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Repression by Ume6 Involves Recruitment of a Complex Containing Sin3 Corepressor and Rpd3 Histone Deacetylase to Target Promoters

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Summary

Sin3 and Rpd3 negatively regulate a diverse set of yeast genes. A mouse Sin3-related protein is a transcriptional corepressor, and a human Rpd3 homolog is a histone deacetylase. Here, we show that Sin3 and Rpd3 are specifically required for transcriptional repression by Ume6, a DNA-binding protein that regulates genes involved in meiosis. A short region of Ume6 is sufficient to repress transcription, and this repression domain mediates a two-hybrid and physical interaction with Sin3. Coimmunoprecipitation and two-hybrid experiments indicate that Sin3 and Rpd3 are associated in a complex distinct from TFIID and Pol II holoenzyme. Rpd3 is specifically required for repression by Sin3, and artificial recruitment of Rpd3 results in repression. These results suggest that repression by Ume6 involves recruitment of a Sin3–Rpd3 complex and targeted histone deacetylation.

Introduction

Understanding of the relationship between chromatin structure and gene expression in eukaryotic organisms has been dramatically enhanced by the identification of nucleosome-modifying activities such as the Swi/Snf and related complexes (Peterson, 1996), histone acetylases Gcn5 (Brownell et al., 1996), P/CAF (Yang et al., 1996b), p300/CBP (Bannister and Kouzarides, 1996; Ogryzko et al., 1996), TAF130/250 (Mizzen et al., 1996), and Rpd3 histone deacetylase (Taunton et al., 1996). Yeast cells that lack Swi/Snf, Gcn5, or Rpd3 are viable but have selective effects on gene expression. An attractive hypothesis is that such selective transcriptional effects are due to the targeting of chromatin-modifying activities to specific promoters. However, it is also possible that these nucleosome-modifying activities have untargeted, genome-wide effects that selectively affect a limited set of promoters with particular configurations of promoter elements (location and quality) and inherent chromatin structures (nucleosome positioning, stability, or density).

In this paper, we define a novel form of transcriptional repression that involves the yeast Sin3 and Rpd3 proteins. Sin3 and Rpd3 negatively regulate a diverse set of genes including those involved in meiosis, cell-type specificity, potassium transport, phosphate metabolism, methionine biosynthesis, and phospholipid metabolism (Vidal and Gaber, 1991; McKenzie et al., 1993; Jackson and Lopes, 1996; Stillman et al., 1994). Sin3 was initially inferred to be a corepressor from the observation that a LexA–Sin3 fusion protein can repress transcription when brought to a heterologous promoter (Wang and Stillman, 1993). Furthermore, a Sin3-related protein from mouse functions as a corepressor for repression by Mad and Mxi1, proteins that bind DNA as heterodimers with Max (Ayer et al., 1995; Schreiber-Agus et al., 1995). However, yeast DNA-binding proteins that specifically require Sin3 and Rpd3 for repression have yet to be identified.

Rpd3 is 60% identical in sequence to a human histone deacetylase (Taunton et al., 1996), and it is likely to possess this activity because *rpd3* mutant strains show increased acetylation of lysines 5 and 12 of histone H4 (Rundlett et al., 1996). Rpd3 is also homologous to a mouse protein that functions as a corepressor for the DNA-binding protein YY1 (Yang et al., 1996a) and a Drosophila protein that affects position-effect variegation (De Rubertis et al., 1996). As deacetylated histones are generally associated with transcriptionally inactive regions (Roth and Allis, 1996; Wolffe and Pruss, 1996), the histone deacetylase activity of Rpd3 is likely to be relevant for its repression function. However, the molecular relationship between Sin3 and Rpd3 and the mechanism by which Rpd3 mediates gene-specific repression are unknown.

Here, we identify a yeast DNA-binding protein, Ume6, that directs transcriptional repression in a manner dependent upon Sin3 and Rpd3. We show that a short region of Ume6 is sufficient to recruit the Sin3 corepressor to promoters, that Sin3 and Rpd3 are physically associated, that Rpd3 is specifically required for repression by the Sin3 corepressor, and that artificial targeting of Rpd3 to promoters inhibits transcription. These observations strongly suggest that repression by Ume6 occurs by targeted histone deacetylation.

Results

URS1 Elements Are Sufficient to Direct Sin3- and Rpd3-Dependent Repression

IME2, a key meiosis regulator, and *INO1*, a gene involved in phospholipid biosynthesis, are negatively regulated by Sin3 (Bowdish and Mitchell, 1993; Slekar and Henry, 1995), and their promoter regions both contain URS1, a GC-rich upstream repression sequence found at a number of yeast promoters. To determine whether URS1 is sufficient to direct repression in a Sin3- and Rpd3-dependent manner, we inserted fragments containing URS1 elements from the *INO1* and *IME2* promoters upstream of the intact *CYC1* promoter and *LacZ* structural gene, and analyzed transcription in wild-type, *sin3*, and *rpd3* strains (Figure 1A). In both cases, the URS1 fragments direct significant levels of repression (9- to 13-fold) in a wild-type strain, whereas repression is nearly abolished in a *sin3* deletion strain and is significantly reduced in a *rpd3* deletion strain. Thus, URS1 elements are sufficient to direct Sin3- and Rpd3-dependent repression from a heterologous promoter.

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A

Strain	UAS LacZ	UAS LacZ	UAS LacZ
WT	103	11	8
Δ*rpd3*	101	36	24
Δ*sin3*	95	56	44
Δ*ume6*	36	45	60

B

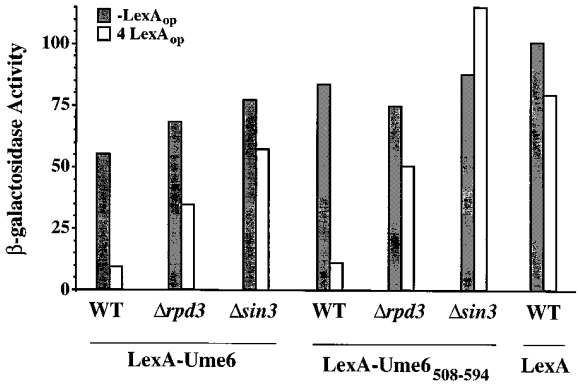

Figure 1. Repression by URS1 Elements and LexA–Ume6 Requires Sin3 and Rpd3

(A) β-galactosidase activities of wild-type and the indicated deletion strains harboring *CYC1* promoter derivatives that do (arrows) or do not contain one copy of the *INO1* oligonucleotide (middle) or two copies of the *IME2* oligonucleotide (right).

(B) β-galactosidase activities of wild-type or the indicated deletion strains expressing LexA–Ume6, LexA–Ume6$_{508-594}$, or LexA. Fold-repression represents the ratio of β-galactosidase activities in strains containing plasmids that either lack (shaded bars) or contain (open bars) 4 LexA operators upstream of the *CYC1* promoter.

Ume6, a DNA-Binding Protein, Directs Repression Mediated by Sin3 and Rpd3

The above results indicate that a URS1-binding protein(s) can repress transcription in a manner dependent on Sin3 and Rpd3. Ume6 was a good candidate for such a DNA-binding protein, because it is required for repression via URS1 in the *IME2*, *CAR1*, and *INO1* promoters (Park et al., 1992; Bowdish and Mitchell, 1993; Jackson and Lopes, 1996), and it binds the URS1 site in the promoter of *SPO13*, another gene subject to Sin3 control (Strich et al., 1994). In accord with these results, repression directed by the isolated URS1 elements is completely abolished in a *ume6* deletion strain (Figure 1A).

We therefore tested whether a LexA–Ume6 fusion protein could repress transcription when artificially recruited to a heterologous promoter (Figure 1B). Indeed, LexA–Ume6 represses transcription by a factor of 6, and this repression is nearly abolished in a *sin3* deletion strain and is significantly reduced in an *rpd3* deletion background. Although LexA–Ume6 can function as a transcriptional activator in cells undergoing meiosis (Bowdish et al., 1995), removal of Sin3 or Rpd3 does not convert LexA–Ume6 from a repressor to an activator (data not shown). Thus, other gene products, such as *IME1* (Bowdish et al., 1995), are required for Ume6 to function as a meiosis-specific transcriptional activator. These results suggest that recruitment of Ume6 to the promoter is sufficient to direct repression in a Sin3- and Rpd3-dependent manner.

Table 1. Localization of the Ume6 Repression Domain

LexA Hybrid Protein	Promoter: 4 LexA$_{op}$	Promoter: −LexA$_{op}$	Fold Repression
Ume6 (1–836)	7	49	7.0
Ume6 (118–836)	9	52	5.8
Ume6 (160–836)	7	49	7.0
Ume6 (508–836)	7	59	8.4
Ume6 (653–836)	72	65	0.9
Ume6 (508–802)	54	79	1.5
Ume6 (508–744)	41	82	2.0
Ume6 (508–651)	50	78	1.6
Ume6 (508–594)	11	73	6.6
Ume6 (1–509, 596–836)	31	56	1.8
LexA	54	69	1.3

β-galactosidase activities of wild-type strains containing the indicated promoters and expressing the indicated proteins (1–836 aa = full length). Fold repression represents the ratio of β-galactosidase activities in strains containing plasmids that either lack or contain four LexA operators upstream of the *CYC1* promoter fused to the *LacZ* structural gene.

A Short Domain of Ume6 That Mediates Transcriptional Repression

The repression function of Ume6 was localized by a deletion analysis of LexA–Ume6 (Table 1). Deletions that removed as many as 507 N-terminal residues behave indistinguishably from the full-length protein, whereas removal of 652 N-terminal residues abolishes repression. Three C-terminal deletions of LexA–Ume6$_{508-836}$ confer little or no repression, but the most extensively deleted derivative (LexA–Ume6$_{508-594}$) directs repression at wild-type levels. Conversely, a LexA–Ume6 derivative lacking residues 510–595 is severely compromised for repression. Thus, an 87–amino acid region (residues 508–594) is necessary and sufficient to mediate the repression function of Ume6; hence, it defines a minimal repression domain. This minimal repression domain shows no significant similarity to the repression domain of Mad. As expected, repression mediated by the minimal Ume6 domain is nearly abolished in a *sin3* deletion strain and is significantly reduced in an *rpd3* deletion strain (Figure 1B), even though the protein is stably expressed in these strains (data not shown). Thus, Sin3 and Rpd3 histone deacetylase are required for the function of the Ume6 repression domain.

Interaction between Sin3 and Ume6

The functional relationship between Ume6 and Sin3 suggested that these proteins might interact with one another. In support of this hypothesis, we detect a two-hybrid interaction between Sin3–VP16 and either LexA–Ume6 or (more strongly) LexA–Ume6$_{508-594}$ (minimal repression domain)(Figure 2). In contrast, the Ume6 derivative lacking the minimal repression domain (Δ510–595) interacts very poorly with Sin3–VP16. Interestingly, the two-hybrid interaction between Sin3 and the repression domain of Ume6 (and full-length Ume6) is enhanced

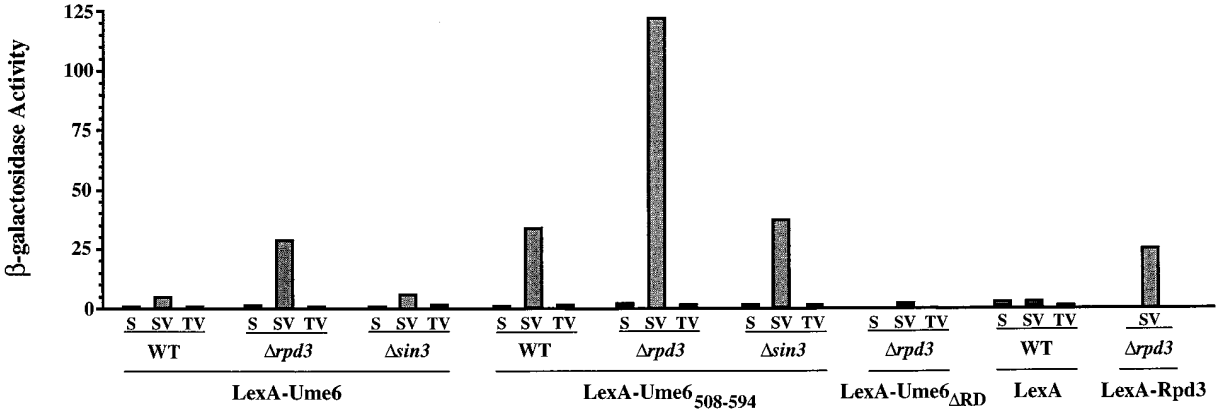

Figure 2. Two-Hybrid Interactions

β-galactosidase activities of wild-type or the indicated deletion strains expressing the indicated LexA, VP16, and control derivatives (S, Sin3; SV, Sin3–VP16; TV, Tup1–VP16); the *LacZ* reporter contains 4 LexA operators upstream of the *GAL1* TATA element. ΔRD indicates the LexA–Ume6 derivative lacking residues 510–595. LexA–Ume6$_{508-594}$ and LexA–Rpd3 do not show two-hybrid interactions with any of approximately 1000 activation domain protein fusions from a library, and Sin3–VP16 does not show two-hybrid interactions with LexA–Tup1, LexA–Cyc8, or LexA–Acr1.

4-fold in an *rpd3* deletion strain. The most likely explanation for this effect is that Sin3–VP16 carries both repression and activation functions and that loss of Rpd3 alleviates the Sin3 transcriptional repression function (see below). However, we can not exclude the possibility that Rpd3 might partially interfere with the interaction between Ume6 and Sin3. By either explanation, Rpd3 is not required for the association of Ume6 and Sin3.

Biochemical confirmation of the Ume6–Sin3 two-hybrid interaction was obtained by affinity chromatography (Figure 3A). Cell-free extracts from strains expressing an epitope (HA-1)-tagged derivative of Sin3 were incubated with beads coupled to glutathione S-transferase (GST) or GST–Ume6$_{508-594}$. Sin3–HA associates strongly with GST–Ume6$_{508-594}$, but not with the GST control. Thus, the Ume6 repression domain interacts with Sin3 in vivo and in vitro, strongly suggesting that repression by Ume6 requires recruitment of the Sin3 corepressor.

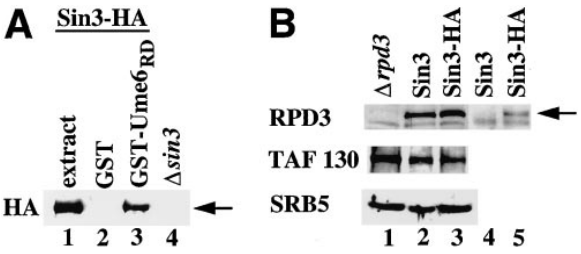

Figure 3. Interaction of Sin3 with Ume6 and Rpd3

(A) Extract from a strain expressing HA-tagged Sin3 (lane 1) was incubated with beads containing GST (lane 2) or GST–Ume6$_{RD}$ (RD = residues 508–594) (lane 3), and the bound proteins analyzed by Western blotting using the HA antibody. Lane 4 is a control extract from a *sin3* deletion strain. The band corresponding to HA-Sin3 is indicated by an arrow.

(B) Extracts from strains expressing Sin3 or HA-tagged Sin3 (lanes 2 and 3) were immunoprecipitated with antibodies to the HA-1 epitope (lanes 4 and 5). The resulting samples were probed with antibodies to Rpd3, TAF130, and Srb5. Lane 1 represents an extract from a control *rpd3* deletion strain. Shown are immunoprecipitations performed at 500 mM (for analysis of Rpd3) or 125 mM (for analysis of TAF130 and Srb5) potassium acetate. The band corresponding to Rpd3 is indicated by an arrow; the band (below Rpd3) observed in lanes 4 and 5 corresponds to the antibody heavy chain that is present in all immunoprecipitations.

Physical Association of Sin3 and Rpd3

The functional relationship between Sin3 and Rpd3 as well as the effect of Rpd3 on the Ume6–Sin3 two-hybrid interaction suggested that Sin3 and Rpd3 might be physically associated. To test this hypothesis, we prepared cell-free extracts from a strain expressing Sin3–HA. Using antibodies to the HA-1 epitope, Rpd3 coimmunoprecipitates with the tagged Sin3 derivative under stringent conditions (500 mM potassium acetate, 1% NP-40) for protein–protein interactions (Figure 3B). This association is specific; components of Pol II holoenzyme (Srb5) and TFIID (TAF130) are not coprecipitated with Sin3, even under less stringent conditions, and Rpd3 is not precipitated from comparable extracts made from a strain expressing nontagged Sin3. As an independent line of evidence, Sin3 and Rpd3 interact strongly in a two-hybrid assay (Figure 2). Taken together, these results indicate that Sin3 and Rpd3 are physically associated in a complex that is distinct from large complexes that comprise the basic Pol II transcription machinery.

Rpd3 Is Specifically Required for Transcriptional Repression by Sin3

In accord with the view that Sin3 is a corepressor that is recruited to promoters by Ume6, artificial recruitment of Sin3 to a heterologous promoter results in repression (Wang and Stillman, 1993). Interestingly, repression by LexA–Sin3 is significantly reduced in an *rpd3* deletion strain (Figure 4). This loss of repression is not due to protein instability because LexA–Sin3 is expressed comparably in wild-type and *rpd3* deletion strains (data not shown). To determine the specificity of Rpd3, we examined whether Rpd3 was required for repression mediated by the Cyc8–Tup1 complex (Keleher et al., 1992;

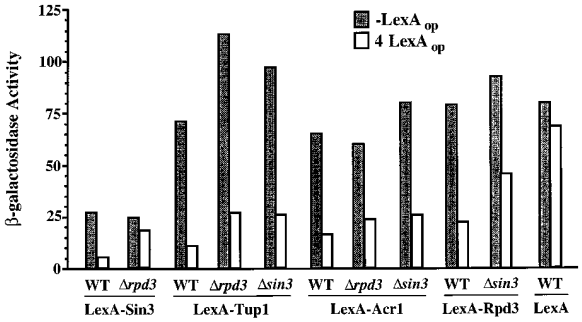

Figure 4. Rpd3 Is Specifically Required for Sin3-Dependent Repression

β-galactosidase activities of wild-type or the indicated deletion strains expressing the indicated LexA proteins. Fold-repression represents the ratio of β-galactosidase activities in strains containing plasmids that either lack (shaded bars) or contain (open bars) 4 LexA operators upstream of the *CYC1* promoter.

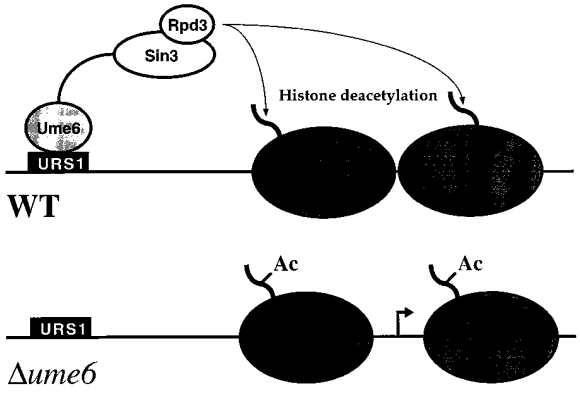

Figure 5. Model for Repression by Ume6

In a wild-type strain (top), Ume6 binds to URS1 and recruits a complex containing Sin3 and Rpd3 histone deacetylase to the promoter. As a consequence, nucleosomes (dark gray ovals) in the vicinity of the promoter have deacetylated histone tails (wavy line), which leads to an "inactive" chromatin structure (depicted as nucleosomes close together) and inhibition of transcription (lack of arrow). In a strain lacking Ume6, URS1 is unoccupied and nucleosomes have acetylated histone tails (Ac), which leads to an "active" chromatin structure (depicted as nucleosomes further apart) and transcription (arrow). Other proteins that might be present in the Sin3–Rpd3 complex or that might be required for the (direct or indirect) interaction between Ume6 and Sin3 are not indicated. Aside from the acetylation state of the histone tails, the model does not specify the molecular nature of inactive and active chromatin structures or the step at which transcriptional repression occurs.

Tzamarias and Struhl, 1994) and by Acr1, an ATF/CREB repressor (Nehlin et al., 1992; Vincent and Struhl, 1992). Unlike the case for LexA–Sin3 or LexA–Ume6, repression by LexA–Tup1 or LexA–Acr1 occurs normally in *rpd3* and *sin3* deletion strains (Figure 4). Conversely, in a *tup1* deletion strain, repression by LexA–Ume6, LexA–Sin3, and LexA–Acr1 occurs normally, whereas repression by LexA–Cyc8 is nearly abolished (data not shown). Finally, LexA–Sin3 differs from LexA–Cyc8 in that repression is unaffected by Srb10 kinase, a component of the Pol II holoenzyme (data not shown). Thus, Rpd3 is required for repression by Sin3, but not for repression by Cyc8–Tup1 or Acr1.

Artificial Recruitment of Rpd3 Histone Deacetylase Represses Transcription

The observations that Rpd3 is required for Sin3-dependent repression and that Rpd3 and Sin3 are physically associated suggested that tethering Rpd3 to a heterologous promoter might inhibit transcription. Because LexA fusions to the N-terminus of Rpd3 interfered with Rpd3 function (assayed by complementation), we fused LexA to the C-terminus. LexA–Rpd3 represses transcription by a factor of 4, and this repression also occurs in a *sin3* deletion strain, albeit with somewhat reduced efficiency (Figure 4). This observation suggests that Sin3 is not absolutely required for repression by Rpd3. The apparent minor role of Sin3 might reflect an inherent repression function and/or an effect on the activity of the associated Rpd3.

Discussion

Ume6 Is a Natural Target of the Sin3 Corepressor

In this paper, we show that Ume6, a URS1-binding protein that negatively regulates genes involved in meiosis and arginine catabolism, is a natural target of the Sin3 corepressor. Ume6 is sufficient to direct Sin3- and Rpd3-dependent repression of a heterologous promoter to an extent comparable to that observed with natural promoters. This requirement for Sin3 and Rpd3 is specific to repression by Ume6; these proteins are not required for repression by Acr1 or Tup1 in the same experimental context. Moreover, a short region of Ume6 is sufficient for repression of a heterologous promoter and for a two-hybrid and physical interaction with Sin3. In fact, fusion of the VP16 activation domain to Sin3 converts Ume6 from a repressor into an activator. These results strongly suggest that Ume6 represses transcription by recruiting the Sin3 corepressor to native yeast promoters. Although recruitment of Sin3 by Ume6 does not require Rpd3, our experiments do not establish whether the interaction between Ume6 and Sin3 is direct or involves another protein(s).

Evidence That Ume6-Dependent Repression Is Mediated by Targeted Histone Deacetylation

Our results strongly support a model in which Ume6, bound at URS1, recruits a Sin3–Rpd3 corepressor complex that mediates transcriptional repression through Rpd3 histone deacetylase (Figure 5). First, repression by Ume6 or URS1 requires Sin3 and Rpd3, and the Ume6 transcriptional repression domain corresponds with the Sin3-interaction domain. Second, coimmunoprecipitation and two-hybrid experiments indicate that Sin3 and Rpd3 are physically associated. Consistent with this observation, complexes containing Sin3, Rpd3, and other proteins have been identified in yeast (David Stillman, personal communication) and mammals (Hassig et al., 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997 [all in this issue of *Cell*]). Third, artificial recruitment of Rpd3 histone deacetylase is sufficient to

mediate repression; such repression can occur in the absence of Sin3, although with reduced efficiency. Fourth, Rpd3 is specifically required for Ume6- and Sin3-dependent repression; Rpd3 does not affect repression by Tup1 or Acr1 even under comparable experimental conditions. This last observation strongly argues that Rpd3 does not mediate repression except under circumstances where it is targeted to promoters via its association with Sin3, which in turn is recruited by Ume6.

Taken together, our results provide strong evidence that repression by Ume6 occurs by targeted histone deacetylation. Decreased histone acetylation is generally correlated with more inaccessible chromatin and decreased transcriptional activity (Roth and Allis, 1996; Wolffe and Pruss, 1996). Thus, the simplest repression mechanism that can be inferred is that targeting of Rpd3 to specific promoters results in local histone deacetylation, thereby directly causing a repressive chromatin structure in the vicinity of the promoter. Such locally repressed chromatin could inhibit the accessibility of activators, TFIID, or Pol II holoenzyme to promoters and/or block the communication between these components of the transcription machinery. However, we can not exclude models in which Rpd3-dependent deacetylation is a signal for interaction with nonnucleosomal repressor proteins or in which Rpd3 mediates its effects in a manner that does not involve histone deacetylation.

In considering the individual roles of Sin3 and Rpd3, the simplest view is that Sin3 acts exclusively to mediate recruitment whereas Rpd3 histone deacetylase mediates repression per se. However, our results do not exclude the possibility that Sin3 may contribute to repression by a separate, although quantitatively minor, mechanism. Although Rpd3 is required for Sin3-dependent repression, we have consistently found that *sin3* deletion strains are slightly more impaired than *rpd3* deletion strains in mediating repression by URS1 and LexA–Ume6. This residual Rpd3-independent repression by Sin3 might be explained by the presence of four Rpd3-related proteins in yeast that may partially substitute for Rpd3 function; one of these proteins, Hda1, is a member of a histone deacetylase complex distinct from that of Rpd3 (Rundlett et al., 1996). Alternatively, Sin3 might utilize an additional mechanism that does not involve histone deacetylation; such a mechanism might account for the reduced repression of LexA–Rpd3 in a *sin3* deletion strain, although other explanations are equally likely.

Concluding Comments

In the past year, tantalizing links between transcription factors and chromatin-modifying activities have been increasingly identified, but the molecular mechanisms remain to be elucidated (Roth and Allis, 1996; Struhl, 1996; Wolffe and Pruss, 1996). Some nucleosome-modifying activities such as the Swi/Snf complex and Gcn5 histone acetylase have gene-specific effects in vivo, but it is unclear if these arise from promoter targeting or promoter-specific responses to genome-wide activity. The histone acetylase activity of TAF130/250, is likely to be targeted to all promoters, and the same may be true for Swi/Snf, which appears to be a component of the Pol II holoenzyme (Wilson et al., 1996); such activities might contribute to the general correlation between an active chromatin structure and gene transcription. The p300/CBP histone acetylase interacts with a wide variety of DNA-binding proteins (Janknecht and Hunter, 1996); hence it, and the associated histone acetylase p/CAF (Yang et al., 1996b), may be recruited to a subset of promoters. However, the promoter specificity of p300/CBP function remains to be clarified, particularly in light of evidence that p300/CBP may be associated with large RNA Pol II complexes (Danny Reinberg, personal communication). Our results, in combination with those obtained in mammalian cells (Hassig et al., 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997), provide strong evidence that Sin3–Rpd3 is an evolutionarily conserved corepressor complex that represses transcription of specific genes by targeting a chromatin-modifying activity to promoters.

Experimental Procedures

Strains and DNAs

All yeast strains were derived from FT5 (α *ura3*-52 *trp1*-Δ63 *his3*-Δ200 *leu2::PET56*) (Tzamarias and Struhl, 1994). The Δ*sin3::HIS3* allele lacks Sin3 residues 498–1204, the Δ*rpd3::HIS3* allele lacks Rpd3 residues 34–302, and the Δ*ume6::LEU2* allele lacks residues 147–159. The parental *LacZ* reporter plasmid used to assay transcriptional repression, pLGΔ312S, contains a *CYC1* fragment (−324 to +141) that includes the two UASs and TATA region (Guarente and Mason, 1983); URS1 elements (underlined) from the *IME2* (two copies of ATCCGTTGTCCAATAATTTATGTTACGGCGGCTATTTGAG) or *INO1* (one copy of ATCCATGCGGAGGCCAAGTATGCGCTTCGGCGGCTAAATGCGG) were cloned into the *Sma*I site immediately upstream of the *CYC1* UASs. The plasmid expressing LexA–Ume6 was obtained by cloning a SmaI-NcoI fragment containing the *UME6* coding region in the YCp91–LexA fusion vector (Tzamarias and Struhl, 1994). Deleted derivatives were generated using naturally occurring restriction sites. LexA(1–87)–Sin3, LexA(1–202)–Rpd3 (C-terminal fusion), and Rpd3 were expressed from the *ADH1* promoter in the multicopy *TRP1* plasmid YEplac112. Sin3 (and derivatives containing the VP16 activation domain or three copies of the HA-1 epitope at the C-terminus) were expressed similarly from the multicopy *LEU2* plasmid YEplac181. Constructs expressing LexA–Cyc8, LexA–Tup1, and Tup1-VP16 have been previously described (Tzamarias and Struhl, 1994). Details of strain and DNA constructions are available upon request.

Transcriptional Repression and Two-Hybrid Assays

The *LacZ* reporter constructs used in LexA-dependent repression assays contain either 4 (JK1621) or no (pLGΔ312S) LexA operator sites upstream of the intact *CYC1* promoter (Keleher et al., 1992). The *LacZ* reporter plasmid for two-hybrid assays was JK103, a multicopy *URA3* plasmid containing four LexA operators upstream of the *GAL1* TATA element (Kamens et al., 1990). Cells were harvested in early log phase ($A_{600} \approx 0.4$), permeabilized with chloroform and SDS, and assayed for β-galactosidase activity. Values are normalized to A_{600} and represent the average of at least six independent transformants; they are accurate to ±10%.

Biochemical Experiments

Whole-cell protein extracts were prepared from *rpd3* or *sin3* deletion strains expressing Sin3 or HA1-tagged Sin3 by lysing cells with glass beads in buffer containing 20 mM HEPES (pH 7.9), 150 mM NaCl, 10 mM $MgCl_2$, 10% glycerol. For each immunoprecipitation, 200 μg of extract was precleared by incubation for 1 hr at 4°C with 25 μl protein A–sepharose beads in buffer A (20 mM HEPES [pH 7.6], 1 mM DTT, 1 mM EDTA, 0.125 M KAc, 20% glycerol, 1% NP-40). The extracts were then incubated overnight at 4°C with 12.5 μl of HA-coupled protein A–sepharose beads in buffer A and washed five times with 1 ml of buffer A (moderate stringency) or once with

1 ml buffer A, three times with 1 ml buffer A (0.5 M KAc), and once again with 1 ml buffer A (high stringency). Finally, the beads were resuspended in Laemmli buffer and boiled to prepare samples for Western analysis. Immunoblots were probed with rabbit polyclonal antibodies to RPD3 (1:2000), SRB5 (1:1000), and TAF130 (1:1500) and secondary AP- or HRP-conjugated anti-rabbit IgG.

Affinity chromatography experiments were performed using glutathione beads coupled to GST–Ume6(508–594) or GST only. Extract (140 μg) from a strain expressing HA-1-tagged Sin3 was incubated with the beads overnight at 4°C and washed in buffer A (containing 25 mM potassium acetate and lacking NP-40). Samples were processed as described above except that immunoblots were probed with mouse antibody to HA-1 (1:20) followed by incubation with a biotinylated anti-mouse secondary antibody and streptavidin-conjugated HRP.

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