Evidence that Set1, a Factor Required for Methylation of Histone H3, Regulates rDNA Silencing in S. cerevisiae by a Sir2-Independent Mechanism

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Summary

Several types of histone modifications have been shown to control transcription [1, 2]. Recent evidence suggests that specific combinations of these modifications determine particular transcription patterns [2–4]. The histone modifications most recently shown to play critical roles in transcription are arginine-specific and lysine-specific methylation [4, 5]. Lysine-specific histone methyltransferases all contain a SET domain, a conserved 130 amino acid motif originally identified in polycomb- and trithorax-group proteins from Drosophila [6, 7]. Members of the SU(VAR)3-9 family of SET-domain proteins methylate K9 of histone H3 [8, 9]. Methylation of H3 has also been shown to occur at K4. Several studies have suggested a correlation between K4-methylated H3 and active transcription [10–13]. In this paper, we provide evidence that K4-methylated H3 is required in a negative role, rDNA silencing in Saccharomyces cerevisiae. In a screen for rDNA silencing mutants, we identified a mutation in SET1, previously shown to regulate silencing at telomeres and HML [14, 15]. Recent work has shown that Set1 is a member of a complex [16] and is required for methylation of K4 of H3 at several genomic loci [17]. In addition, we demonstrate that a K4R change in H3, which prevents K4 methylation, impairs rDNA silencing, indicating that Set1 regulates rDNA silencing, directly or indirectly, via H3 methylation. Furthermore, we present several lines of evidence that the role of Set1 in rDNA silencing is distinct from that of the histone deacetylase Sir2. Together, these results suggest that Set1-dependent H3 methylation is required for rDNA silencing in a Sir2-independent fashion.

Results

Set1 Is Required for rDNA Silencing

To gain further insight into possible mechanisms of rDNA silencing, we screened for new mutations that increase transposition of a silenced Ty1 element in rDNA. This screen used a yeast strain, MBY1198 (Table S1), containing two genetically marked Ty1 elements, one within rDNA (Ty1his3AI-236r), and the other outside of rDNA (Ty1ade2AI-S15) (Figure S1A). Each of these Ty1 elements can be assayed for transposition by a simple growth test (Figure S1B; see the Experimental Procedures in the Supplementary Material available with this article online). We screened for mutations that increase the transposition of the Ty1 element within rDNA but do not alter the transposition of the Ty1 element outside rDNA (see the Experimental Procedures). From approximately 4400 mutagenized colonies, 3 mutants exhibited a significant increase in transposition of Ty1his3AI-236r without an increase in transposition of Ty1ade2AI-S15. This paper focuses on one of these mutants.

To identify the mutant gene, the site of insertion of the Tn3::LEU2 cassette was determined (Experimental Procedures). The insertion is located in the SET1 gene, previously shown to control expression of DNA repair genes and to control transcriptional silencing at telomeres and at the HML locus [14, 15, 18, 19]. Transposition assays demonstrated that a complete deletion of SET1, set1Δ, causes loss of the rDNA-silencing phenotype, similar to that caused by the original set1::Tn3 mutation (Figure 1A). All subsequent experiments have been performed using set1Δ strains.

To determine if the increased Ty1his3AI transposition is caused at the transcriptional level, we performed Northern analysis, measuring the mRNA level produced from the Ty1his3AI element in rDNA and the total Ty1 mRNA level. Our results (Figure 1B) show that the level of Ty1his3AI mRNA is increased significantly, approximately 3.3-fold, in the set1Δ mutants compared to the SET1 strains. In contrast, the total Ty1 mRNA level is not increased in the set1Δ mutants. In addition, in set1Δ mutants, Ty1 transposition is increased greatly for those elements within rDNA, but is not significantly affected for Ty1 elements outside of rDNA (Tables S2 and S3). Therefore, Set1 is required for rDNA silencing at the level of transcription.

We also employed a second rDNA-silencing assay that measures expression of a modified URA3 gene, mURA3, and the LEU2 gene when integrated either in the rDNA or outside the rDNA [20]. As previously demonstrated, in SET1 strains, expression of the mURA3 and LEU2 genes in the rDNA is reduced relative to their expression when integrated at leu2Δ (Figure 1C), reflecting transcriptional silencing in rDNA [20]. In contrast, in set1Δ strains, expression of mURA3 or LEU2 in the rDNA is approximately 100-fold higher than in the SET1 strain. This experiment verifies that Set1 is required for the silencing of multiple RNA pol II-transcribed genes when located in the rDNA.

Set1 Does Not Regulate the Mitotic Stability of Ty1 Elements in the rDNA

Previous studies have shown that mutations that impair rDNA silencing also derepress mitotic recombination at
Figure 1. Silencing Defects of set1 Mutants

(A) Transposition patch assay of SET1, set1::Tn3, and set1Δ strains on SC-His medium (see the Experimental Procedures).

(B) Effect of the set1Δ mutation on Ty1his3AI and total Ty1 RNA levels. Total RNA from SET1 and set1Δ strains was hybridized to a 32P-labeled sense strand HIS3 riboprobe to detect Ty1his3AI RNA (top), an antisense Ty1 riboprobe to detect total Ty1 RNA (middle), and an antisense PYK1 riboprobe as a loading control (bottom). These data are representative of three RNA blots. The set1Δ/SET1 ratio of Ty1his3AI RNA normalized to PYK1 RNA is 3.3 ± 0.2. The set1Δ/SET1 ratio of total Ty1 RNA normalized to PYK1 RNA is 1.0 ± 0.1.

(C) mURA3-LEU2 rDNA-silencing assay. Ten-fold serial dilutions of stationary-phase cultures of SET1 or set1Δ strains containing the mURA3-LEU2 cassette at the rDNA or at leu2Δ1 were spotted onto SC-Leu, 5-FOA, or SC medium to monitor expression of the mURA3-LEU2 cassette. The slightly smaller colony size of the SET1 strain JS210-1 (mURA3-LEU2 at leu2Δ1), compared to the set1Δ strain MBY1317 on SC-Leu medium is most probably caused by the trp1Δ63 mutation present in JS210-1.

The Silenced Ty1 Element in rDNA Is Associated with K4-Methylated H3

Recent studies have demonstrated that Set1 is required for the methylation of histone H3 on K4 and that this modification is present in the rDNA [17]. To determine if this modification is also present over the silenced Ty1 element, we performed ChIP experiments. Our results (Figure 3A) show that the Ty1 promoter is indeed associated with K4-methylated H3 in a Set1-dependent fashion. Deletion of SET1 Does Not Alter the Association of Sir2 or Net1 with the rDNA

The best-understood mechanism of rDNA transcriptional silencing involves the histone deacetylase Sir2 [24]. Sir2’s localization to the nucleolus and association with the rDNA [25, 26] are dependent on its interactions with Net1, which plays a critical role in nucleolar structure and function [27–29]. We determined if set1Δ affects the association of Net1 or Sir2 with the rDNA by chromatin immunoprecipitation (ChIP) experiments. Our results (Figure 2) show that both Net1 and Sir2 associate with the rDNA nontranscribed spacer (NTS) at wild-type levels in set1Δ mutants. Additionally, we examined the localization of Sir2 and NET1 fused to the coding region of green fluorescent protein (GFP). Their localization in wild-type and set1Δ strains was indistinguishable (data not shown). From these results, we conclude that Set1 is not required for the association of Net1 or Sir2 with the rDNA.

The Silenced Ty1 Element in rDNA Is Associated with K4-Methylated H3

Recent studies have demonstrated that Set1 is required for the methylation of histone H3 on K4 and that this modification is present in the rDNA [17]. To determine if this modification is also present over the silenced Ty1 element, we performed ChIP experiments. Our results (Figure 3A) show that the Ty1 promoter is indeed associated with K4-methylated H3 in a Set1-dependent fashion. Thus, in the case of the Ty1 in rDNA, there is a correlation between K4-methylated H3 and the mRNA level.

The Sir2-Dependent Acetylation Levels in rDNA Are Not Altered in a set1Δ Mutant

To test for any relationship between Set1-dependent histone methylation and Sir2-dependent histone deacetylation in the rDNA, we also performed ChIP experiments. Our results show that K4-methylated H3 is present in the sir2Δ mutant (Figure 3B). In this mutant, the level of K4-methylated H3 is modestly increased at the rDNA, is unchanged at SPT15, and is increased approxi-
We conclude that the Sir2-dependent hypoacetylation K4R mutant causes a similar increase in Ty1 SPT15 mRNA levels. In these experiments, we measured the frequency of transposition of a histone H3-Ty1 element located in the rDNA. The average (n = 22–27) frequency of transposition (/H11006) of the rDNA NTS for NET1/TEL-VIR ranged from 9; in the set1 mutant, it was 1.1 (±0.1) × 10^−7; and in the set1/sir2:hisG double mutant, it was 3.8 (±0.6) × 10^−7. Therefore, the silencing defect is significantly increased in the double null mutant, indicating that Set1 and Sir2 contribute independently to rDNA silencing.

Analysis of rDNA Silencing in the Presence of an Unmethylated Form of Histone H3

If the rDNA-silencing defect in set1Δ mutants is caused by a defect in histone H3 K4 methylation, then an H3 mutant that cannot be methylated should cause the same rDNA-silencing defect as a set1Δ mutation. To test this model, we measured rDNA silencing in an H3 mutant with a substitution of arginine for lysine at position 4 (K4R) that is not methylated in vivo [17]. Our results (Figure 4) show that either a set1Δ mutation or the H3 K4R mutant causes a similar increase in Ty1/His3A1 mRNA levels. Significantly, in the set1Δ H3-K4R double mutant, there is no greater increase in mRNA levels, strongly suggesting that each mutation impairs silencing by a common mechanism. In these experiments, we also observed an effect of both set1Δ and H3-K4R on total Ty1 mRNA levels, in contrast to what was observed for set1Δ in strains with a wild-type configuration of histone genes (Figure 1B). This effect is not understood, although it may be due to the reduced number of H3-H4-encoding genes present in the strains used for this experiment (Experimental Procedures). We have observed that some set1Δ phenotypes are strengthened
Figure 3. set1Δ Results in Loss of K4-Methylated Histone H3 at Several Loci

(A) Chromatin immunoprecipitation analysis of wild-type and set1Δ strains using antisera specific for the K4-methylated form of histone H3 by quantitative radioactive PCR shows that Set1 is necessary for the presence of K4-methylated H3 in the promoter region of the silent Ty1his3AI element in the rDNA. The average %IP of the rDNA-Ty1 promoter region in the SET1 strain is 2.5%, and, in the set1Δ strain, it is 0.1%. The average %IP of the GAL1 UAS region in the SET1 strain is 3.2%, and, in the set1Δ strain, it is 0.1%. The %IP values from two independent experiments are: for SET1 at the rDNA-Ty1: 3.5, 1.4; at the GAL1 UAS: 4.4, 2.0; for set1Δ at the rDNA-Ty1 promoter: 0.1, 0.1; and at the GAL1 UAS: 0.1, 0.1. Slanted triangles indicate a 2-fold increase in the amount of DNA used in the PCR reactions.

(B) Chromatin immunoprecipitation analysis of SET1 SIR2, set1Δ SIR2, and SET1 sir2Δ cells using antisera specific for the K4-methylated form of histone H3 show that SET1 is required for the presence of K4-methylated histone H3 at these loci. The average ratio of the %IP for SET1/set1Δ are: for rDNA NTS, 23; for SPT15, 97; and for TEL-VIR, 24. The %IP values from two independent experiments are: for SET1 SIR2 at the rDNA NTS: 1.7, 1.1; at SPT15: 6.6, 2.1; and at TEL-VIR: 0.6, 0.6; for set1Δ SIR2 at the rDNA NTS: 0.07, 0.05, at SPT15: 0.04, 0.05, and at TEL-VIR: 0.01, 0.04; and for SET1 sir2Δ at the rDNA NTS: 3.1, 1.9; at SPT15: 5.4, 2.1; and at TEL-VIR: 3.2, 2.2. Chromatin immunoprecipitation analysis using antisera specific for acetylated (K9, K14) histone H3 show that set1Δ does not alter the levels of acetylated histone H3 at the rDNA NTS, the SPT15 ORF, and TEL-VIR. Control immunoprecipitations show that sir2Δ causes an increased level of acetylated histone H3 at the rDNA (average 3-fold increase, n/H11005 2) and at TEL-VIR (4.3-fold increase, n/H11005 2). The average SET1/set1Δ ratio of the %IP with the anti-Ac histone H3 antisera are: for the rDNA NTS, 0.7; for SPT15, 0.8; and for TEL-VIR, 1.0. The average SIR2'/sir2Δ ratio of the %IP with the anti-Ac histone H3 antisera are: for the rDNA NTS, 0.3; for SPT15, 1.2; and for TEL-VIR, 0.2. The %IP values from two independent experiments in the SET1 SIR2 strain at rDNA NTS: 0.88, 0.72; at SPT15: 1.1, 1.2; and at TEL-VIR: 0.4, 0.6; in the set1Δ SIR2 strain at rDNA NTS: 1.4, 0.9; at SPT15: 1.8, 1.2; and at TEL-VIR: 0.5, 0.5; in the SET1 sir2Δ strain at rDNA NTS: 2.7, 2.0; at SPT15: 1.3, 0.7; and at TEL-VIR: 2.4, 1.9. Additional control reactions (with no primary antibody) show the specificity of the protein-A sepharose beads for the antibody-bound chromatin. Slanted triangles indicate dilutions (1:25, 1:50, 1:100) of input DNA.

in this genetic background (M.B. and F.W., unpublished data). The common phenotypes of set1Δ, H3-K4R, and set1Δ H3-K4R mutants strongly suggest that set1Δ phenotypes are due to a loss of methylation at K4 of H3.

Discussion

In summary, our results demonstrate that Set1, a factor previously shown to be important for transcriptional silencing at the HML and telomeric loci [15], also regulates transcriptional silencing in the rDNA. As reported recently [17], Set1 is required for the methylation of histone H3 at position K4. These results, taken together with our analysis of set1Δ and H3 K4R mutants, suggest that Set1 is required for rDNA silencing due to its activity as an H3 K4 methyltransferase.

Our results do not yet distinguish whether Set1 and K4-methylated H3 act directly or indirectly to control rDNA silencing. However, our results provide strong evidence that the Set1-dependent mechanism acts independently of Sir2. First, set1Δ causes no detectable defect in the association of Sir2 and Net1 with the rDNA.
Consistent with this observation, set1Δ does not change SIR2 mRNA levels (data not shown). Second, set1Δ does not alter the Sir2-dependent level of acetylated H3 at the rDNA, showing that Sir2’s function at rDNA is independent of Set1. Third, in a set1 sir2 double null mutant, the rDNA silencing defect is greater than in either single mutant. Fourth, in contrast to sir2Δ, set1Δ does not alter recombination levels in the rDNA. Finally, in contrast to net1Δ [29], set1Δ does not cause a defect in RNA pol I transcription (Figure S2). These findings support the existence of a Set1-dependent mechanism for rDNA transcriptional silencing that acts independently of Sir2. Many factors that are required for rDNA silencing appear to function via Sir2; however, others may participate via either a Set1-dependent or other type of mechanism [22, 23, 32, 33].

Our analysis of sir2Δ and set1Δ mutants strongly suggests that the transcription of genes in rDNA and at telomeres is not controlled by the absolute level of either H3 methylation or acetylation, but rather by a specific combination of the two types of modifications. In wild-type strains, in which both rDNA and telomeric silencing occurs, the H3 amino-terminal tail is hypoacetylated, but it is methylated at position K4 (data shown here; [30, 31]). In both sir2Δ and set1Δ mutants, silencing is impaired; yet, the H3 modifications are strikingly different between the two mutants. In sir2Δ mutants, both H3 acetylation and H3 K4 methylation levels are increased; in contrast, in set1Δ mutants, H3 is still hypoacetylated and H3 K4 methylation is abolished. Thus, opposite changes in H3 modification levels each confer loss of silencing. These findings suggest that the silencing mechanism at both rDNA and telomeres relies on a particular pattern of acetylation plus methylation on H3. However, different perturbations of this pattern are each permissive for transcription at these loci. Finally, the increased level of H3 K4 methylation in sir2Δ mutants suggests that hyperacetylation of H3 enhances H3 K4 methylation. These results suggest a possible functional relationship between H3 acetylation and methylation.

Supplementary Material

The Experimental Procedures and Supplementary Results can be found with this article online at http://images.cellpress.com/supmat/supmatin.htm.

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mediate gene silencing in Drosophila heterochromatin and at S. cerevisiae telomeres. EMBO J. 16, 3219–3232.


Note Added in Proof
Supplementary Material

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Supplementary Experimental Procedures

Media, Yeast Strains, Plasmids, and Genetic Procedures

Standard yeast media were prepared as described [S1]. YPD is YM0 medium supplemented with L-tryptophan (20 mg/L) and adenine sulfate (20 mg/L). Medium containing the chemical 5-fluoroorotic acid (5-FOA, 1 mg/L) [S2] was used to measure the level of expression of the mura3 gene located in the rDNA or at the ade2Δ1 (see below) loci. All yeast strains used in this study are described in Table S1. Yeast strains JC236 and JC242 [S3] and JS210-1 and JS215-10 [S4] have been described previously. All other strains were constructed for this study. The SET1-coding region was deleted and replaced with the TRP1 gene from pRS404 (set1Δ1) or the KANMX4 cassette [S5] from pRS400 [S6] (set1Δ2) using a PCR-mediated gene disruption technique [S7]. Both set1Δ mutations cause identical phenotypes and are referred to as set1Δ in Results. The specific alleles are designated in Table S1. A previous study demonstrated that a set1Δ in the W303a genetic background causes several mutant phenotypes, including growth defects, morphological abnormalities, and sporulation defects [S8]. In contrast, we observed that a set1Δ mutation in our genetic background does not cause these phenotypes, the mutants exhibiting only mild flocculence. Two different sir2Δ null mutations are used in our studies. First, the sir2Δ-coding region was deleted and replaced with the KANMX4 cassette, as described above. Second, we used sir2Δ::hisG [S9]. The sir2Δ null mutations were verified by a nonamating phenotype and by Southern blot analysis or PCR. The his3Δ1 marker in the rDNA Ty1his3Δ1 element in strains MBY1198 and MBY1217 was replaced with a HIS3 marker by integrative transformation using the ClaI fragment of pGEM-HIS3, as described [S9]. NET1-HA3-LEU2 from plasmid pDM239 [S10] was integrated into the NET1 locus in strains MBY1198 and MBY1217. Deletions of HHT1-HHF1 and HHT2-HHF2 were introduced into strains MBY1198 and MBY1217 by standard genetic crosses. The plasmid containing HHT2-HHF2 is described in [S11], and the hht2K4R-HHF2 plasmid was a gift from Sharon Y. R. Dent and Judith K. Davie [S12].

Transposon Mutagenesis

Haploid strain MBY1198 (Table S1, Figure S1) was mutagenized by transformation [S13] with NotI-digested DNA from an insertional mutagen library based on the transposon Tn3::lacZ::LEU2 [S14]. Transformed cells were plated on SC-Leu medium to yield approximately 200 transformants per plate and were grown for 3 days at 30°C. Transformants were picked and grown in patches on fresh SC-Leu plates for 2 days at 30°C. Then patches were transferred to YPD plates that were incubated at room temperature (RT) for 4 days. Temperatures in the range of 20°C–23°C are permissive for Ty1 transposition. These patches were replica plated to medium lacking histidine (SC-His) and medium lacking adenine (SC-Ade) to measure the levels of transposition of the genetically marked Ty1 elements. Transposition of Ty1his3Δ1-236r in the rDNA gives rise to His+ papillae on SC-His, and transposition of Ty1ade2Δ1-515 located outside of the rDNA gives rise to Ade+ papillae on SC-Ade. Mutants defective for rDNA silencing are expected to have an increased number of His+ papillae without a change in the number of Ade+ papillae. Such candidates were colony purified on SC-Leu medium. For each of these mutants, a single Leu+ colony was patched to YPD, grown at RT for 4 days, and then replica plated to SC-His and SC-Ade to recheck the hypertransposition phenotype. The site of insertion of the Tn3::LEU2 in mutant 26.1 was determined by rescuing the insertion and flanking DNA onto a plasmid as described [S14], and DNA sequencing was performed at the Biopolymers facility of the Genetics Department at Harvard Medical School. To determine if the insertion mutation in 26.1 causes the rDNA-silencing defect, a genetic cross was performed by strain MBY1155 (data not shown). In 12 of 12 tetrads, the rDNA-silencing defect and the Tn3::LEU2 insertion cosegregated 2:2, demonstrating that the silencing defect is caused by a single mutation that is the Tn3::LEU2 insertion.

Transposition Assays

The rate of transposition (number of transposition events/cell/generation) of Ty1 elements marked with the his3Δ1 retrotransposition indicator gene is directly proportional to the rate of His+ prototroph formation [S3]. Likewise, the rate of transposition of Ty1ade2Δ1 and of Ty1ade2Δ1/kanAI elements is proportional to the rate of formation of Ade+ prototrophs and G418-resistant colonies, respectively. The rate of transposition of genetically marked Ty1 elements (Ty1his3Δ1A, Ty1ade2Δ1A, or Ty1ade2Δ1/kanAI) was determined by the maximum-likelihood method [S15], as previously described [S9]. SET1 and set1Δ strain pairs were always tested in the same experiment. The rate of transposition of the Ty1ade2Δ1-515 element is variable, which is attributable to a high degree of variability in Ade+ colony formation because of growth suppression by cotransformed Ade− cells. Therefore, several strains with different Ty1 elements were tested to determine the effect of set1Δ on transposition of Ty1 elements outside the rDNA. The frequency of Ty1his3Δ1A transposition was determined by calculating the average number of His+ papillae per cell in 2 ml YPADT cultures grown to saturation at 20°C. Transposition patch assays were performed as described above. Student’s t-tests were performed to determine the probability that the frequency of transposition in the wild-type, set1Δ, sir2Δ::hisG, and set1Δ sir2Δ::hisG strains were the same. In each case, the Student’s t test showed that the transposition frequencies of the strains were significantly different. The Student’s t test values are: wild-type and set1Δ, 2.0 × 10−2; wild-type and sir2Δ::hisG, 2.9 × 10−1; wild-type and set1Δ sir2Δ::hisG, 4.9 × 10−1; sir2Δ::hisG and set1Δ, 5.6 × 10−1; and set1Δ and set1Δ sir2Δ::hisG, 3.2 × 10−1.

Phenotypic Assay for Expression of mURA3-LEU2

Cultures containing 10 ml YPD medium were seeded with SET1 and set1Δ strains containing the mura3Δ-LEU2 marker in the rDNA or at the ade2Δ1 locus and were grown to saturation at 30°C. Ten-fold serial dilutions of each culture were made in sterile H2O, and 5 μl of each dilution was spotted onto SC, SC-Leu, and 5-FOA agar. Plates were photographed after 2 days of incubation at 30°C.

Northern Blot Analysis

Total RNA was isolated from yeast cells as described previously [S9]. Northern analysis was performed as described [S16]. 32P-labeled RNA probes were used to detect Ty1his3Δ1A, total Ty1, and PYK1 transcripts [S17]. Quantification was performed on a Storm 8600 phosphor imager (Molecular Dynamics) using ImageQuant software. The levels of Ty1his3Δ1A mRNA and total Ty1 mRNA were normalized to the PYK1 mRNA loading control.
Mitotic Stability of Ty1HIS3 Elements

The mitotic stability of Ty1HIS3 elements was measured as described [S9]. A control culture containing a mixture of His+ and His− cells was included in each experiment to check that neither cell type was lost during the extended growth period in YPADT medium. The average ratio of His+/His− cells for the control cultures in the two experiments was: before growth, 49% His+; after growth, 49% His+51% His−. Since HIS3 is not silenced in the Ty1HIS3 element in the rDNA, this assay accurately measures mitotic recombination in the rDNA.

S1 Nuclease Protection Analysis

RNA pol I transcription was measured using an oligonucleotide probe that is complementary to the rapidly processed 3′ end of the precurso 25S RNA (see [S18] and references within, sequence available upon request). The oligonucleotide used to measure the loading control U6 RNA was described previously [S19]. Analysis with the two oligonucleotides was performed in separate reactions because the products of the hybrids are close in size. To perform S1 analysis, total RNA (2.5, 5, or 10 µg) isolated from cells grown to log phase (1 x 10^6/mL) was hybridized to ^32P-labeled oligonucleotide specific for the precursor rRNA or U6 RNA in 0.3 M NaCl, 1 mM EDTA, 38 mM HEPES (pH 7.0), and 0.1% Triton X-100 at 55°C for at least 12 hr. Unhybridized nucleic acids were digested at 37°C for 30 min by adding 50 U S1 nuclease (from nuclease) in 0.33 M NaCl, 66 mM sodium acetate (pH 4.6), 2.2 mM ZnCl2, and 0.01% Triton X-100. The reaction was stopped by the addition of EDTA (to 5 mM) and tRNA (50 µg), and nucleic acids were precipitated in ethanol. Pellets were resuspended in 0.1 M NaOH and 80% formamide loading dye and heated to 90°C for 3 min prior to separation by electrophoresis on 10% polyacrylamide/8 M urea gels. Quantification of the amount of precipitated RNA was performed on a Storm 8600 phosphorimager (Molecular Dynamics) using ImageQuant software.

Chromatin Immunoprecipitation

Cells were grown, and lysates were prepared as described [S20], with slight modifications noted below. The chromatin-containing fraction of the lysate was sonicated 12 times for 20 s each at power level 4 using a Fisher dismembranator, resulting in chromatin fragments of an average size of 0.3−1.0 kb. The sonicated lysate was clarified by centrifugation at 14,000 rpm for 30 min at 4°C. Immunoprecipitations were performed in a total volume of 500 µL. An aliquot of sonicated lysate (250−500 µL) was incubated with −1 µg of antisera (anti-Sir2, 1 µL; anti-HA1 [Babco], 1 µL α-methylated [lys4] H3 [S12], 1.5 µL α-acetylated [lys9, 14] H3 [Upstate Biotechns], 1.5 µL) or 12CA5-conjugated protein-A Sepharose beads (40 µL of a 50% suspension of protein-A or protein-G sepharose beads equilibrated in ice-cold lysis buffer) for 12 hr at 4°C or a Nutator rocker. For two-step immunoprecipitations, 40 µL of a 50% suspension of protein-A or protein-G sepharose beads equilibrated in ice-cold lysis buffer was added, and incubation was continued for 2 hr at 4°C. After washing, the precipitate was eluted from beads by adding 100 µL 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% SDS, and incubating at 65°C for 15 min. Beads were pelleted briefly, and the eluate was transferred to a fresh tube. Beads were washed with 150 µL TE/0.67% SDS, and the wash was pooled with the eluate. The bead eluate and a 50 µL aliquot of crosslinked chromatin (to serve as input DNA) were incubated at 65°C overnight to reverse the formaldehyde crosslinks. Then, the chromatin solution was treated with 100 µg proteinase K for 2 hr at 37°C. A total of 5 µg glyoxyl and lithium chloride (to 0.4 M) were added to the DNA solution, and the DNA was purified by extracting with phenol-chloroform (1:1) and chloroform, prior to precipitation with ethanol. The precipitated chromatin pellet was washed with 1 ml 70% ethanol and resuspended in 100 µL TE (pH 8.0).

Analysis of Chromatin Immunoprecipitations

Chromatin immunoprecipitation reactions were analyzed by quantitative radioactive PCR or slot blot. Quantitative radioactive PCR analysis of chromatin immunoprecipitations was used to determine if the promoter region of the silent Ty1his3AI element in the rDNA contained K4-methylated H3. A total of 4 µL of a 1:100 and a 1:50 dilution of input DNA and 2 and 4 µL of a 1:4 dilution of the immunoprecipitated DNA (using anti-Methyllys4)H3 antisera or no antibody) were amplified using two sets of primers. The rDNA-specific primers were complementary to the TyA ORF of the silent Ty1 element (5′-CGGATCTTGATTGTGACGTCCC-3′) and to a region downstream of the 5S rRNA gene (5′-CTTCTCCAGTAAGCTGTTTC-3′).
Figure S1. Ty1 Retrotransposition Used to Assay rDNA Silencing

(A) Ty1 retrotransposition indicator genes present in the mutagenesis strain. Strain MBY1198 contains a single Ty1his3AI element in the rDNA subject to rDNA silencing. Transposition of this element can generate His+ prototrophs. The strain also contains a Ty1ade2AI-515 element outside of the rDNA not subject to rDNA silencing. Transposition of Ty1ade2AI-515 can generate Ade+ prototrophs.

(B) Transposition can generate a HIS3+ gene. Ty1his3AI-236r is marked with a mutant HIS3 gene (gray rectangle) in the opposite transcriptional orientation to Ty1. Coding sequences of the HIS3 gene are interrupted by an artificial intron (AI) in the antisense orientation relative to the HIS3-coding sequence. The AI is not spliceable from transcripts initiating from the HIS3 promoter; however, it is in the correct orientation to be spliced from transcripts initiating from the Ty1 long terminal repeat. When a spliced transcript from Ty1his3AI is used for reverse transcription and cDNA integration, the newly transposed element will contain a HIS3+ gene [S3]. Tripartite boxes joined by a thick line represent the genomic Ty1 element; thin double lines with a dot represent the chromosome.

CTT-3') and amplify a 540-bp fragment containing the Ty1 promoter. A second set of primers that amplify a 260-bp fragment flanking the GAL1 UAS was included to examine a locus outside of the rDNA (sequences available by request). Reactions were separated on 6% polyacrylamide gels. The relative fraction of specific DNA immunoprecipitated was determined by quantitation on a Storm 8600 phosphorimager (Molecular Dynamics) using ImageQuant software. The amount of hybridization of probes to the no-antibody reaction that represents nonspecific binding of DNA to the sepharose beads was subtracted from the appropriate IP values prior to any calculations.

Supplementary References


Table S2. set1 Δ Reduces Transcriptional Silencing of rDNA-Ty1his3Δ1 Elements

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ty1 Element</th>
<th>Location of Ty1 Element</th>
<th>Relevant Genotype</th>
<th>Transposition Rate b</th>
<th>Ratio of Rates (set1 Δ/SET1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBY1198</td>
<td>Ty1his3Δ1-236 rDNA</td>
<td>rDNA</td>
<td>SET1</td>
<td>7.0 (±3.5) × 10^-6</td>
<td>13.4</td>
</tr>
<tr>
<td>MBY1217</td>
<td>Ty1his3Δ1-236 rDNA</td>
<td>rDNA</td>
<td>set1 Δ</td>
<td>9.4 (±4.1) × 10^-6</td>
<td>13.4</td>
</tr>
<tr>
<td>MBY1198</td>
<td>Ty1ade2Δ1-515 outside</td>
<td>outside</td>
<td>SET1</td>
<td>1.6 (±8.4) × 10^-6</td>
<td>3.3</td>
</tr>
<tr>
<td>MBY1217</td>
<td>Ty1ade2Δ1-515 outside</td>
<td>outside</td>
<td>set1 Δ</td>
<td>5.2 (±1.4) × 10^-6</td>
<td>3.3</td>
</tr>
<tr>
<td>MBY1269</td>
<td>Ty1his3Δ1-236 rDNA</td>
<td>outside</td>
<td>SET1</td>
<td>7.8 (±5.0) × 10^-7</td>
<td>17.4</td>
</tr>
<tr>
<td>MBY1322</td>
<td>Ty1his3Δ1-236 rDNA</td>
<td>outside</td>
<td>set1 Δ</td>
<td>1.4 (±0.9) × 10^-7</td>
<td>17.4</td>
</tr>
<tr>
<td>MBY1269</td>
<td>Ty1pTEF-kanΔ1-2910 outside</td>
<td>outside</td>
<td>SET1</td>
<td>1.1 (±0.4) × 10^-7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Location, in or outside the rDNA.

The rate of Hisa prototroph formation per cell per generation (±SD) was determined using the method of the medians [S15]. The data for MBY1198 and MBY1217 are from four independent experiments. The data for MBY1269 are the average of two independent experiments. The data for MBY1322 are from a single experiment and represent the median rate values determined after five independent measurements using strains derived from the same cross as MBY1322 (range of transposition rates of Ty1his3Δ1-236 rDNA, 2.5 × 10^-4 to 2.5 × 10^-7; of Ty1pTEF-kanΔ1-2910, 4.8 × 10^-4 to 3.2 × 10^-7).

Table S3. Frequency of Transposition of Marked Ty1 Elements in SET1 and set1 Δ Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ty1 Element</th>
<th>Location of Ty1 Element</th>
<th>Relevant Genotype</th>
<th>Transposition Frequency a</th>
<th>Ratio of Frequencies (set1 Δ/SET1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC236</td>
<td>Ty1his3Δ1-236 rDNA</td>
<td>rDNA</td>
<td>SET1</td>
<td>4.1 (±3.1) × 10^-5</td>
<td>7.6</td>
</tr>
<tr>
<td>MBY1290</td>
<td>Ty1his3Δ1-236 rDNA</td>
<td>rDNA</td>
<td>set1 Δ</td>
<td>3.1 (±2.0) × 10^-5</td>
<td>7.6</td>
</tr>
<tr>
<td>JC242</td>
<td>Ty1his3Δ1-242 outside</td>
<td>outside</td>
<td>SET1</td>
<td>4.4 (±2.6) × 10^-7</td>
<td>1.3</td>
</tr>
<tr>
<td>MBY1293</td>
<td>Ty1his3Δ1-242 outside</td>
<td>outside</td>
<td>set1 Δ</td>
<td>5.6 (±3.4) × 10^-7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Location, in or outside the rDNA.

The frequency of Hisa prototroph formation per cell (±SD) was determined in five or six independent experiments, as described in the Experimental Procedures.
Table S4. Deletion of SET1 Does Not Alter Levels of Mitotic Recombination in the rDNA

<table>
<thead>
<tr>
<th>Strain Ty1 Element (Location)</th>
<th>Relevant Genotype</th>
<th>His&lt;sup&gt;+&lt;/sup&gt; Auxotrophs/Total Cells Analyzed</th>
<th>Loss of HIS3 per Generation&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBY1445 Ty1/HIS3-236r (in rDNA)</td>
<td>SET1</td>
<td>273/2181</td>
<td>1.0 (±0.45) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBY1410 Ty1/HIS3-236r (in rDNA)</td>
<td>set1Δ</td>
<td>163/1177</td>
<td>1.2 (±0.33) × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>The rate of mitotic recombination (± SD) was determined by measuring the rate of loss of the HIS3 marker (His<sup>+</sup> auxotrophs/total number of cells analyzed) after 120 generations of growth in nonselective media (see the Experimental Procedures).