Ume1p Represses Meiotic Gene Transcription in *Saccharomyces cerevisiae* through Interaction with the Histone Deacetylase Rpd3p

Received for publication, August 5, 2003, and in revised form, September 2, 2003 Published, JBC Papers in Press, September 2, 2003 DOI 10.1074/jbc.M308632200

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Ume1p is a member of a conserved protein family including RbAp48 that associates with histone deacetylases. Consistent with this finding, Ume1p is required for the full repression of a subset of meiotic genes during vegetative growth in budding yeast. In addition to mitotic cell division, this report describes a new role for Ume1p in meiotic gene repression in precommitment sporulating cultures returning to vegetative growth. However, Ume1p is not required to re-establish repression as part of the meiotic transient transcription program. Mutational analysis revealed that two conserved domains (NEX box and a WD repeat motif) are required for Ume1p-dependent repression. Co-immunoprecipitation studies revealed that both the NEX box and the WD repeat motif are essential for normal Rpd3p binding. Finally, Ume1p-Rpd3p association is dependent on the global co-repressor Sin3p. Moreover, this activity was localized to one of the four paired amphipathic-helix domains of Sin3p shown previously to be required for transcriptional repression. These findings support a model that Ume1p binding to Rpd3p is required for its repression activity. In addition, these results suggest that Rpd3p-Ume1p-Sin3p comprises an interdependent complex required for mediating transcriptional repression.

In the budding yeast *Saccharomyces cerevisiae*, the induction of meiosis is controlled by a combination of cell type and environmental cues. Specifically, only strains heterozygous at the MAT locus that are deprived of nitrogen and a fermentable carbon source will initiate meiotic development (for a review, see Refs. 1 and 2). Many genes required for meiosis are expressed during vegetative growth but exhibit a precise pattern of mRNA accumulation and decline during specific junctures in this process (3, 4). Six genes were identified (UME1–6) that are required for the vegetative repression of several genes normally transcribed early in meiosis (e.g. SPO11, SPO13) (5, 6). Subsequent studies revealed that these factors can be divided into two repression systems. One system is composed of a cyclin-cyclin-dependent kinase (Ume6p/Srb11p-Ume5p/Srb10p) (7–10) that co-localizes with the RNA polymerase II holoenzyme mediator complex (10).

The second repression system operates through the URS1 promoter element found in several early meiotic genes (11–14). Ume6p binds URS1 directly (15) and represses early meiotic gene expression through recruitment of the histone deacetylase (HDAC)1 Rpd3p (16) and the Isw2 chromatin remodeling complex (17, 18). Chromatin immunoprecipitation studies have confirmed the role of histone deacetylation in Ume6p-dependent repression (19, 20). Recruitment of Rpd3p by Ume6p is mediated through the Ume4p/Sin3p co-repressor (16). Sin3p contains four paired amphipathic helix (PAH) domains that direct protein-protein interactions (21). Deletion analysis found that of these domains, only PAH3 was required for repression of many mitotic genes (22). A more recent study found that PAH2 directs Ume6p binding (23), indicating that these domains are performing independent, but coordinated, functions. However, Ume6p does not regulate many genes that are controlled by Rpd3p or Isw2 (17, 18). Therefore, the early meiotic genes represent a select subset of loci that are controlled by all six of the *UME* factors.

As indicated above, Rpd3p, Ume6p, and Isw2 activities have been studied in some detail. However, little is known about the role of the co-repressor Ume1p in controlling meiotic gene expression. Ume1p binds Rpd3p in vivo (24), suggesting that it plays a role in chromatin deacetylation. However, the functional significance of this association has not been determined. The present study analyzes the requirement of Ume1p for meiotic gene repression at different stages of the yeast life cycle. In addition to mitotic cell division, Ume1p is also necessary for the rapid repression of precommitment meiotic cells returning to growth. Structure/function analysis revealed that two conserved domains shared between Ume1p and the human HDAC-binding protein p48 are required for repression and Rpd3p binding in vivo. The stable association between Rpd3p and Ume1p requires PAH3 of the Sin3p co-repressor. These studies provide a functional role for the conserved domains in the p48-Ume1p protein family. In addition, our findings suggest that Ume1p-Rpd3p-Sin3p comprise an interdependent complex that mediates transcriptional repression.

**EXPERIMENTAL PROCEDURES**

*Strains and Media*—The strains used in this study are listed in Table I. All growth and sporulation procedures have been described (25). Meiotic progression was followed by staining samples with the DNA-specific stain 4',6-diamidino-2-phenylindole as described (26). The completion of meiosis I and meiosis II was determined by the appearance of binucleated and tetranucleated cells, respectively.

*Plasmids and S1 Protection Probes*—The plasmid containing the spo13-lacZ reporter gene (p*spo1328*) has been described elsewhere.

† This work was supported in part by Grant MCB-9513479 from the National Science Foundation and GM57842 from the National Institutes of Health (to R. S.), Grant CA-08927 from the National Cancer Institute, and an appropriation from the Commonwealth of Pennsylvania. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: HDAC, histone deacetylase; PAH, paired amphipathic helix; HA, hemagglutinin; ARE, auxiliary repression element.
Characterization of UME1

Table I

| Strain | Genotype |
|--------|----------|
| RSY10  | MATa ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 |
| RSY287 | MATa ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-3 sin3::HIS3 |
| MMY1   | MATaMATa cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z leu2::HisG/LEU2 lys2::Lys2 trp1::HisG/TPR1 ura3/ -
|        | ar3-3 ade6-1-UME1 |
| MMY2–2A| MATa cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z leu2::HisG/LEU2 lys2::Lys2 trp1::HisG/TPR1 ura3/ - |
| RSY33  | MATaMATa cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z leu2::HisG/LEU2 trp1::HisG/TPR1 ura3/ - |
| RSY35  | MATaMATa cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z cyt2-zyh2-z leu2::HisG/LEU2 trp1::HisG/TPR1 ura3/ - |
| RSY81  | MATa ade6 can1-100 his4-C leu2-3,112 trp1-1 ura3-1 |
|        | Ref. 5 |

RESULTS

Isolation of UME1—A DNA fragment containing the coding region of UME1 was isolated by complementing the ura1-1 mutant phenotype (see "Experimental Procedures" for details). The nucleotide sequence of the minimum complementary fragment (pMM112) was determined. Analysis of the DNA sequence revealed a single large open reading frame encoding a 460-amino-acid protein (Ume1p) of a predicted molecular mass of 51 kDa. Three pieces of evidence indicate that the authentic UME1 gene was isolated. A strain carrying a deletion for the only large open reading frame in this clone was mated to a ura1-1 strain containing a spo13-lacZ reporter ρspo13128. The resulting diploid strain still displayed the merc1 phenotype as determined by the aberrant mitotic expression of the reporter (i.e. the recessive merc1-1 allele was not complemented by the null strain). This diploid was then sporulated and subjected to tetrad analysis. Of 10 tetrads analyzed, all of the meiotic products still exhibited aberrant mitotic spo13-lacZ β-galactosidase activity, indicating that no recombination between merc1-1 and the deletion allele occurred. Finally, if the UME1 gene was isolated, then the merc1-1 locus should carry a mutation. To address this issue, the merc1-1 allele was isolated and subjected to DNA sequence analysis (see “Experimental Procedures”). Sequence analysis of three independent isolates from RSY81 revealed an identical nucleotide deletion at position +1431, which resulted in a frameshift and stop codon one residue later at amino acid 215. The combined genetic and molecular results demonstrate that the UME1 gene was isolated.

Ume1p Represses SPO13 Transcription in Cultures Utilizing Fermentable or Non-fermentable Carbon Sources—To further
analyze the role of UME1 in meiotic gene expression, a null allele was constructed (ume1–2, see “Experimental Procedures” for details). Viable spores harboring this allele were recovered, indicating that UME1 is dispensable for vegetative growth. Moreover, growth curves generated from wild-type and ume1–2 cultures revealed no difference in generation times in medium containing either a fermentable or a non-fermentable carbon source (data not shown). To determine the effects on SPO13 expression in a null versus a ume1-1 strain, S1 protection assays were performed on isogenic UME1 and ume1–2 strains. These experiments revealed an elevated level of SPO13 mRNA in mutant vegetative cultures as compared with wild type (Fig. 1A). Quantitation of these experiments revealed a 6-fold increase in SPO13 mRNA levels, a value observed previously for the ume1-1 allele (5). These results suggest that truncation of Ume1p past amino acid 215 destroys the activity of this factor (see below). The derepression associated with loss of Ume1p activity is ~20-fold below the fully induced meiotic levels (Fig. 1A, mei lane), indicating that additional UME repression activity is still active.

A previous report found that UME2 and UME5 repress SPO13 expression in the presence of glucose but not in acetate medium (8). To investigate the role of glucose in UME1 activity, we measured SPO13 expression in wild-type and ume1 mutant strains growing in medium containing glucose or acetate as the carbon source. At least three independent UME1 (RSY333) and ume1–2 (MMY2-2A) cultures harboring a spo13-lacZ reporter gene (pspo13lacz28) were grown to mid-log phase in either synthetic dextrose or synthetic acetate medium. The cells were harvested, and β-galactosidase activities from the spo13-lacZ reporter gene were measured (see “Experimental Procedures” for details). The UME1 strain exhibited a 3-fold increase in β-galactosidase activity when the cultures were grown in acetate medium as compared with glucose (Fig. 1B). In glucose medium, the ume1–2 mutants exhibited an approximate 4-fold increase in spo13-lacZ expression as compared with wild type. A similar difference in β-galactosidase activity was observed between the mutant and wild-type strains with acetate as the carbon source. These results indicate that, unlike Ume2p and Ume5p, Ume1p still actively represses SPO13 in non-glucose medium.

Ume1p Is Not Required to Re-establish Repression of Early Meiotic Genes—Previous studies have found that sin3 (5), rpd3 (33), and ume6 (15) mutants all arrest prior to meiosis I, whereas ume3/srb11 mutants exhibit more subtle meiotic phenotypes (34). To investigate the role of UME1 in meiosis, both molecular and cytological parameters were examined in homozygous ume1–2/ume1–2 (MMY2) and heterozygous UME1/ume1–2 (MMY1) diploids induced to undergo synchronous meiotic divisions. No significant differences were observed in the timing of either the first or the second meiotic divisions as determined by the appearance of bi- and tetranucleated cells during the meiotic time course (data not shown). MMY2 produced ascii at a rate similar to wild type although spore viability was slightly reduced from 95% in the wild type as compared with 81% for the mutant. These results suggest that Ume1p may play a modest role in spore viability.

To evaluate the impact of Ume1p on early meiotic gene expression, the levels of SPO13 mRNA were monitored during a meiotic time course experiment. Samples were taken prior to the shift to sporulation medium (0 h) and continued throughout meiosis and spore formation. SPO13 mRNA levels were determined from total RNA preparations using S1 nuclease protection assays as described in Fig. 1. These S1 protection assays were not exposed to film sufficiently to detect the aberrant vegetative SPO13 transcripts as these experiments were intended to observe the mRNA accumulation kinetics during meiosis. These experiments found that SPO13 expression patterns were similar between the wild type and mutant (Fig. 2A). A small, but reproducible, elevation in SPO13 mRNA levels was observed in the mutant as compared with wild type in early time points (e.g. 3 h). These results suggest that SPO13 induction occurs more rapidly in the ume1 mutant. However, no differences were observed in the kinetics of SPO13 re-repression (9–24 h). These results suggest that the repression system utilized to silence early meiotic genes is different in vegetative cultures than in cells completing the meiotic program.

UME1 Is Required for the Normal Return-to-Growth Response in Precommitment Meiotic Cells—Early meiotic genes are rapidly repressed when glucose is added to meiotic cultures prior to the commitment point (29). To examine whether Ume1p plays a role in this response, isogenic diploids harboring either the wild-type or the null allele of UME1 were induced to enter meiosis but then challenged with glucose (2% final concentration) 6 h following shift to sporulation medium. This time point represents the peak of SPO13 mRNA accumulation (Fig. 2A) and is before the acquisition of commitment. Samples were taken prior to (0 min) and at intervals following the addition of glucose. Total RNA was prepared from these samples, and the levels of SPO13 mRNA were determined by S1 analysis (Fig. 2B). In the wild type, the half-life of SPO13 mRNA following the addition of glucose was calculated to be 4.2 min (three experiments, Fig. 2C). A similar value was reported previously (29). However, the ume1–2 mutant exhibited a 2.2-fold increase (9.3 min, three experiments) in the half-life of SPO13 mRNA. Previous studies reported that Ume1p does not affect SPO13 half-life under these conditions (29). Therefore, these results indicate that Ume1p is involved in the rapid
repression of SPO13 transcription when precommitment meiotic cells return to vegetative growth.

Glucose Stimulates UME1 Transcript Levels—Ume1p represses meiotic gene expression in vegetatively growing cells and in precommitment meiotic cells returning to growth. To determine whether this factor is itself subject to transcriptional regulation, UME1 mRNA levels were measured when SPO13 expression switched from an induced to a repressed state. UME1 mRNA levels were followed when precommitment meiotic cultures were challenged with glucose. The results from these experiments indicated that UME1 mRNA levels increased 2-fold coincident with the decline of SPO13 mRNA (Fig. 3A, top panel, quantitated in Fig. 3B). The elevated levels of UME1 transcript remained constant following the complete repression of SPO13 and during subsequent mitotic cell divisions (data not shown). Currently, it is not clear whether the increase in UME1 mRNA levels represents an increase in transcription initiation, stability, or both (see “Discussion”). The rapid induction of UME1 co-incident with the down-regulation of SPO13 suggests a role for this factor in the establishment, as well as the maintenance, of full transcriptional repression of SPO13. These results indicate that, unlike other UME factors studies (7, 8, 15, 35), UME1 is transcriptionally regulated. However, placing UME1 on a high-copy plasmid did not alter the transcription kinetics of SPO13 during meiosis or in return-to-growth assays (data not shown). These results suggest that although UME1 mRNA levels increase during periods of peak repression activity, Ume1p levels do not appear to be rate-limiting for this control.

Ume1p Association with the Histone Deacetylase Rpd3p Requires the Conserved NEE Box Motif—Previous studies have found that Ume1p and p48 associate with Rpd3p and the human histone deacetylase HDAC1, respectively (24, 36). Ume1p shares homology to a family of proteins including p48, a factor that associates with the retinoblastoma tumor suppressor protein Rb (37), the human histone deacetylase HDAC1 (36), and the chromatin assembly factor CAF-1 (38). Additional members of this family include a human protein of unknown function (p46) and a yeast protein (Msi1p) that, when overexpressed, is able to suppress the phenotypes associated with constitutively active Ras2p (39). A high degree of similarity between these proteins is observed in the amino-terminal region (Fig. 4A) with one region (NEEYKIKKK, indicated by bracket) particularly well conserved among these family members. To determine whether this region is required for Ume1p-Rpd3p interaction, co-immunoprecipitation studies were performed with a derivative lacking these core residues (NEE box, see “Experimental Procedures” for details). The UME1-HA and UME1NEE-HA expression plasmids were introduced into a wild-type strain (Rsy10), and extracts prepared from transformants were harvested during mid-log phase growth. As described elsewhere (24), Ume1p is able to co-immunoprecipitate with Rpd3p (Fig. 4B, top panel). However, the NEE box mutant was not able to co-immunoprecipitate with Rpd3p. Control
Western blots revealed that Ume1p_{\text{NEE}} levels were similar to wild-type Ume1p (second panel). Taken together, these results indicate that the conserved NEE box is required for Rpd3p association in vivo.

The other conserved feature within this family is a repeated WD motif (40) that directs specific protein-protein interactions (41). WD repeats were originally observed in β-transducin (42) but have subsequently been found in a variety of proteins involved in diverse cellular functions (reviewed in Ref. 43). To investigate the role of these conserved regions, the most conserved WD residues, amino acids 306–307, were replaced with alanines (WD_{\text{A}}). Similar to the NEE box mutant, the WD_{\text{A}} mutant failed to co-immunoprecipitate with Rpd3p (Fig. 4A, top panel). However, control studies revealed that the WD_{\text{A}} mutant levels were significantly reduced as compared with the wild type. To elevate the levels of this protein, the UME1_{\text{WD}} gene was placed on a high-copy plasmid to increase protein levels.

Sin3p PAH3 Is Required for Ume1p-Rpd3p Association in Vivo—Sin3p is the global co-repressor that tethers Rpd3p to the DNA-binding protein Ume6p (16). Sin3p directs repression through one of its four PAH domains, PAH3 (22). One model consistent with these results is that association of Sin3p to the Ume1p-Rpd3p complex is important for repression activity. As a first step to investigate this possibility, we determined whether any of the PAH domains are required for the stable association of Ume1p and Rpd3p. SIN3 was deleted in the RSY10 background, and the plasmids containing wild-type SIN3 or the various PAH deletion derivatives were introduced (22), a gift from D. Stillman, University of Utah. Co-immunoprecipitation studies were performed as just described probing for Ume1p in Rpd3p immunoprecipitates. Interestingly, the Rpd3p-Ume1p interaction was markedly reduced in the strain harboring the PAH3 mutant (Δ3) as compared with the wild type or other PAH mutant constructs (Fig. 5). The protein levels of either Rpd3p or Ume1p remained unchanged in these mutant strains as determined by Western blot analysis. These results indicate a new role for Sin3p in the normal association of Ume1p and Rpd3p.

Ume1p-dependent Repression Requires Both the NEE Box and the WD Domain Repeat—The results just described indi-
cate that the NEE box, and to a lesser extent the WD repeat, are required for Ume1p-Rpd3p association in vivo. We next addressed the impact that these mutations had on Ume1p-dependent repression. The UME1\textsuperscript{NEE,HA} and UME1\textsuperscript{WD,HA} expression plasmids were introduced into a \textit{ume1Δ} mutant strain harboring the \	extit{spo13-lacZ} reporter gene. As described above, the UME1\textsuperscript{WD,HA} mutant was expressed from a high-copy plasmid to produce levels similar to protein of Ume1p\textsuperscript{NEE} and the wild type. Controls for this experiment included the wild-type UME1-HA on a single copy plasmid and the vector without an insert. The loss in Ume1p activity resulted in a 3–4-fold increase in vegetative expression of the spo13-lacZ reporter gene (Fig. 6). Interestingly, reporter gene activity was also elevated in the strains expressing either of the mutant constructs, indicating that repressor function was lost. These results demonstrate that two motifs conserved among the Ume1-p48 protein family are required for transcriptional repression. Furthermore, combined with the co-immunoprecipitation studies, the Ume1p interactions may be complex, with associations occurring with factors other than Rpd3p (see “Discussion”).

**DISCUSSION**

This report describes the characterization of the transcriptional repressor Ume1p. Ume1p binds the histone deacetylase Rpd3p and is required for the repression of early meiotic genes during vegetative growth. We demonstrate that Ume1p is required for early meiotic gene repression in vegetative cultures regardless of the carbon source and in precommitment meiotic cells returning to mitotic cell division. Our finding that Ume1p is not required to re-establish repression following meiotic induction of these genes suggests that Ume1p functions primarily in cells undergoing mitotic cell division. Ume1p contains two functional motifs (NEE box and WD repeat) that are conserved with the human histone deacetylase HDAC1-binding protein p48 (36). Although both elements are required for normal association with Rpd3p, the NEE box is essential for this interaction. In addition, both mutants are defective for the vegetative repression of the early meiotic gene \textit{SPO13}. These findings suggest that Rpd3p association is important for Ume1p repression activity. Finally, we demonstrate an unexpected requirement of the global co-repressor Sin3p in maintenance of the Ume1p-Rpd3p complex in vivo. These results suggest the existence of a multisubunit repression complex whose integrity requires the participation of all its members.

How is \textit{UME1} itself regulated? \textit{UME1} mRNA levels increase ~2-fold when sporulating cells are exposed to glucose. Many genes, including those encoding glycolytic enzymes and ribosomal proteins, exhibit a 2–100-fold glucose-dependent transcriptional activation (for a review, see Ref. 44). Two transcription factors that mediate this response (Rap1p and Gcr1p), and the cis elements through which they function, have been identified (45, 46). Rap1p binds a divergent sequence (RMACCCAN-NCAYY) found in glycolytic gene promoters (47), whereas Gcr1p has been shown to bind a pentimer CTTCC (48). In the \textit{PGK} promoter, one copy of the Rap1p recognition site coupled with three repeats of the Gcr1p binding motif are required for normal glucose induction (49). A similar organization is found in the \textit{UME1} promoter. Two copies of near consensus matches of the Rap1p binding site are present in the \textit{UME1} 5′ promoter region (−741 and −198 with respect to the initiator ATG). In close proximity to the first Rap1p site, five CTTCC pentamers are found. These findings suggest that, similar to other glucose responsive genes, \textit{UME1} is controlled by a combination of the transcription factors. Our finding that \textit{UME1} overexpression does not alter the \textit{SPO13} meiotic expression profile indicates that transcription is not a rate-limiting step controlling Ume1p activity. These observations therefore suggest that additional, posttranslational mechanisms, may also regulate Ume1p.

Individually, Rpd3p, Sin3p, and Ume6p regulate diverse gene sets (13, 50–52). However, only a small number of genes including those transcribed early in meiosis have been shown to be regulated by the Ume1p-Sin3p-Rpd3p-Ume6p histone deacetylase complex (5, 15, 24, 33, 53). These findings suggest that, similar to other systems (54), meiosis-specific gene expression profiles are generated through the use of unique combinations of common factors. To correctly assemble these complexes, signals must be present to direct specific protein-protein interactions. Previous studies have revealed a central role for the function of the PAH domains of Sin3p in organizing this complex. PAH3 and an adjacent region of Sin3p mediate the interaction with the HDAC (Fig. 7) (36) and are required for most of the Sin3p-dependent repression studied to date (22). A more recent study found that PAH2 is required for Ume6p association (23). Moreover, Sin3p mutants lacking PAH2 are defective for \textit{SPO13} repression in vegetative cultures.\footnote{M. J. Mallory and R. Strich, unpublished results.}

**Fig. 5.** The Sin3p PAH3 domain is required for Ume1p-Rpd3p interaction. Co-immunoprecipitation (IP) experiments were conducted as described in the legend for Fig. 4 except that the host strain was deleted for \textit{SIN3} but harboring the PAH deletion derivatives as indicated. The \textit{rdp3} mutant strain was used to control for non-specific cross reactivity with the Rpd3-specific polyclonal antibody. Levels of Rpd3p (straight Western blot) and Ume1p-HA (immunoprecipitation) are shown in the various extracts.

**Fig. 6.** The conserved NEE box and WD motifs are required for Ume1p-dependent repression. β-Galactosidase activity from a \textit{spo13-lacZ} reporter in a \textit{ume1Δ} strain harboring the vector (vec), wild type (\textit{UME1}), \textit{WD} → AA substitution (\textit{WDΔ}), or the \textit{neeΔ} box deletion (\textit{NEEΔ}). The values depicted represent the average of at least three samples. The \textit{UME1WDΔ} allele was maintained on a high copy plasmid to increase protein levels similar to wild type. Error bars are given. The protein levels for the wild-type protein and the two mutants were determined by Western blots of immunoprecipitates (250 µg).
Characterization of UME1

Fig. 7. Model for Rpd3p-Ume1p-Sin3p interactions at the SPO13 promoter. Rpd3p is tethered to the SPO13 promoter by Ume6p via Sin3p. The Sin3p PAH domains required for these interactions are depicted by the arrows. The association of this domain with nucleosomes suggests that Ume1p targets Rpd3p deacetylation activity. Alternative binding targets include Sin3p itself, Rpd3p, or the unknown protein(s) (14); ARE sequence, –101–GAAATA, (13); see “Discussion” for details.

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