Recruitment of the Yeast Tup1p-Ssn6p Repressor Is Associated with Localized Decreases in Histone Acetylation*

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Posttranslational acetylation of histones is an important element of transcriptional regulation. The yeast Tup1p repressor is one of only a few non-enzyme proteins known to interact directly with the amino-terminal tail domains of histones H3 and H4 that are subject to acetylation. We demonstrated previously that Tup1p interacts poorly with more highly acetylated isoforms of these histones in vitro. Here we show that two separate classes of promoters repressed by Tup1p are associated with underacetylated histones in vivo. This decreased histone acetylation is dependent upon Tup1p and its partner Ssn6p and is localized to sequences near the point of Tup1p-Ssn6p recruitment. Increased acetylation of histones H3 and H4 is observed upon activation of these genes, but this increase is not dependent on transcription per se. Direct recruitment of Tup1p-Ssn6p complexes via fusion of Tup1p to the lexA DNA binding domain is sufficient to confer repression and induce decreased acetylation of H3 and H4 at a target promoter. Taken together, our results suggest that stable decreases in histone acetylation levels are directed and/or maintained by the Tup1p-Ssn6p repressor complex.

The yeast Tup1p-Ssn6p repressor complex provides a novel paradigm for transcriptional repression and for the role of chromatin in this process. TUP1 and SSN6 are required for the repression of several diverse families of genes in yeast, including cell type-specific genes (regulated by the o2 and a1/a2 repressors), as well as genes responsive to different physiological conditions including SUC2 (responsive to change in carbon source), RNR3 (responsive to DNA damage), and ANB1 (responsive to oxygen levels), among others (see Ref. 1 for review and Ref. 2). Neither Ssn6p nor Tup1p binds directly to DNA, but these proteins are recruited to promoters through interactions with sequence-specific DNA binding factors such as the o2 repressor (3, 4) and Ctr1p (2). Bypass of the DNA binding factor by fusion of Ssn6p or Tup1p to a heterologous lexA DNA binding domain demonstrates that these proteins can directly orchestrate repression. Ssn6p-lexA fusions require Tup1p (5) to confer repression of an artificial promoter containing lexA operator sequences. However, Tup1p-lexA fusions can confer repression in the absence of Ssn6p (6), suggesting that Tup1p is the dominant repressor moiety in the complex.

The ability of Tup1p-Ssn6p to regulate many diverse genes indicates that these complexes may interact with some common promoter component, such as a basal transcription factor or a component of chromatin. Indeed, two models have been proposed to explain how Tup1p-Ssn6p complexes confer repression. The first is based on studies that indicate that a number of other factors necessary for repression, including Sin4p (7, 8), Sin3p/Rpd1p (9), Rpd3p (10), Srb10p/Are1p/Ssn3p, Srb11p/Ssn8p (11–13), and Srb8p/Are2p (14), are associated with subcomplexes within the RNA polymerase II holoenzyme (13). Thus, Tup1p-Ssn6p may inhibit transcription through direct interactions with one or more of these transcription factors. Accordingly, partial repression (2–4-fold) can be achieved in vitro in the presence of just the basal transcription machinery, Ssn6p, and Tup1p (15, 16).

An alternative model suggests that Tup1p-Ssn6p directs repression through modulation of chromatin structure. Tup1p interacts directly with the amino-terminal tail domains of histones H3 and H4 in vitro (17), and mutations in these histone domains synergistically reduce repression of multiple classes of Tup1p-regulated genes in vivo (17, 18). Moreover, the domain in Tup1p that is required for interaction with the histones overlaps independently defined repression domains (6). Thus, Tup1p-Ssn6p may affect repression through interactions with components of chromatin that lead to decreased accessibility of promoter regions.

These two models for Tup1p-Ssn6p repression may coincide. Complete repression could require interactions with both the basal transcription machinery and the histones. For example, Tup1p-Ssn6p complexes might first halt transcription by altering the activity of the basal apparatus and then maintain the repressed state through organization of chromatin.

One aspect of chromatin that is often modulated in active and repressed chromatin domains is histone acetylation. Lysine residues in the amino-terminal domains of all four core histones are subject to this posttranslational modification. Increased histone acetylation is often correlated with increased gene expression, whereas decreased acetylation is correlated with decreased transcription. The identification of several transcriptional regulators as histone acetylases or deacetylases in the last few years has provided a molecular basis for this correlation (see Refs. 19–23 for recent reviews).

We have shown previously that Tup1p binding to the amino-terminal tails of histones H3 and H4 in vitro is inhibited by high levels of acetylation (17, 24). These findings suggest that Tup1p binding, and thus repression, may be modulated by changes in histone acetylation in vivo. If so, then genes regulated by Tup1p should be associated with different levels of acetylated histones under conditions of repression and activation. Consistent with this idea, we report here that genes

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The abbreviation used is: PCR, polymerase chain reaction.

repressed by Tup1p are associated with underacetylated forms of histones H3 and H4 in vivo. These same genes are associated with more highly acetylated histones when repression is relieved, and the genes are activated. The difference in these acetylation states is not dependent upon transcription per se but does require Tup1p. We have also found that deacetylation of histones H3 and H4 accompanies reporter gene repression upon targeted recruitment of Tup1p. These data indicate that Tup1p repression is linked mechanistically to changes in histone acetylation.

EXPERIMENTAL PROCEDURES

Yeast Strains and Genetic Methods—The strains FY23, FY24, FY716, FY24Atup1, and FY24ssn6 have been described previously (25, 26). Strains FY23 and FY24 carrying the TALS plasmid (FY297T and FY247T) have been described previously (26). The FY716atsn1 strain was created by transformation of strain FY716 with the plasmid tup1Δ406 cut with AvrII (26) followed by selection for uracil auxotrophy. Disruption of the TUP1 gene was confirmed by Southern blot. All strains were grown according to standard procedures (27) in either rich media or selective media, synthetic dextrose media supplemented with the appropriate auxotrophic requirements.

Total yeast RNA was prepared as described previously (27), and specific transcript was quantitated with a Storm 840 PhosphorImager (Molecular Dynamics, Inc.), 330 mM NaCl, 66 mM sodium acetate, pH 4.6, 2.2 mM ZnCl2, and 0.01% Triton X-100. The reaction was halted by the addition of 5 μl of 0.5 M EDTA and 5 μl of 10 mg/ml yeast RNA. Protected fragments were ethanol-precipitated and separated on an 8% denaturing acrylamide gel. The protected band corresponding to the SUC2 transcript was quantitated with a Storm 840 PhosphorImager (Molecular Dynamics) and normalized relative to the levels of ACT1 RNA.

Chromatin Immunoprecipitation—Chromatin immunoprecipitations were performed essentially as described previously (32). Antibodies specific to H3 Ac9,14 were kindly provided by C. D. Allis (University of Virginia, Charlottesville, VA) (33). Antibodies specific to H3 Ac9,18 and unacetylated H3 have been described previously (17). Antibodies specific for unacylated histone H4 are described in Ref. 34. Antibodies recognizing acetylated histone H4 were purchased from Upstate Biotechnology. Antibody incubations were carried out overnight at 4 °C with rotation. Immune complexes were purified after a 1-h incubation (4 °C with rotation) with 60 μl of protein A-Sepharose beads (1:1 slurry; Pharmacia CL4B). Washes and DNA purification were performed as described previously (32).

Quantitation of Immunoprecipitated DNA—DNA purified from immunoprecipitated complexes was slot-blotted onto nylon membrane (GeneScreen Plus; DuPont) according to the manufacturer’s instructions. The hybridization probe, specific for the random primed labeling technique (36) substituting sequence-specific primers for random hexamers. DNA on slot blots was quantitated with a Storm 840 PhosphorImager. Data presented were normalized against input DNA.

Quantitation of MF2.1 DNA was carried out by PCR essentially as described in Ref. 32. The promoter region of MF2.1 (nucleotides –286 to –68, primer sequences are available upon request) adjacent to the lexA operator was amplified from DNA derived from chromatin immunoprecipitation experiments. PCR products were run on 7% acrylamide gels and stained with SYBR Green 1 (Molecular Probes) at a dilution of 1:10,000 (in 1× Tria borate-EDTA buffer, pH 7.5) for 30 min. Quantitation was carried out after scanning the gel on a Storm 840 PhosphorImager. Multiple dilutions were performed to ensure the linearity of the assay, and data presented were normalized relative to input DNA.

RESULTS

Acetylation of TALS Chromatin Is Markedly Lower under Conditions of Tup1p-Ssn6p Repression—We examined histone acetylation levels associated with Tup1p-Ssn6p-regulated genes under conditions of repression and activation using chromatin immunoprecipitation (32, 37–39). Cells were grown under conditions of repression or activation specific for each gene and then fixed with formaldehyde to cross-link proteins to DNA. The cross-linked chromatin was reduced to an average length of 150–300 base pairs. The protein-DNA complexes were then immunoprecipitated using antibodies recognizing specific acetylated isoforms of histone H3, unacetylated H3, acetylated H4, or unacetylated H4. Immunoprecipitated DNA was analyzed by slot blot hybridization using gene-specific probes. This method allows quantitation of small changes in histone acetylation at a specific locus that accompany alterations in transcriptional competence. Our initial experiments involved analysis of the acetylation state of the TALS plasmid, a yeast episome containing the α2/Mcm1 operon inserted into the TRP1/ARS1 plasmid upstream of the TRP1 gene. The α2/Mcm1 operon, which is derived from the promoter of the α cell-specific STE6 gene, is sufficient to confer Tup1p-Ssn6p-
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Decreased acetylation of histones H3 and H4 in TALS plasmid chromatin in a cells. A, schematic representation of the TALS plasmid. The letters A–F indicate the DNA fragments (thin lines) generated by PCR to probe specific regions of the plasmid in chromatin immunoprecipitation experiments. Also indicated are the sites of the α2/Mcm1 operator, the autonomous replication sequence (ARS1), and the TRP1 gene. The filled circles represent nucleosomes that are positioned in a cells. B, top, slot blot data gathered from chromatin immunoprecipitation experiments examining acetylation of histone H3 at the region corresponding to TALS probe A. Immunoprecipitations were carried out using chromatin isolated from strains FY23T (a), FY24T (α), and FY24TΔ tup1 (αΔtup1), with antisera specific for unacetylated histone H3 (H3) or histone H3 acetylated at lysine 9 and/or 18 (acetyl H3). Quantitation of the slot blot data is shown as a bar graph below the blot. C, summary of data from chromatin immunoprecipitation experiments examining the acetylation of histone H3 using probes specific for all regions of the TALS plasmid (probes A–E) and chromatin from strain FY23 (a, III) or strain FY24 (α, □). The positions of the α2/Mcm1 operator and the TRP1 gene relative to probes A–F are indicated. D, top, slot blot data gathered from chromatin immunoprecipitation experiments examining the acetylation state of histone H4 in TALS chromatin at the region corresponding to probe A. Immunoprecipitations were carried out using chromatin isolated from strains FY23T (a), FY24T (α), and FY24TΔ tup1 (αΔtup1), with antisera specific for unacetylated histone H4 (H4) or acetylated histone H4. Quantitation of the slot blot data is shown as a bar graph below the blot.

mediated repression to downstream genes in α cells (40, 41). The chromatin structure of the TALS plasmid has been extensively mapped in a and α cells (41), and its structure and regulation are representative of natural a cell-specific genes (42).

Immunoprecipitations were performed using chromatin fragments isolated from a cells (condition of activation), α cells (condition of repression), or α cells bearing a disruption of the TUP1 gene (which abolishes repression in α cells). The immunoprecipitated DNA was probed with specific regions of the TALS plasmid (Fig. 1, probes A–F). Data obtained with probe A indicate that sequences adjacent to the α2/Mcm1 operator are enriched in H3 acetylated at lysines 9 and/or 18 in α cells relative to α cells (Fig. 1B). We consistently observe that acetylation of histones associated with this region of TALS is about three times higher in a cells than in α cells (4.5 versus 1.5, Fig. 1, B and C). The same trend and relative degree of acetylation change were observed in immunoprecipitations using an antiserum specific for H3 acetylated at lysine 9 and/or 14 (data not shown). Additionally, deletion of TUP1 leads to hyperacetylation of this region of TALS in α cells to an extent even greater than that observed in wild-type a cells (increase in histone acetylation relative to wild-type α cells = 7.3; 11 versus 1.5, Fig. 1B). A difference in acetylation of histone H3 between a and α cells is not observed at the ACT1 locus, a gene not subject to regulation by Tup1p (data not shown). This indicates that the changes we see at TALS do not simply reflect a global, cell type-specific difference in acetylation levels.

To determine the extent of the region of altered H3 acetylation relative to the location of the α2/Mcm1 operator (the point of Tup1p-Ssn6p recruitment), immunoprecipitated DNA was probed with sequences specific for other regions of the TALS plasmid (Fig. 1C). In α cells (Fig. 1C, □), the lowest levels of H3 acetylation are associated with sequences adjacent to either side of the α2/Mcm1 operator (probes A, F, and E), whereas increased acetylation is associated with more distal sequences (probes B, C, and D). Increased acetylation is observed for all probes (with the exception of probe E) in chromatin isolated from a cells, with the highest levels occurring in the region of the TRP1 gene (probes A–C). The largest changes in relative acetylation between a and α cells occur at regions of TALS directly adjacent to the α2/Mcm1 operator (probes A and F, Fig. 1C). We have also observed similar decreases in acetylation of histone H3 associated with sequences proximal to the α2/Mcm1 operator in the promoters of the endogenous STE6 and MFA2 genes (data not shown). Thus, Tup1p-Ssn6p-dependent repression of cell type-specific genes is associated with localized decreases in histone H3 acetylation.

Decreased acetylation of histone H4 has been observed concomitant with transcriptional repression mediated via other yeast repressor complexes (43, 44). Using chromatin immunoprecipitation, we examined the acetylation state of histone H4 in TALS chromatin purified from either a cells, α cells, or a cells containing a deletion of the TUP1 gene. Similar to the loss of H3 acetylation observed above, we detected a reproducible 2-fold decrease in acetylation of histone H4 in TALS chromatin (region A) from a cells as compared with a cells (Fig. 1D). Again, as observed for H3, this change in acetylation was contingent upon the TUP1 gene (Fig. 1D). These data suggest that recruitment of Tup1p-Ssn6p in a cells leads to localized decreases in both H3 and H4 acetylation levels.

SUC2 Exhibits Transcription-independent Changes in Acetylation of Histone H3—To further examine the link between Tup1p-Ssn6p repression and decreased histone acetylation, we examined a non-cell type-specific gene regulated by this corepressor. SUC2 encodes the enzyme invertase, which catalyzes the hydrolysis of sucrose and is repressed when yeast are grown in high concentrations of glucose (Fig. 2A, lanes 1 and 3; Refs. 45 and 46). Glucose repression of SUC2 is mediated by a sequence-specific DNA-binding protein, Mig1p, which recruits Tup1p and Ssn6p to the SUC2 promoter (47). SUC2 is expressed constitutively in strains lacking either TUP1 or SSN6, even when strains are grown under repressing conditions (Fig. 2A, lanes 5 and 6; Refs. 48–53).

Consistent with the results described above, decreased levels of H3 acetylation are associated with SUC2 repression. Chromatin immunoprecipitation experiments demonstrate that a fragment of the SUC2 promoter adjacent to the Mig1p binding site is associated with decreased acetylation of H3 (approximately 2-fold lower) under repressing conditions (high glucose) as compared with derepressing conditions (low glucose), coincident with activation of SUC2 (Fig. 2B).

As observed in TALS chromatin, the changes in acetylation that are observed at the SUC2 locus are localized to a region proximal to the site of Tup1p-Ssn6p recruitment. Use of a probe specific for a portion of the SUC2 coding sequence does not reveal a change in acetylation of H3 in chromatin more distal to
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Fig. 2. Decreased acetylation of SUC2 in glucose is dependent on TUP1. A, S1 analysis of SUC2 RNA levels in strains FY24 (lanes 1 and 3), FY716 (lanes 2 and 4), FY24Δtup1 (lane 5), or FY24Δssn6 (lane 6) grown under repressing conditions (2.0% glucose, lanes 1, 2, 5, and 6) or switched to derepressing conditions (0.05% glucose) for 2.75 h (lanes 3 and 4). The amount of SUC2 RNA detected relative to ACT1 RNA levels (ACT1 data are not shown) is shown below each lane. B, summary of data from chromatin immunoprecipitation experiments analyzing acetylation of histone H3 at the SUC2 promoter proximal to the Mig1p binding site. Chromatin was isolated from strains FY24 (WT), FY716 (ΔTATA), FY24Δtup1 (Δtup1), and FY24Δssn6 (Δssn6) grown under derepressing conditions (low glucose, ■) or repressing conditions (high glucose, □). C, summary of data from chromatin immunoprecipitation experiments analyzing acetylation of histone H3 at the SUC2 promoter distal to the Mig1p binding site. Chromatin was isolated from strains FY24 (WT) and FY716 (ΔTATA) grown under derepressing conditions (low glucose, ■) or repressing conditions (high glucose, □). Data presented in B and C were normalized against input DNA (% Input).

Histone acetylation is thought to alter gene expression by altering DNA-histone interactions within single nucleosomes and by altering nucleosome-nucleosome interactions involved in higher order chromatin packing. Histone acetylation is likely to affect interactions of non-histone regulatory proteins with chromatin as well, although such effects are understudied at present. Tup1p is one of only a few proteins determined to interact directly with the amino-terminal tails of histones H3 and H4. The changes in acetylation we observe might be due to an active increase in H3 acetylation upon transcription of SUC2, to an active decrease in acetylation upon recruitment of Tup1p-Ssn6p, or both. To distinguish between these possibilities, we examined H3 acetylation in a strain (FY716) containing a nontranscribed allele of SUC2, suc2-104, that contains a mutated TATA box (Fig. 2A, lanes 2 and 4; Ref. 25). We found that acetylation of H3 is still increased in the promoter region of this allele under derepressing conditions (Fig. 2B, ΔTATA). Indeed, the levels of H3 acetylation observed for this allele in both high and low glucose are nearly identical to those observed for a wild-type SUC2. Thus, the increased acetylation of H3 observed at the SUC2 promoter in low glucose is not dependent upon transcription per se.

To determine whether the decreased H3 acetylation observed upon SUC2 repression in high glucose is dependent on Tup1p and Ssn6p, we examined acetylation of H3 associated with the SUC2 promoter in strains lacking either TUP1 or SSN6. We found increased acetylation of H3 in this region in both of these mutant strains, even when strains were grown under repressing, high glucose conditions (Fig. 2B). Again, this increased acetylation is not caused by increased transcription because acetylation of the nontranscribed suc2-104 allele is also increased in the absence of Tup1p (data not shown).

Direct Recruitment of Ssn6p or Tup1p Is Sufficient to Induce Repression of a cell-specific Promoter and Decreased Acetylation of H3 and H4—The above-mentioned data raise the possibility that changes in histone acetylation may be directed by the corepressor complex. Alternatively, changes in acetylation might occur before Tup1p-Ssn6p recruitment, perhaps directed by other proteins required for repression, such as α2/Mcm1 or Mig1p. Others have shown that these recruiting proteins can be bypassed by the fusion of Ssn6p or Tup1p to the lexA DNA binding domain. Ssn6p-lexA and Tup1p-lexA fusion proteins can repress artificial promoters containing multiple lexA operators. Here we examined whether direct recruitment of such fusion proteins is sufficient to direct repression of a more natural α cell-specific promoter containing a single lexA operator, and, if so, whether decreased acetylation of H3 and H4 accompanies this repression.

Sequences corresponding to the lexA operator were inserted just upstream of the α2/Mcm1 operator in the promoter region of the endogenous MFA2 gene (see “Experimental Procedures”). This derivative (MFA2.1) is still susceptible to Tup1p-Ssn6p repression in α cells but is not repressed in a cells, as expected. Insertion of the lexA operator sequences therefore did not disrupt the natural function or regulation of this promoter.

To test the ability of Ssn6p or Tup1p to direct changes in the expression of MFA2.1, we expressed Ssn6p-lexA or Tup1p-lexA fusion proteins in α cells. As a control, the lexA DNA binding domain alone (lexA-DBD) was expressed separately in these same cells. The lexA DNA binding domain could not repress MFA2.1 under any conditions, as expected (Fig. 3B). However, Tup1p-lexA was able to confer efficient repression of this gene even in the absence of endogenous TUP1 or SSN6 (Fig. 3B). In contrast, Ssn6p-lexA could only partially direct repression of MFA2.1, and this repression required endogenous TUP1 but not SSN6 (Fig. 3B). These findings are consistent with previous reports by others, which indicate that repression by Ssn6p requires Tup1p, but Tup1p-mediated repression can occur independently of Ssn6p (6).

To determine whether direct recruitment of Tup1p is sufficient to induce decreased acetylation of H3 and H4, we again performed chromatin immunoprecipitations. Decreased acetylation of H3 and H4 associated with MFA2.1 promoter sequences was observed upon recruitment of Tup1p-lexA (Fig. 4, □) relative to recruitment of the lexA DNA binding domain alone (■), and this decreased acetylation did not require endogenous Tup1p. Direct recruitment of Ssn6p, however, was not sufficient to confer a stable decrease in H3 or H4 acetylation at the MFA2.1 promoter, consistent with the inefficient repression conferred by this fusion protein (data not shown). Taken together, these data demonstrate that direct recruitment of Tup1p is sufficient to induce decreased histone acetylation associated with repression of target promoters, furthering the link between repression and chromatin remodeling in vivo.

DISCUSSION

Histone acetylation is thought to alter gene expression by altering DNA-histone interactions within single nucleosomes and by altering nucleosome-nucleosome interactions involved in higher order chromatin packing. Histone acetylation is likely to affect interactions of non-histone regulatory proteins with chromatin as well, although such effects are understudied at present. Tup1p is one of only a few proteins determined to interact directly with the amino-terminal tails of histones H3 and H4.
disrupt Tup1p-histone interactions synergistically compromise Tup1p repression (17, 24). These synergistic effects support an important, if redundant, role for these histones in Tup1p functions in vitro. Acetylation of H3 and H4 appears to antagonize Tup1p functions. Tup1p binds most favorably to underacetylated forms of H3 and H4 in vitro, and loss of class I histone deacetylase activities that alter the acetylation state of these histones leads to loss of repression in vivo (17, 24, 54). Moreover, we show here that underacetylation of histones H3 and H4 accompanies Tup1p repression of both an a cell-specific gene and SUC2. We have observed a similar decrease in acetylation of these histones associated with repression of the DNA damage-inducible gene RNR3 by Tup1p-Ssn6p.3 Thus, at least three distinct classes of Tup1p-regulated genes exhibit a change in the acetylation state of associated histones. Our finding that direct recruitment of Tup1p leads to decreased histone acetylation of target promoters is also consistent with our recent discovery that the corepressor complex is associated with multiple histone deacetylase activities in vivo (54).

The differential acetylation we observe associated with the nontranscribed suc2-104 allele indicates that changes in acetylation of histones associated with Tup1p-regulated genes do not result from a change in transcription states but rather occur at an upstream step. The increased acetylation we observe under activating conditions might be directed by histone acetyltransferase activities that are recruited by transactivators that bind to the upstream activating sequences in the SUC2 promoter even in the absence of a functional TATA box. Even so, the Tup1p-Ssn6p-dependent decrease in histone acetylation that we observe under repressing conditions indicates that these activities are either limited in function by Tup1p-Ssn6p or counteracted by opposing histone deacetylase activities recruited by the corepressor complex. The underacetylated state might be further stabilized by Tup1p-histone interactions, which could limit reacetylation of the histone tails. Histone deacetylase recruitment and protection of the tails would account for our finding that changes in acetylation are greatest proximal to the site of Tup1p-Ssn6p recruitment.

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and H4. Others include Sir3p and Sir4p, which are thought to facilitate formation of heterochromatin-like structures associated with gene silencing in yeast. Proteins like Tup1p and the Sir proteins may be critical in establishing the architecture and functions of particular chromatin domains.

Mutations in the amino-terminal regions of H3 or H4 that

![Fig. 3. Transcriptional repression mediated by artificial recruitment of Tup1p or Ssn6p. A, schematic of the MFA2.1 reporter gene located at the endogenous MFA2 locus. The promoter region ( ), the MFA2 gene ( ), and the lacZ gene ( ) are shown. The positions of the lexA operator, the α2MCM1 operator, the TATA box, and the start of translation (+1) are indicated. The region corresponding to the PCR product amplified by specific primers for quantitation of MFA2.1 sequences in chromatin immunoprecipitation experiments is also depicted. Positions (in base pairs) are relative to the start of translation. B, summary of results obtained from β-galactosidase assays examining the effect of targeting either Tup1p (Tup1p-lexA) or Ssn6p (Ssn6p-lexA) to the MFA2.1 reporter gene. Results are expressed as a percentage of the β-galactosidase activity observed in MFA2.1 tup1 expressing only Tup1p-lexA. The genotype of the FY716 strain containing the suc2-104 allele indicates that changes in acetylation of histones associated with Tup1p-regulated genes do not result from a change in transcription states but rather occur at an upstream step. The increased acetylation we observe under activating conditions might be directed by histone acetyltransferase activities that are recruited by transactivators that bind to the upstream activating sequences in the SUC2 promoter even in the absence of a functional TATA box. Even so, the Tup1p-Ssn6p-dependent decrease in histone acetylation that we observe under repressing conditions indicates that these activities are either limited in function by Tup1p-Ssn6p or counteracted by opposing histone deacetylase activities recruited by the corepressor complex. The underacetylated state might be further stabilized by Tup1p-histone interactions, which could limit reacetylation of the histone tails. Histone deacetylase recruitment and protection of the tails would account for our finding that changes in acetylation are greatest proximal to the site of Tup1p-Ssn6p recruitment.

![Fig. 4. Decreased acetylation of the MFA2.1 gene upon targeting of Tup1p. Chart summarizing results of chromatin immunoprecipitation assays examining the acetylation of histone H3 (lysines 9 and 18) and histone H4 in a strain in which the endogenous Tup1p gene has been deleted. Results of PCR quantitation for a strain expressing only the lexA DNA binding domain ( ) were compared with those from a strain expressing a Tup1p-lexA fusion protein ( ). Experiments were repeated, and the results were averaged. All values are normalized to the amount of PCR product generated using DNA isolated from a fraction of the chromatin used in the immunoprecipitation.

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