Published in final edited form as:
J Biol Chem. 2005 August 5; 280(31): 28761–28765. doi:10.1074/jbc.C500097200.

Global Loss of Set1-mediated H3 Lys^4 Trimethylation Is Associated with Silencing Defects in *Saccharomyces cerevisiae*[*]

Ian M. Fingerman[‡,§], **Chia-Ling Wu**[‡,§], **Bradley D. Wilson**[‡,§], and **Scott D. Briggs**[‡,§,¶]

[‡]Department of Biochemistry and the Cancer Center, Purdue University, West Lafayette, Indiana 47907

[§]Walther Cancer Institute, Indianapolis, Indiana 46202

Abstract

Post-translational histone modifications, such as acetylation, phosphorylation, ubiquitination, and methylation, have been correlated with regulation of gene expression. In *Saccharomyces cerevisiae*, Set1 has been identified as the sole histone methyltransferase required for histone H3 lysine 4 (Lys^4) methylation. Yeast cells that do not express Set1 have several apparent phenotypes, including slow growth and defects in telomere, *HML*, and rDNA silencing. However, the mechanism by which the Set1 methyltransferase mediates differential histone H3 methylation (mono-, di-, and tri-) is still not understood, and the involvement of domains or regions in Set1 contributing to H3 Lys^4 methylation has not been well characterized. In this study, the N terminus of Set1 was shown to be important for global and gene specific histone H3 trimethylation. We show that Set1 trimethyl-defective mutants can rescue a *set1*Δ slow growth defect. In contrast, Set1 trimethyl mutants were defective in telomere, rDNA, *HML*, and *HMR* silencing. Taken together, these data suggest that histone H3 Lys^4 trimethylation is required for proper silencing, while mono- and/or dimethylation is sufficient for cell growth.

The existence of histone methylation has been known for over 30 years (1) and recently site specific histone methylation and the corresponding methyltransferases have been identified (2,3). The catalytic core for most histone lysine methyltransferases resides in the SET domain (2,4). The SET domain is an evolutionarily conserved motif, with homologues present in organisms ranging from yeast to humans (5). In *Saccharomyces cerevisiae*, Set1 is the sole methyltransferase responsible for histone H3 lysine 4 (H3 Lys^4) methylation and catalyzes the addition of up to three methyl groups to its substrate resulting in mono-, di-, or trimethylated lysine 4 (6-10). Cells that do not express Set1 have several apparent phenotypes, including slow growth and defects in telomere, *HML*, and rDNA silencing (6,10-13).

Purification of Set1 indicates that Set1 is in a high molecular weight complex with seven other proteins (Swd1, Swd2, Swd3, Bre2, Sdc1, Spp1, and Shg1) and together with Set1 is referred to as COMPASS or SET1C (8,9,14). Several of the Set1-associated proteins are also critical for Set1-mediated histone H3 Lys^4 methylation suggesting that their interactions with Set1 regulate enzymatic activity or targeting of Set1 to chromatin (8-10). More recently, human homologues of Set1 (MLL1, MLL2, and KIAA0339) have also been purified as protein

[*]This work was supported by the Walther Cancer Institute, Purdue Cancer Center, American Cancer Society Institutional Grant, Indiana Elks Charities, Inc., and the Purdue Department of Biochemistry. This is journal paper number 17682 from the Purdue University Agricultural Experiment Station.

¶A Sidney Kimmel Scholar. To whom correspondence should be addressed: Dept. of Biochemistry, Purdue University, 175 S. University, West Lafayette, IN 47907. Tel.: 765-494-0112; Fax: 765-494-7897; sdbriggs@purdue.edu.

complexes and have been shown to have histone H3 Lys^4 methyltransferase activity (15-18). Furthermore, several of their complex members have been identified as the human homologues of the proteins associated with Set1 (14,16,17).

In budding yeast, H3 Lys^4 trimethylation and Set1 are highly concentrated at chromatin near the 5′ ends of open reading frames and is associated with transcriptionally active chromatin (7,19,20). In contrast, H3 Lys^4 dimethylation can be located at intergenic regions, promoters, and open reading frames (13,19,21). Therefore, it is thought that H3 Lys^4 dimethylation is more global and is located at regions of chromatin that are transcriptionally active, competent for transcription, or transcriptionally repressed (7,13,19-21). These results indicate that the different forms of H3 Lys^4 methylation may have distinct and/or overlapping biological roles. However, the mechanism by which Set1 methyltransferase differentially methylates H3 (mono-, di-, and tri-) is still not understood, and potential domains within Set1 that regulate H3 Lys^4 methylation have not been well characterized.

Set1 and COMPASS subunits have also been found to interact with the CTD of RNA polymerase II when it is phosphorylated at serine 5, and this interaction is dependent on the Paf1 elongation complex (19,21). In addition, yeast deletion strains of Paf1 complex members lack both H3 Lys^4 and Lys^{79} methylation (19,21,22). Based on these observations and chromatin localization studies of Set1 and Lys^4 methylation, it has been proposed that H3 Lys^4 methylation may play a role in transcription activation and/or elongation (19,21). However, the exact mechanism by which Set1 and H3 Lys^4 methylation functions in these processes is still poorly understood.

In this study, we generated N-terminal Set1 deletion mutants that lack global and gene specific H3 Lys^4 trimethylation while maintaining normal levels of mono- and dimethylated H3 Lys^4. We determined that the putative RNA recognition motif (RRM) domain in the N terminus of Set1 is needed for histone H3 Lys^4 trimethylation. Using Set1 trimethyl-defective mutants, we showed that histone H3 Lys^4 trimethylation alone is required for proper silencing of telomeres, rDNA, *HML*, and *HMR* loci, while only mono- and dimethylation were sufficient for proper cell growth suggesting that the different forms of histone H3 Lys^4 methylation are playing distinct biological roles.

MATERIALS AND METHODS

Yeast Plasmids and Strains

Construction of the plasmid expressing FLAG-tagged full-length Set1 (1–1080) and Set1 (780–1080) has been described previously (6). The N-terminal truncation of Set1 (829–1080) was made by PCR amplification of the desired region of Set1 using the full-length *SET1* plasmid as template. All constructs were engineered with a single FLAG epitope at the N terminus and subcloned into a yeast expression plasmid under the control of the *ADH1* promoter and containing a *URA3* or *TRP1* selectable marker. The RRM deletion mutant (1–1080 ΔRRM) was made via site-directed deletion using the Stratagene Quikchange site-directed mutagenesis kit. Briefly, complementary oligonucleotides were designed that had homology both to the regions immediately upstream and downstream of the region to be deleted. PCR amplification of the template (Set1 1–1080) and subsequent steps were performed as described by the manufacturer. The following strains were used: MBY1198 (*MATα his3Δ200 ade2*::*hisG leu2Δ0 ura2Δ0 met15Δ0 trp1Δ63* Ty1*his3AI*-236r, Ty1*ade2AI*515), MBY1217 (*MATα his3Δ200 ade2*::*hisG leu2Δ0 ura2Δ0 met15Δ0 trp1Δ63* Ty1*his3AI*-236r, Ty1*ade2AI*515 *set1Δ*::*TRP1*) (6), MSY421 (*MATα ura3–52 leu2–3,112 trp1 his3* Δ[*HHT1-HHF1*] Δ[*HHT2-HHF2*] pMS329 copy I (*URA3, HHT1-HHF-1*) (23), MBY1587 (*MATα ura3–52 leu2–3,112 trp1 his3* Δ[*HHT1-HHF1*] Δ[*HHT2-HHF2*] pMS329 copy I (*URA3, HHT1-HHF-1) set1Δ*::KanMX4) (6), UCC506 (*MATa ade2–101 his3-Δ200 leu2-Δ1 lys2–801 trp1-Δ1 ura3–*

52 URA::*Tel-V-R*) (24) UCC506 *set1*Δ (isogenic to UCC506, containing *set1*Δ::KanMX4; this study). UCC7262 (*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1*::*LEU2 hhf2-hht2*::*MET15 ADE2-TEL-VR hmr*::*URA3*,pMP9) (25), UCC7262 *set1*Δ (isogenic to UCC7262, containing *set1*Δ::KanMX4; this study). UCC7266 (*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1*::*LEU2 hhf2-hht2*::*MET15 ADE2-TEL-VR hml*::*URA3*,pMP9) (25), UCC7266 *set1*Δ (isogenic to UCC7262, containing *set1*Δ::KanMX4; this study). UCC1188 (MAT*α leu2*-Δ*1 lys2–801 trp1 ura3 hhf1-hht1*::*LEU2 hhf2-hht2*::*HIS3 RDN1*::*URA3*,pMP9) (25), UCC1188 *set1*Δ (isogenic to UCC1188, containing *set1*Δ::KanMX4; this study).

Preparation of Yeast Whole Cell Extracts and Immunoprecipitations

For analysis of yeast histones, yeast whole cell extracts were prepared as follows: 5-ml cultures of yeast were grown to mid-log phase (A_{600} = 1.0). Cells were harvested, washed with water and resuspended in 250 μl of 2 M NaOH with 8% β-mercaptoethanol. Cells were incubated on ice for 5 min and then pelleted at 13,000 rpm for 2 min at 4 °C. Cell pellets were resuspended gently in 250 μl of Buffer A (40 mM HEPES-KOH, pH 7.5, 350 mM NaCl, 0.1% Tween 20, 10% glycerol, 1 μg/ml leupeptin, aprotinin, and pepstatin A, 1 mM phenylmethylsulfonyl fluoride) and pelleted as described above. Cell pellets were resuspended in 180 μl of 2× SDS-sample buffer. Five μl or 10 μl of each sample were loaded per lane for Western blotting. For immunoprecipitation of yeast Set1, 50-ml cultures of MBY1198 and MBY1217 expressing blank vector or the indicated FLAG-Set1 (1–1080) or FLAG-tagged Set1 mutants (1–1080 ΔRRM, 780–1080 and 829–1080) were grown to mid-log phase and harvested. Cells were washed with water, resuspended in Buffer A (see above) and lysed with glass beads using a mini-bead beater (Biospec Products). Lysates were clarified by centrifugation and removed to a new tube. M2 resin (Sigma) was added (10 μl) to each lysate and rotated at 4 °C for 2 h. M2 resin was pelleted by spinning at 3000 rpm in a microcentrifuge and washed two times for 5 min each with 1 ml of Buffer A. Immunoprecipitated Set1 was eluted from the M2-resin by the addition of 10 μl of 2× SDS-Laemmli sample buffer.

Electrophoresis and Western Blotting

Western blot analysis to detect methylated histones was performed as described previously (6). To detect FLAG-Set1, immunoprecipitates were resolved on 8% or 10% SDS-PAGE gels, transferred to PVDF[1] membrane, and immunoblotted with monoclonal anti-FLAG antibody. Western blots directed against glyceraldehyde-3-phosphate dehydrogenase were also performed on the IP input to control for protein levels (data not shown).

Chromatin Immunoprecipitations

For analysis of *PYK1*, 100-ml cultures were grown as described (19). Briefly, cultures were grown in YP-ethanol (1% yeast extract, 2% peptone, 2% ethanol) to mid-log phase (A_{600} = 1.0) and split. Cells were washed and resuspended in either 50 ml of YP-ethanol or YPD (1% yeast extract, 2% peptone, 2% glucose) to induce expression of *PYK1*. Cells were induced for 1.5 h, harvested, and cross-linked with 1% formaldehyde. For analysis of *GAL10*, cultures were grown as described previously (26). Briefly, 100-ml cultures were grown in SC media (0.67% (w/v) yeast nitrogen base supplemented with amino acids, 2% glucose) lacking uracil overnight and split. Cells were harvested and resuspended in either SC-Ura or SC-Ura containing 0.5% glucose and 2% galactose. Cells were induced for 4 h, harvested, and cross-linked with 1% formaldehyde.

Chromatin immunoprecipitations were performed as described previously using H3 Lys^4 dimethyl- and H3 Lys^4 trimethyl-specific antibodies (Upstate Biotechnology) (27).

[1]The abbreviations used are: PVDF, polyvinylidene fluoride; 5-FOA, 5-fluoroorotic acid; RRM, RNA recognition motif.

Immunoprecipitated DNA was analyzed by PCR using primers that have been described previously to analyze the promoter, 5′ and 3′ regions of *PYK1* and *GAL10* (19). Two additional oligonucleotides were designed to analyze a region of the *GAL10* promoter (−290 to −40 bp). Their sequences are as follows: 5′-CACGGAGGAGAGTCTTCCGTCGGAG-3′ and 5′-GGACGCAAAGAAGTTTAATAATCAT-3′.

Growth and Silencing Assay

Growth assays were performed as follows. Yeast strains MSY421 and MBY1587 were transformed with plasmids to express wild type, epitope-tagged, FLAG-Set1 (1–1080), FLAG-Set1 ΔRRM (1–1080 ΔRRM), FLAG-Set1 (780–1080), FLAG-Set1 (829–1080), or blank vector. Cells were grown to mid-log phase (A_{600} = 1.0), serially diluted 5-fold, and plated on SC-Trp plates. Cells were photographed after 36 h at 30 °C. Telomere silencing assays were performed as described previously (11). Briefly, strains UCC506 (*MATa ade2–101 his3-Δ200 leu2-Δ1 lys2–801 trp1-Δ1 ura3–52 URA∷Tel-V-R*) and UCC506 *set1*Δ were transformed with the above plasmids. Individual isolates were grown 3 days to saturation in SC-Trp, normalized for A_{600}, serially diluted (2-fold), and spotted (5 μl/spot) on SC-Trp or SC-Trp plates containing 5-fluoroorotic acid (5-FOA) (100 μg/ml, Bio 101, Inc.). Cell growth was monitored over time at 30 °C. Cells on SC-Trp plates were photographed after 24 h, and cells on SC-Trp + 5-FOA were photographed after 60 h. rDNA, *HML*, and *HMR* silencing were performed as described previously (13,25). For rDNA silencing cells were grown 5 days to saturation, normalized for A_{600}, serially diluted (4-fold), and spotted on SC-Trp or SC-Trp plates containing 5-FOA (100 μg/ml, Bio 101, Inc.). For *HML* and *HMR* silencing cells were grown overnight, diluted (5-fold), and spotted as described above. Cells on SC-Trp plates were photographed after 24 h. 5-FOA plates were photographed at 48 h.

RESULTS AND DISCUSSION

To investigate the importance of the N terminus of Set1 in regulating H3 Lys^4 methylation, two FLAG-tagged N-terminal *set1* deletion constructs (780–1080 and 829–1080) were generated (Fig. 1*A*) and expressed in a yeast strain in which the endogenous *SET1* was deleted (*set1*Δ). To determine the histone H3 Lys^4 methylation status in these cells, yeast *set1*Δ strains containing blank plasmid (*Vector*) and the two N-terminal *set1* deletion constructs (780–1080 and 829–1080) were grown to an A_{600} = 1.0 and extracted with 2× SDS-Laemmli sample buffer. Clarified supernatants were run on SDS-polyacrylamide gels, transferred to a PVDF membrane, and probed with methyl-specific antibodies (Upstate Biotechnology) to mono-, di-, and trimethylated forms of histone H3 Lys^4. As shown in Fig. 1*B*, H3 Lys^4 trimethylation was nearly abolished in *set1*Δ strains expressing N-terminal *set1* deletion constructs (780–1080 and 829–1080). Trace amounts of H3 Lys^4 trimethylation were detected after longer exposure time to film (Fig. 1*B*). Surprisingly, H3 Lys^4 mono- and dimethylation were restored to similar levels to that of a wild-type isogenic strain, indicating that the N terminus of Set1 is specifically required for H3 Lys^4 trimethylation. To confirm that the Set1 N-terminal deletion mutants (780–1080 and 829–1080) were expressed, mutant yeast strains were grown to an A_{600} = 1.0 and lysed using glass beads. Clarified supernatants were immunoprecipitated with an anti-FLAG affinity resin (M2 resin, Sigma). Immunoprecipitates were run on SDS-polyacrylamide gels, transferred to a PVDF membrane, and probed with anti-FLAG monoclonal antibodies (M2, Sigma). Western blots indicated equal expression of the two N-terminal *set1* deletion constructs (780–1080 and 829–1080) (Fig. 1*B*). No FLAG-Set1 deletion mutants were detected in the unbound fraction (data not shown).

Besides the n-SET, SET, and post-SET domains of Set1, which are required for methyltransferase activity, the only other known domain found in Set1 is its putative RRM. The RRM is a domain commonly found in RNA-binding proteins and has been implicated in

binding RNA, single-stranded DNA, as well as proteins (28). To test whether the putative RRM domain might be the region in the N terminus of Set1 that regulates H3 Lys^4 trimethylation, we constructed a FLAG-tagged Set1 expression construct that lacked this domain (Fig. 1*A*). This deletion construct was expressed in the *set1*Δ strain and examined for H3 Lys^4 methylation status. As shown in Fig. 1*C*, the Set1 ΔRRM (1–1080 ΔRRM) mutant restored H3 Lys^4 mono- and dimethylation levels similar to wild-type cells and full-length Set1 (1–1080). Similar to the N-terminal Set1 deletion mutants (780–1080 and 829–1080), H3 Lys^4 trimethylation was nearly abolished in the Set1 ΔRRM (1–1080 ΔRRM) mutant strain (Fig. 1*C*). Again, longer exposure time to film indicated the presence of trace amounts of H3 Lys^4 trimethylation (Fig. 1*C*). Together these data suggest the RRM domain is at least one domain within the N terminus of Set1 that is needed for trimethylation. In further support of our data, Schlichter and Cairns (29) recently published a similar result showing that the RRM domain of Set1 is required for H3 Lys^4 trimethylation. To determine the levels of protein expression, full-length FLAG-Set1 (1–1080) and FLAG-Set1 ΔRRM (1–1080 ΔRRM) were immunoprecipitated from whole cell extracts and immunoblotted with anti-FLAG antibodies (Fig. 1*C*). Our results showed expression of both full-length Set1 (1–1080) and Set1 ΔRRM (1–1080 ΔRRM). However, lower levels of Set1 ΔRRM (1–1080 ΔRRM) mutant protein were detected when compared with full-length Set1 (1–1080) (Fig. 1*C*). Again, no FLAG-Set1 (1–1080) or Set1 ΔRRM (1–1080 ΔRRM) was detected in the unbound fraction (data not shown). Together these data may indicate that the Set1 ΔRRM (1–1080 ΔRRM) mutant protein is less stable. Interestingly, Swd2, a Set1-associated protein, has been implicated in the protein stability of Set1 suggesting the possibility that Swd2 or other Set1-associated proteins (Swd1, Swd3, Spp1, Bre2, Sdc1, or Shg1) interact with the RRM domain to stabilize the protein levels of Set1 (30).

To examine the effects Set1 trimethyl-deficient mutants at the gene level, chromatin immunoprecipitations were preformed using H3 Lys^4 di- and trimethyl-specific antibodies. The *GAL10* and *PYK1* loci, which are known targets of Set1 and H3 Lys^4 methylation, were examined (19,31,32). A complete loss of H3 Lys^4 trimethylation was observed at these loci in our *set1*Δ strain expressing Set1 829–1080 under conditions in which *GAL10* and *PYK1* were either induced (Fig. 2) or uninduced (data not shown). Importantly, the level of H3 Lys^4 dimethylation was similar to that of wild type cells (Fig. 2). Together these data suggest that our Set1 trimethyl-deficient mutants are still targeted to chromatin and are competent to dimethylate chromatin templates.

With the establishment of Set1 mutants that are defective for H3 Lys^4 trimethylation but not mono- or dimethylation, we wanted to determine whether differential methylation of H3 Lys^4 played a distinct biological function. Deletion of *SET1* in *S. cerevisiae* leads to the loss of telomere, *HML*, and rDNA silencing (6,8,10-13). In addition, some yeast strains deleted for *SET1* also demonstrate a slow growth phenotype (6). To examine the extent of H3 Lys^4 methylation (mono-, di-, or trimethylation) associated with these known *set1*Δ phenotypes, we expressed full-length Set1 (1–1080), Set1 ΔRRM (1–1080 ΔRRM), and both N-terminal Set1 deletions (780–1080 and 829–1080) in *set1*Δ strains that either have growth or silencing defects (6,8,10,11).

To determine whether slow growth is the result of a loss in H3 Lys^4 mono-, di-, or trimethylation, the yeast strain background, MSY421, was used. This strain was previously shown to have a significant growth defect when *SET1* was deleted (6). Both full-length Set1 (1–1080), Set1 ΔRRM (1–1080 ΔRRM), and the two N-terminal Set1 deletion mutants (780–1080 and 829–1080) were expressed in the MSY421 *set1*Δ strain. All cells were grown to A_{600} = 1.0, and 5-fold serial dilutions were spotted on plates containing synthetic complete media lacking uracil (SC-Ura, Bio 101, Inc.) and incubated at 30 °C. Interestingly, *set1*Δ strains expressing Set1 trimethylation mutants (1–1080 ΔRRM, 780–1080, and 829–1080) rescued the slow growth phenotype similar to that of full-length Set1 (Fig. 3). Furthermore, these data

would suggest that mono- or dimethylation of H3 Lys^4 or a combination of both is sufficient for proper cell growth.

To determine whether the observed loss of telomere silencing in a *set1*Δ strain is the result of losing H3 Lys^4 mono-, di-, or trimethylation, *SET1* was deleted in a strain where a *URA3* gene has been integrated at a subtelomere locus (24). Surprisingly, we observed that the *set1*Δ cells expressing Set1 ΔRRM (1–1080 ΔRRM) or N-terminal Set1 deletions (780–1080 and 829–1080) showed a dramatic sensitivity to 5-FOA (Bio 101, Inc.) as compared with both wild-type cells transformed with blank plasmid (*Vector*) or *set1*Δ cells expressing full-length Set1 (1–1080) (Fig. 4). Interestingly, cells expressing Set1 trimethylation mutants (1–1080 ΔRRM, 780–1080, and 829–1080) will eventually grow on 5-FOA plates, albeit in a diminished capacity, upon longer incubation periods at 30 °C (data not shown). This is consistent with our data indicating trace amount of H3 Lys^4 trimethylation in cells expressing trimethyl-defective mutants (Fig. 1, *B* and *C*). Since these cells contain proper H3 Lys^4 mono- and dimethylation, our data suggest that H3 Lys^4 trimethylation is needed for proper telomere silencing.

To determine whether silencing at rDNA, *HML*, and *HMR* loci is dependent upon H3 Lys^4 trimethylation, similar assays, as described above, were performed in strains lacking Set1 and containing a *URA3* gene integrated at the rDNA locus and the *HMR* and *HML* silent mating type loci (24,25). In all instances, cells that expressed Set1 mutants that are deficient for H3 Lys^4 trimethylation exhibit sensitivity to 5-FOA as compared with both wild-type cells transformed with blank plasmid (*Vector*) or *set1*Δ cells expressing full-length Set1 (1–1080) (Fig. 4). This data again suggests that H3 Lys^4 trimethylation is also needed for proper silencing at the rDNA, *HML*, and *HMR* loci.

Interestingly, at the different silent loci, silencing seems to be compromised at varying degrees when *SET1* is deleted or when expressing the Set1 trimethyl-deficient mutants in a *set1*Δ. Although the reason for this difference is not known, this has been commonly observed among other protein factors that disrupt silencing. However, various explanations could account for our observed differences. For example, the differences between *HML* and *HMR* silencing could be a consequence on how the *URA3* gene was previously integrated in these strains. It has been shown previously that the *URA3* gene is silenced better at the *HML* than *HMR* locus due to the way the gene was inserted (33). Therefore, *HMR* silencing in this strain has been shown to be more sensitive to silencing defects than *HML* (33). Another strain difference is that *HMR*, *HML*, and rDNA strains contain one gene copy of histones H3 and H4, therefore histone amounts may also contribute to these silencing differences. Although we speculate that some of these differences are due to subtle strain differences, it is also possible these differences are due to the type or amount of protein factors (*e.g.* Sir proteins) that are required for silencing at each of these distinct loci (34).

Our results demonstrate a strong correlation between H3 Lys^4 trimethylation and silencing. Although, the mechanism by which H3 Lys^4 trimethylation regulates silencing is still unclear, it has been proposed that loss of histone methylation at H3 Lys^4 and/or H3 Lys^{79} methylation allows promiscuous binding of Sir proteins to euchromatic regions, which results in titrating away Sir proteins from silent loci (35). In support of this model, it has recently been shown that Sir3 localization is disrupted in a yeast strain expressing a catalytically inactive mutant of Set1 and that Sir3 can bind to unmodified histone peptides but not peptides trimethylated at H3 Lys^4 (12). It will now be interesting to determine whether similar results are observed using yeast strains lacking only H3 Lys^4 trimethylation. However, other possibilities could still exist. For example, protein-protein interactions at the N terminus of Set1 may be needed for proper gene silencing, or Set1-mediated trimethylation may regulate expression of a known or unknown silencing factor(s).

It has been indicated by several groups that H3 Lys^4 trimethylation plays a role in transcriptional activation and/or elongation (7,19-21,36). To determine the role of Set1 trimethyl-deficient mutants in transcription, mRNA levels of known Set1 target genes *GAL1, GAL10,* and *PYK1* (19,31,32,37) were examined under uninduced and induced conditions. Surprisingly, reverse transcription-PCR and quantitative real time PCR analysis revealed no significant changes in *GAL1, GAL10,* and *PYK1* steady state mRNA levels under uninduced or induced conditions in either *set1*Δ cells or *set1*Δ cells expressing Set1 trimethyl-deficient mutants (data not shown). These data suggest that specific loss of H3 Lys^4 trimethylation is not sufficient to disrupt transcription of these Set1 targeted genes. In addition, *set1*Δ and Set1 trimethyl-deficient mutant strains do not show a hypersensitive phenotype when plated on media containing 6-azauracil, suggesting they are not defective in transcriptional elongation. [2] Further investigation will be needed to assess the precise role of Set1-mediated Lys^4 trimethylation in transcriptional activation and elongation.

In summary, this study demonstrates that a region outside the SET domain (*i.e.* the RRM domain or other domains in the N terminus of Set1) is required for regulation of Set1-mediated trimethylation. This is in contrast to Set7/9 and Dim5 histone methyltransferases in which a conserved tyrosine or phenylalanine residue within the SET domain mediates the degree of histone methylation (38,39). In addition, Set1 trimethyl-specific mutants have allowed us to discover that differential H3 Lys^4 methylation is required for distinct biological functions such as proper cell growth and telomere, rDNA, *HML,* and *HMR* silencing. Surprisingly, we were unable to determine a role for H3 Lys^4 trimethylation in transcription. Further investigation will be needed to determine precise mechanism of how Set1-mediated Lys^4 mono-, di-, and trimethylation can regulate these biological processes. Since Set1 exists in a high molecular weight complex, it is likely that Set1-associated factors will play a significant role in mediating the distinct forms of H3 Lys^4 methylation (8,9,14). These and other Set1 methyl-specific mutants will be useful in dissecting out the biological mechanism of how the different methylation states in the eukaryotic genome effects chromatin structure and function.

Acknowledgments

We thank Ann Kirchmaier, Joe Ogas, and Harry Charbonneau for helpful discussions; Dan Gottschling for the UCC506, UCC7266, UCC7262, and UCC1188 yeast silencing strains; Mary Bryk for the MBY1198, 1217 and 1587 yeast strains; and Peter Cheung and Doug Mersman for critical review of the manuscript.

References

1. Murray K. Biochemistry 1964;127:10–15. [PubMed: 14114491]
2. Lachner M, O'Sullivan RJ, Jenuwein T. J Cell Sci 2003;116:2117–2124. [PubMed: 12730288]
3. Peterson CL, Laniel MA. Curr Biol 2004;14:R546–R551. [PubMed: 15268870]
4. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T. Nature 2000;406:593–599. [PubMed: 10949293]
5. Jenuwein T, Laible G, Dorn R, Reuter G. Cell Mol Life Sci 1998;54:80–93. [PubMed: 9487389]
6. Briggs SD, Bryk M, Strahl BD, Cheung WL, Davie JK, Dent SYR, Winston F, Allis CD. Genes Dev 2001;15:3286–3295. [PubMed: 11751634]
7. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NCT, Schreiber SL, Mellor J, Kouzarides T. Nature 2002;419:407–411. [PubMed: 12353038]
8. Nagy PL, Griesenbeck J, Kornberg RD, Cleary ML. Proc Natl Acad Sci U S A 2002;99:90–94. [PubMed: 11752412]
9. Roguev A, Schaft D, Shevchenko A, Pijnappel WWMP, Wilm M, Aasland R, Stewart AF. EMBO J 2001;20:7137–7148. [PubMed: 11742990]

[2] I. M. Fingerman and S. D. Briggs, unpublished observation.

10. Krogan NJ, Dover J, Khorrami S, Greenblatt JF, Schneider J, Johnston M, Shilatifard A. J Biol Chem 2002;277:10753–10755. [PubMed: 11805083]
11. Nislow C, Ray E, Pillus L. Mol Biol Cell 1997;8:2421–2436. [PubMed: 9398665]
12. Santos-Rosa H, Bannister AJ, Dehe PM, Geli V, Kouzarides T. J Biol Chem 2004;279:47506–47512. [PubMed: 15280381]
13. Bryk M, Briggs SD, Strahl BD, Curcio MJ, Allis CD, Winston F. Curr Biol 2002;12:165–170. [PubMed: 11818070]
14. Miller T, Krogan NJ, Dover J, Erdjument-Bromage H, Tempst P, Johnston M, Greenblatt JF, Shilatifard A. Proc Natl Acad Sci U S A 2001;98:12902–12907. [PubMed: 11687631]
15. Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, Hess JL. Mol Cell 2002;10:1107–1117. [PubMed: 12453418]
16. Yokoyama A, Wang Z, Wysocka J, Sanyal M, Aufiero DJ, Kitabayashi I, Herr W, Cleary ML. Mol Cell Biol 2004;24:5639–5649. [PubMed: 15199122]
17. Wysocka J, Myers MP, Laherty CD, Eisenman RN, Herr W. Genes Dev 2003;17:896–911. [PubMed: 12670868]
18. Hughes CM, Rozenblatt-Rosen O, Milne TA, Copeland TD, Levine SS, Lee JC, Hayes DN, Shanmugam KS, Bhattacharjee A, Biondi CA, Kay GF, Hayward NK, Hess JL, Meyerson M. Mol Cell 2004;13:587–597. [PubMed: 14992727]
19. Ng HH, Robert F, Young RA, Struhl K. Mol Cell 2003;11:709–719. [PubMed: 12667453]
20. Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bouman P, Liu JS, Kouzarides T, Schreiber SL. Proc Natl Acad Sci U S A 2002;99:8695–8700. [PubMed: 12060701]
21. Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, Boateng MA, Dean K, Ryan OW, Golshani A, Johnston M, Greenblatt JF, Shilatifard A. Mol Cell 2003;11:721–729. [PubMed: 12667454]
22. Xiao T, Kao CF, Krogan NJ, Sun ZW, Greenblatt JF, Osley MA, Strahl BD. Mol Cell Biol 2005;25:637–651. [PubMed: 15632065]
23. Morgan BA, Mittman BA, Smith MM. Mol Cell Biol 1991;11:4111–4120. [PubMed: 2072911]
24. Gottschling DE, Aparicio OM, Billington BL, Zakian VA. Cell 1990;63:751–762. [PubMed: 2225075]
25. van Leeuwen F, Gafken PR, Gottschling DE. Cell 2002;109:745–756. [PubMed: 12086673]
26. Carvin CD, Kladde MP. J Biol Chem 2004;279:33057–33062. [PubMed: 15180994]
27. Kuo MH, Allis CD. Methods 1999;19:425–433. [PubMed: 10579938]
28. Stefl R, Skrisovska L, Allain FH. EMBO Rep 2005;6:33–38. [PubMed: 15643449]
29. Schlichter A, Cairns BR. EMBO J 2005;24:1222–1231. [PubMed: 15775977]
30. Dichtl B, Aasland R, Keller W. RNA (N Y) 2004;10:965–977.
31. Ezhkova E, Tansey WP. Mol Cell 2004;13:435–442. [PubMed: 14967150]
32. Daniel JA, Torok MS, Sun ZW, Schieltz D, Allis CD, Yates JR III, Grant PA. J Biol Chem 2004;279:1867–1871. [PubMed: 14660634]
33. Singer MS, Kahana A, Wolf AJ, Meisinger LL, Peterson SE, Goggin C, Mahowald M, Gottschling DE. Genetics 1998;150:613–632. [PubMed: 9755194]
34. Rusche LN, Kirchmaier AL, Rine J. Annu Rev Biochem 2003;72:481–516. [PubMed: 12676793]
35. van Leeuwen F, Gottschling DE. Curr Opin Cell Biol 2002;14:756–762. [PubMed: 12473351]
36. Hampsey M, Reinberg D. Cell 2003;113:429–432. [PubMed: 12757703]
37. Henry KW, Wyce A, Lo WS, Duggan LJ, Emre NC, Kao CF, Pillus L, Shilatifard A, Osley MA, Berger SL. Genes Dev 2003;17:2648–2663. [PubMed: 14563679]
38. Zhang X, Yang Z, Khan SI, Horton JR, Tamaru H, Selker EU, Cheng X. Mol Cell 2003;12:177–185. [PubMed: 12887903]
39. Collins RE, Tachibana M, Tamaru H, Smith KM, Jia D, Zhang X, Selker EU, Shinkai Y, Cheng X. J Biol Chem 2005;280:5563–5570. [PubMed: 15590646]

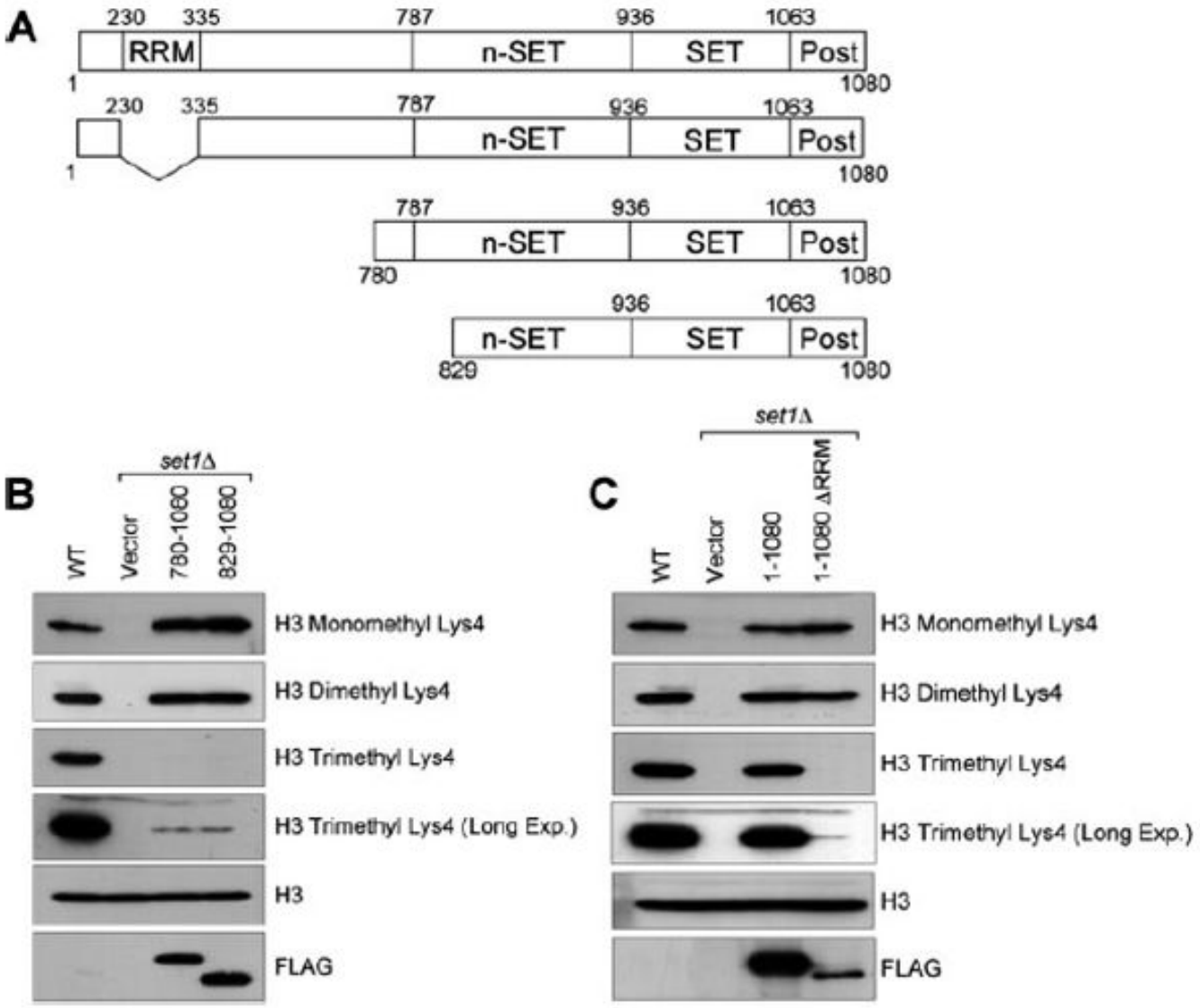

Fig. 1. The N terminus of Set1 is required for global histone H3 Lys^4 trimethylation

A, schematic representation of Set1 and Set1 deletion constructs used in this study. Amino acid positions of the RRM domain, n-SET, SET, and post-SET domains are indicated. All constructs are N-terminally tagged with a single FLAG epitope. *B* and *C*, Western blots using methyl-specific antibodies shows the methylation status of H3 Lys^4 in yeast strains expressing full-length Set1 (1–1080) and Set1 mutants (1–1080 ΔRRM, 780–1080, and 829–1080). Long exposure (*Long Exp.*) represents the same trimethyl immunoblot (above) but 15× longer exposure to film. Antibodies directed against general histone H3 (Abcam) were used as a loading control. Anti-FLAG immunoblots were performed to examine the protein levels of the Set1 mutants.

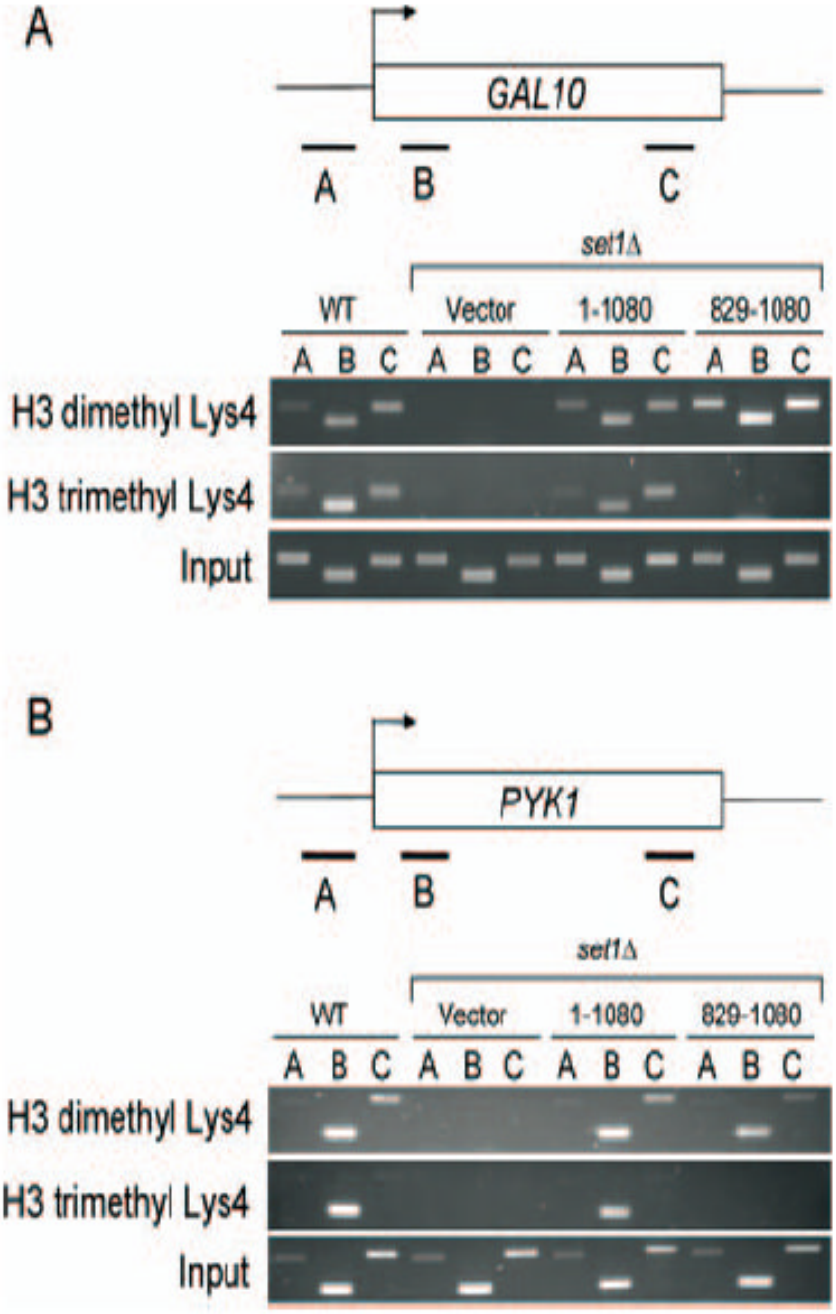

Fig. 2. Set1 trimethyl-defective mutants show gene-specific loss of H3 Lys^4 trimethylation but not H3 Lys^4 dimethylation

Chromatin immunoprecipitations were performed at *GAL10* and *PYK1* using antibodies specific to di- or trimethylated Lys^4. *A* and *B*, chromatin immunoprecipitation eluates were examined by PCR at the indicated regions (*A–C*) and analyzed by agarose gel electrophoresis. Chromatin immunoprecipitation data shown represent both *PYK1* and *GAL10* under induced conditions.

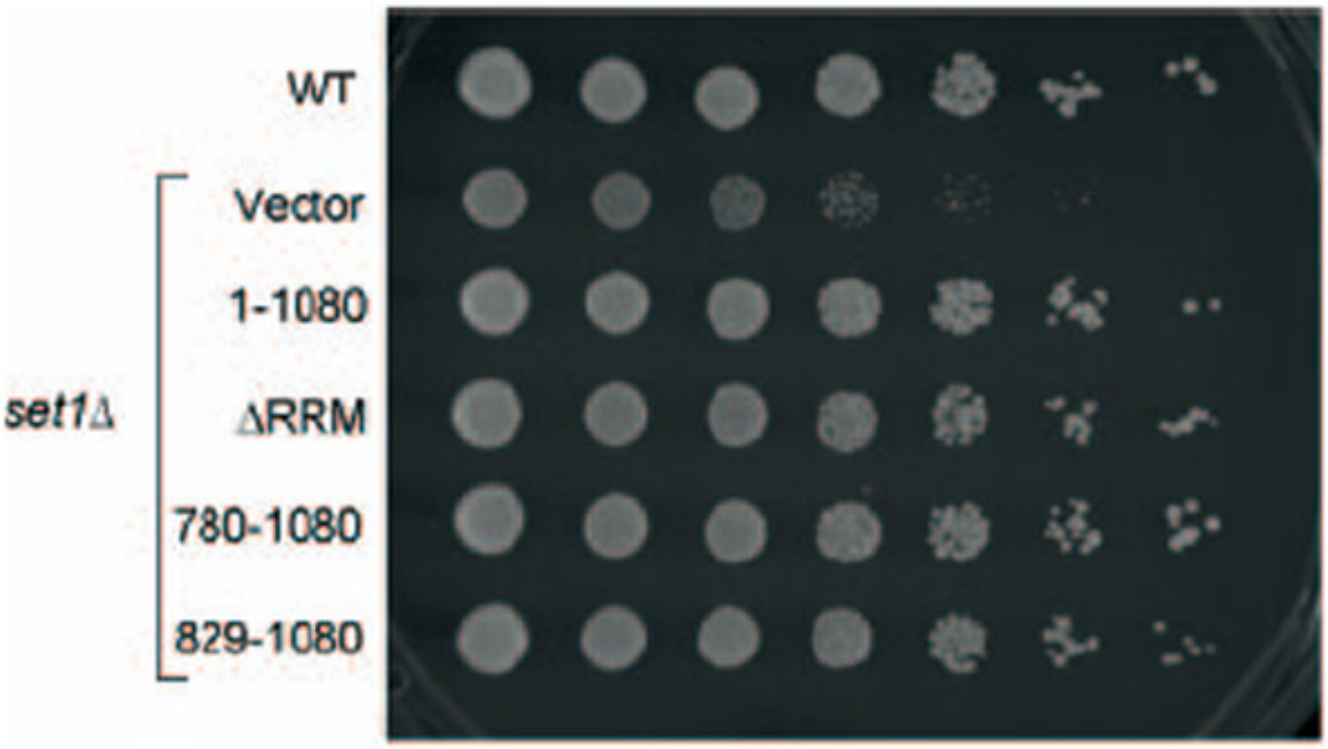

Fig. 3. The N terminus of Set1 is not essential for cell growth

Yeast strains MSY421 and MBY1587 were transformed with the indicated plasmids to determine whether H3 Lys^4 trimethylation is essential for growth. Cells were grown to mid-log phase (A_{600} = 1.0), serially diluted 5-fold, and plated on SC-Trp plates. Cells were photographed after 36 h of incubation at 30 °C.

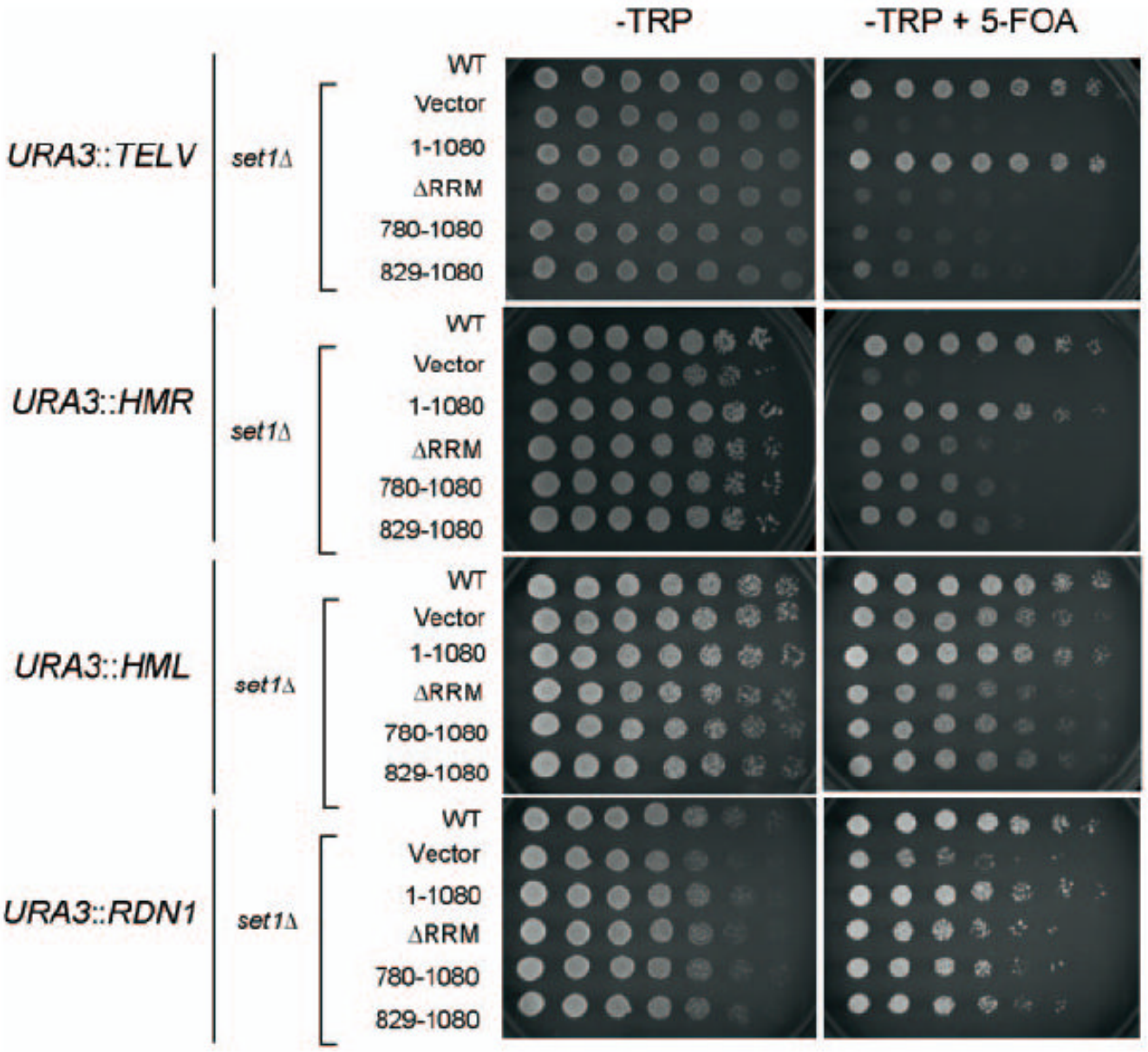

Fig. 4. The N terminus of Set1 is required for silencing

Strains UCC506 and UCC506 *set1*Δ(*TEL-V*), UCC7262 and UCC7262 *set1*Δ(*HMR*), UCC7266 and UCC7266 *set1*Δ(*HML*), and UCC1188 and UCC1188 *set1*Δ(rDNA) were transformed with the indicated plasmids. For silencing assays, individual isolates were grown to saturation, normalized for A_{600}, and serially diluted and spotted on SC-Trp or SC-Trp/5-FOA plates. Cell growth was monitored at 30 °C. Cells on SC-Trp plates were photographed after 24 h, and cells on SC-Trp + 5-FOA were photographed after 48 h (*HMR*, *HML*, rDNA) or after 60 h (TEL).