in their 5′ regions (reviewed in 1). Ume6p recruits the histone deacetylase (HDAC) complex Sin3p-Rpd3p to exert repression (2,3). It is well established that the recruitment of Rpd3p generates a localized histone deacetylation domain over a range of one to two nucleosomes in the targeted promoter (4–7). Ume6p also recruits the Isw2p chromatin remodeling complex, which promotes the formation of a nucleosome-inaccessible chromatin structure proximal to the URS1 site at target promoters (8,9). Both Sin3p-Rpd3p and Isw2p are required for full repression by Ume6p, thus supporting the idea that nucleosome modification and position together govern promoter function (8–10).

Though effects of the Ume6p complex on local chromatin structure are well documented (8,9), the transcription factors that are ultimate targets of repression are uncertain. Histone deacetylation in a TATA-proximal nucleosome inhibits TATA box-binding protein (TBP) binding at the human pS2 promoter (11), but inhibits activator Adr1p binding at the yeast ADH2 promoter (12). Histone deacetylation at Polycomb-repressed Drosophila promoters does not affect TBP binding, but may inhibit RNA polymerase II recruitment (13). SIR-generated repressive chromatin is permissive for both activator and TBP binding (14). Based on these precedents, Ume6p may repress transcription through inhibition of activator or TBP binding or by inhibition of a later step in transcription initiation.

Here we report analysis of Ume6p-repressed promoters through in vivo footprinting and chromatin immunoprecipitation (ChIP) assays. Our findings argue that inhibition of TBP binding is a critical step in Ume6p-dependent repression.

MATERIALS AND METHODS

Yeast strains and plasmids

Yeast strains used were SK1 derivatives, have genetic markers ura3 trpl1 leu2 lys2 ho::LYS2 and are isogenic except as noted below: AMP107 (MATα), AMP1779 (MATα hap1::ZC-TBP-TRP1), AMP1780 (MATα hap1::LEU2), MHS21 (MATα hap1::ZC-TBP-TRP1 ume6Δ2), MHS22 (MATα hap1::LEU2 ume6Δ2), MHS24 (MATα ume6Δ2), xy268 (MATα ume6Δ1::TRP1 gal80::LEU2 his3Δ), xy423 (MATα gal80::LEU2 his3Δ). MHS21, 22 and 24, which are ume6Δ2 strains, carry a ume6Δ allele lacking codons 159–836. Construction of the ume6Δ1::TRP1 mutation in xy268 was described previously (15).

ZC-TBP is an in-frame fusion protein in which the zinc cluster DNA binding domain of Hap1p (amino acids 1–247) (16) is fused to the N-terminus of TBP. It was created through several steps in the genome of strain AMP1779 as follows. A DNA fragment containing the TBP open reading frame (ORF) (called SPT15 in S.cerevisiae) and 300 bp of its 3′ untranslated region was cloned between the Spel and Xhol sites of pRS304, forming plasmid pAD6. PCR was then performed with pAD6 as template using a 5′-primer (5′-AGT AAC GGA ACC ATC CAC TTA GGT GCC ACC CAC TGG TGG TCT ATC ATG AAA GGT GAC CCG ATG GCC GAT GAG GAA CGT TTA) which corresponds to nucleotide positions 682–741 of the HAP1 coding strand and positions 1–21 of the SPT15 coding strand and a 3′-primer (5′-GTT

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AGA CAC GTC CTG GCT GGT TGG AAT GGT AGC GTT TAA TTG AGG AAA ATT ATC AGG CGG CAT CAG AGC AGA TTG T-3′ which corresponds to positions 2160–2100 of the HAP1 non-coding strand and positions 151–169 of pRS306. The amplified fragment was integrated into the hap1::LEU2 locus of AMP1780 to express the Hap1p DNA binding domain fused with the N-terminus of TBP (referred to as ZC-TBP).

Epitope-tagged TBP was constructed in a TRP1 centromeric plasmid pRS314 as follows. To express HA epitope-tagged TBP (HA3-TBP) from its natural promotor, the SPT15/ TBP1 gene with 5′-flanking sequences (~840 to -1) and 3′-flanking sequences (the stop codon to 300 bp downstream) was cloned between the KpnI and SpeI sites of pRS314 and a NotI site was created after the first codon of TBP. Then, a DNA fragment that encodes three copies of HA epitope was obtained by digestion of pGTEP1 with NotI (17) and was inserted into the NotI site after the first codon of TBP, in-frame with the TBP ORF.

CYC1-lacZ plasmids pKB112, pKB143 and pLGΔ312ΔRS were constructed previously (18,19). Plasmid pKT5-1 was constructed from pAV79B (generously provided by A. Vershon; 20) as follows: the region −131 to −114 of the HOP1 promoter in pAV79B was replaced by the CYC1 UAS1 sequence (5′-GGC CGG TTA CGG ACG-3′), forming pKT5-1.

All the expression and reporter plasmids constructed here were verified by DNA sequencing.

Culture conditions, media, strain construction, transformations and the β-galactosidase assay followed standard recipes and protocols as described (21).

In vivo footprinting

In vivo UV and DMS footprintings by primer extension were performed as described (22,23). Primer sequences are available upon request.

Chromatin immunoprecipitation assay

Chromatin-containing whole cell extract was prepared from 100 ml of log phase culture as described (24–26). Chromatin corresponding to 2 × 10⁸ cell equivalents of the whole cell extract (an average size of ~500 bp) was combined in a final volume of 0.2 ml with 10 μl of monoclonal anti-HA antibody (5 mg/ml 12CA5; Roche). Immunoprecipitated DNA was analyzed by quantitative PCR using primer sets for specific regions, which were designed to 24±28mers. Primer sequences are available upon request. PCR was first performed with decreasing amounts of DNA templates to determine the linear range. The PCR conditions were 94°C for 5 min, followed by 27 cycles of 94°C for 30 s, 40°C for 30 s and 72°C for 30 s, then 72°C for 5 min. PCR products were resolved by 2% agarose gel electrophoresis, stained with SYBR-Green, visualized and quantitated with a FujiFilm Luminescent Image Analyzer LAS-1000plus.

RESULTS AND DISCUSSION

The Ume6p repressor complex does not affect activator binding in the CYC1 and HOP1 promoters

To understand the mechanism of Ume6p-dependent repression, we compared binding of transcriptional activators to repressed and derepressed promoters in vivo. We first used the well characterized CYC1-lacZ promoter region. This hybrid gene is activated by the transcription factor Hap1p, which binds to the UAS1 site. Binding of Hap1p results in UV hypersensitivity within UAS1 (23) and is thus detectable by UV photofootprinting. CYC1-lacZ is not normally repressed by Ume6p, so we created the hybrid CYC1-URS1-lacZ gene, which has the Ume6p binding site (URS1) inserted between UAS1 and the TATA region (19). We verified that CYC1-URS1-lacZ expression was repressed about 20-fold by Ume6p (Fig. 1A), in agreement with previous reports (2,19). We then analyzed activator Hap1p binding in the CYC1-URS1-lacZ promoter by in vivo UV photofootprinting (Fig. 1B). Hap1p- UAS1 binding was readily detectable at CYC1-URS1-lacZ in the presence or absence of Ume6p. Therefore, repression of this hybrid promoter does not result from inhibition of activator binding.

We also examined the HOP1 promoter, a natural Ume6p repression target. The HOP1 promoter is activated by binding of the transcription factor Abf1p to an upstream site (27). HOP1 is normally repressed in mitotic cells by Ume6p (20) and expressed only in meiotic cells when Ume6p repression is
Artificial recruitment of TBP relieves repression by Ume6p at the HOP1 promoter

Inhibition of TBP binding may be the cause of repression by Ume6p at HOP1 or it may be an indirect consequence of the repressed state. The former model predicts that expression of a TBP derivative which is able to bind to the HOP1 TATA region, independently of Ume6p, will relieve repression. To create such a derivative, we made use of the observation above that the activator Hap1p binds to CYC1 UAS1 independently of Ume6p (Fig. 1B). We fused the zinc-cluster DNA binding domain of Hap1p (16) to the N-terminus of TBP to create ZC-TBP (in strains lacking intact Hap1p). We also introduced the Hap1p binding site, CYC1 UAS1, upstream of the HOP1 TATA region to create the HOP1-UAS1 promoter. We reasoned that ZC-TBP would bind to HOP1-UAS1 with increased affinity because it can make both TBP-TATA and ZC-UAS1 protein–DNA contacts. In vivo UV photofootprinting verified that ZC-TBP is bound to the HOP1-UAS1 promoter in both UME6 and ume6Δ strains (data not shown).

We then examined expression of HOP1 and HOP1-UAS1 in the presence and absence of ZC-TBP (Fig. 4A). In the absence of ZC-TBP, the HOP1 and HOP1-UAS1 promoters were expressed at equivalent levels; Ume6p caused several hundred-fold repression. In the presence of ZC-TBP, the two promoters were expressed at similar derepressed levels (ume6Δ strain). However, whereas the HOP1 promoter (WT TATA) was repressed over 600-fold, the HOP1-UAS1 promoter (UAS1+WT TATA) was repressed only 2.5-fold. As a control, we prepared an additional strain expressing ZC alone and found that Ume6p repression was not relieved by the expression of ZC (data not shown). Thus artificial recruitment of TBP by ZC at the HOP1 TATA region relieves repression by Ume6p (Fig. 4B). This finding supports the model that Ume6p causes repression through inhibition of TBP binding at the HOP1 promoter.
Ume6p repression: mechanistic implications

The localized histone deacetylation domain established by the Ume6p-Sin3p-Rpd3p complex (4–7) has been proposed to inhibit the binding of activators and/or TFIIID to their cognate sites (4,5,29,30). This model is based upon a prevailing idea for transcriptional regulation by histone acetylation: acetylation weakens histone–DNA interaction and allows trans-acting factors access to cis-elements; deacetylation prevents access (30). Our findings indicate that Ume6p does not inhibit access of activators to CYC1-URS1 and HOP1, but that it impairs TBP binding at HOP1. Furthermore, at the HOP1 promoter, the relief of repression by tethered TBP argues that reduced TBP binding is the major cause of repression.

One simple model for repression is that the Ume6p repressor complex interferes with TBP binding through deacetylation of histones in a nucleosome which occludes the TATA region. This explanation is consistent with the finding that a deacetylated nucleosome can block TBP binding in vitro and in vivo (11,31,32). It is also consistent with the finding that lsw2p complex recruitment by Ume6p generates a local nuclease-inaccessible chromatin structure (8–10). These precedents argue that Ume6p-dependent chromatin modification can inhibit TBP binding directly.

A second possibility is that Ume6p affects TBP-promoter binding indirectly. For example, inhibition of the SAGA complex or another coactivator would lead to reduced TBP recruitment (33,34). According to this model, inhibition of activator function would then reduce TBP binding (25,35) and may be bypassed by tethering of TBP (36–39). Although we cannot rule out indirect models, our study narrows the target of Ume6p repression to an event between post-activator binding and TBP recruitment at the HOP1 promoter.

Another possibility is that Ume6p has multiple repression targets, since Ume6p interacts with both the Sin3p-Rpd3p and lsw2p complexes. This may explain the observation that the difference in TBP occupancy is not as great as the difference in repression we observed.

Very recently, similar results were published for artificial his3 promoters with or without IME2 URS1 sites (40). Deckert and Struhl (40) showed that binding of activators to their cognate sites, which were introduced to the his3 promoter, was unaffected by Rpd3p recruitment, whereas TBP occupancy was reduced upon Rpd3p recruitment in the range 2- to 6-fold, both in the artificial promoters and in the natural promoters INO1, CAR1, CAR2, SPO11 and SPO13. They also showed that Rpd3 repression at the his3 promoter can be bypassed by artificial recruitment of TFIIID components (40). Our results for the natural HOP1 and artificial CYC1 promoters agree with the earlier study (40), though promoter structures and properties are different, indicating that blocking of TBP binding but not activator binding is a common mechanism for repression of Ume6p-regulated genes.

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