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Biochemical and Biophysical Research Communications 349 (2006) 463–470
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Different roles of histone H3 lysine 4 methylation in chromatin maintenance

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Received 22 July 2006
Available online 31 August 2006

Abstract

Histone H3 methyltransferases are involved in the epigenetic control of transcription and heterochromatin maintenance. In *Saccharomyces cerevisiae*, deletion of a histone H3 methyltransferase *SET1* leads to the induction of a subset of stress responsive genes in a Rad53 dependent manner. This type of induction was observed only in the absence of *SET1* and not in the absence of other histone methyltransferases, *SET2* or *DOT1*. We show that the increased expression of the stress responsive genes results from a lack of histone H3 lysine (K) 4 methylation. The loss of mono-methylation on H3 K4 is necessary to increase the expression of the stress responsive genes, while the loss of di- or tri-methylation induced by deletion of either RRM domain of Set1 or the upstream effector molecules hardly affected their expression. These results suggest that mono- and multiple methylation of H3 K4 have different roles. The mono-methylation of H3 K4 might be required for the global integrity of chromatin structure, which is normally monitored by the Rad53 dependent chromatin surveillance system.

Keywords: Chromatin; Histone methylation; Histone methyltransferase; *SET1*; *RAD53*

In eukaryotic cells, DNA associates with histones and non-histone proteins to form highly organized chromatin structures. The basic element of chromatin, the nucleosome, consists of 146 bp of supercoiled DNA wrapped almost twice around an octamer of two copies of each of the four histone proteins: H2A, H2B, H3, and H4 [1]. The post-translational modifications of histones, including acetylation, phosphorylation, methylation, ubiquitination, and sumoylation, regulate chromatin structure and function [2–4].

In *Saccharomyces cerevisiae*, lysine (K) 4, K36, and K79 are the most characterized methylation sites on histone H3. H3 K4 methylation by Set1 [5–7] and H3 K79 methylation by Dot1 [8–10] are important for maintaining chromatin structure to keep heterochromatic genes silent. The elimination of H3 K4 or H3 K79 methylation abolishes the telomere position effect (TPE) by spreading Sir proteins from a silenced region into a non-silenced region [10–12]. Meanwhile, H3 K36 methylation by Set2 does not affect the heterochromatic gene silencing, but is implicated in the activation and repression of transcription [13–18]. Not only the methylation of specific histone residues but also the extent of methylation on each residue is important. Multiple methylation must have a distinct biological function as it is regulated in many ways. The subunits within the Set1 complex (COMPASS) regulate K4 di- and tri-methylation [19,20]. The mono-ubiquitination of H2B on K123 by Rad6 and Bre1 regulates di- and tri-methylation of histone H3 K4 and H3 K79 as well [19,21]. In addition to Rad6/Bre1, the Paf1 protein complex and BUR kinase complex (*BUR1*/*BUR2*), associated with the positive transcription

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doi:10.1016/j.bbrc.2006.08.122

elongation, are important for di- and tri-methylation of H3 K4, which means that multiple methylation of H3 K4 is tightly coupled to the transcription [22,23]. Although the mechanism of regulation of H3 K4 methylation has been understood, it is unclear whether different numbers of methyl groups on K4 have different biological functions.

Recently, H3 K79 methylation has been reported to play a key role in the response to DNA damage. The checkpoint protein, 53BP1 (Rad9 in budding yeast), directly binds the methylated K79 via its conserved tudor domain to activate a checkpoint pathway [24]. In contrast to *DOT1*, a *SET1* deletion mutation is not directly involved in the DNA damage checkpoint. Interestingly, loss of *SET1* increases cell viability upon DNA damage of the checkpoint mutants such as *mec3*, *rad9*, *rad17*, and *rad24* by inducing a Mec1/Tel1 independent but Rad53 dependent novel signaling pathway [25]. Rad53 kinase activity is essential for this pathway in that it leads to the constitutive expression of Rfa2 (the middle subunit of replication protein A) dependent repair genes [25]. However, it is not clear how *SET1* deletion leads to the activation of Rad53 to induce Rfa2 regulated gene expression. Although Rad53 is required for the typical DNA damage response, its additional role in maintaining the normal genome integrity is not clearly understood [26].

The aim of this study was to investigate different roles of mono-, di-, and tri-methylation of histone H3 K4. Di- and tri-methylation of histone H3 K4 is enriched in actively transcribed regions and correlated with the activity of transcription, whereas mono-methylation is rather evenly distributed over the genome, suggesting that mono-methylation might have a more generalized role. Since *SET1* deletion enhances the repair capacity in a Rad53 dependent manner, we investigated the possibility that the altered chromatin structure induced by the loss of histone methylation could be monitored through the chromatin surveillance pathways mediated by Rad53.

Here, we report that a subset of stress responsive genes is induced by *SET1* deletion in a Rad53 dependent manner because of the loss of histone H3 K4 methylation. This molecular phenotype enabled us to differentiate the role of histone H3 K4 mono-methylation because none of the upstream effector mutations responsible for di- and tri-methylation or RRM mutant of Set1 that abolishes tri-methylation induced stress responsive genes. This suggests that the loss of di- and tri-methylation of H3 K4 is well tolerated, but further loss of H3 K4 mono-methylation is detected by the Rad53 dependent chromatin surveillance system as a form of stress.

Materials and methods

Yeast strains and plasmids. The yeast strains used in this study are summarized in Table 1. The histone H3 mutants were constructed by shuffling each plasmid containing histone H3 (WT or mutant alleles) and H4 into WZY42 (from Dent-Roth) to make YC85 (wild-type, pWZ414-F13), YC86 (pRS314-H3(K4A)-H4), YC87 (pRS314-H3(K36A)-H4), YC77 (pRS314-H3(K79A)-H4), and YC79 (pRS314-H3(K4A/K79A)-H4). Wild-type, K4A, and K36A mutant plasmids were gifts from Buratowski. An immunoblotting assay was performed to confirm the strains. YC81 and YC83 were constructed by amplifying a DNA fragment encompassing the *set1Δ*::*KanMX4* region from the genomic DNA of UCC1001 *set1Δ* strain (from Kouzarides) and using it to transform either W3031A or U960-5C (from Verreault).

RNA analysis. The yeasts were grown to the exponential phase ($OD_{600} = 1.0$) and used for RNA isolation. The total RNA was prepared using the Trizol method according to the manufacturer's instructions (Invitrogen). Before RT-PCR, the RNA was usually treated with DNase I (Promega) to remove any residual chromosomal DNA in the sample. Double-stranded cDNA was made from 1 μg of the total RNA using reverse transcriptase (Promega). Semi-quantitative PCR amplification of the target regions from the various genes was performed in a 10 μl reaction mixture using the following program: 5 min at 94 °C followed by 18 cycles with 45 s at 94 °C, 45 s at 50 °C or 55 °C, and 45 s at 72 °C. The reaction was finished by an extra extension step at 72 °C for 5 min. Signals were quantified by Chemi-digital image analysis system using Labworks software (UVP, Upland, Calif.). The oligonucleotide primers used in RT-PCR are as follows: *ADH1* 5′-TTCAACCAAGTCAAGTCCATCTCTA-3′ (forward), 5′-ATTTGACCCTTTTCCATCTTTTCGTAA-3′ (reverse); *CPA2* 5′-ACGTATCTACAGGAACACATCAAA-3′ (forward), 5′-TGAGGTTCATTGAACAATGGGATA-3′ (reverse); *DDR48* 5′-AAAGTGAAGCAATTTGCTAACAGC-3′ (forward), 5′-TTAGAACCATATGAGTCGTTGTTG-3′ (reverse); *ECM4* 5′-CAGAAGCTGACATTAGATTGTATA-3′ (forward), 5′-TAATTCCCAAGGGGTTGATCCTT-3′; *FLR1* 5′-CCGTTTTTGCAGGCAATGGTTTTTG-3′ (forward), 5′-CTTGTACGTAATGATGGCCCATAC-3′ (reverse); *GTT2* 5′-CGACAAAGCCCTACATGGAATG-3′ (forward), 5′-CGGATTTCTAGCAGTTTCTTCACG-3′ (reverse); *SED1* 5′-AACTACATCTTTGCCACCA-3′ (forward), 5′-GTGGTAGTGCCCTTAGATT-3′ (reverse); *SSU1* 5′-AGAGAGTTTATACCTTCCACAAAGG-3′ (forward), 5′-ATACTCATGCAATGTGCATAAAAGGC-3′ (reverse).

PCR-mediated mutagenesis. The histone H3 mutant alleles (H3K79A and H3K4A/79A) were constructed by PCR-mediated site-directed mutagenesis using pWZ414-F13 [27] or pRS314-H3(K4A)-H4 as the starting material. PCR was performed with mutagenic primers, 5′-CGCTCAAGATTTCGCGACCGACTTGAGA-3′ (forward) and 5′-TCTCAAGTCGGTCGCGAAATCTTGAGCG-3′ (reverse). The PCR conditions were: 1 min at 95 °C followed by 17 cycles of 30 s at 95 °C, 1 min at 55 °C, and 15 min at 68 °C. The final PCR cycle was 1 min at 94 °C, 1 min at 55 °C, and 10 min at 72 °C. The PCR-amplified DNA was treated with 10 U of *Dpn*I (New England Biolabs) for 1 h at 37 °C to remove the template plasmid DNA before *Escherichia coli* transformation. A mutation of the intended location was confirmed by DNA sequencing.

Immunoblotting assay. Yeast cells were grown overnight in either YPD or the appropriate selective medium. To prepare the bulk histones, 50 μl of a cracking buffer (8 M urea, 5% SDS, 40 mM Tris–HCl (pH 8.0), 0.1 M EDTA, 0.4 mg/ml bromophenol blue, 10 μl β-mercaptoethanol, plus the protease inhibitors and phosphatase inhibitors) and 30 μl of 0.5 mm glass beads (Biospec Products) were added to the yeast cell pellets. Samples were immediately heated at 100 °C for 10 min followed by vigorous vortexing for 1 min. The supernatant obtained after centrifuging (13,000 rpm for 5 min) contained the histones. An immunoblotting assay was performed with the mono-methyl-H3 K4 (07-436), di-methyl-H3 K4 (07-030), tri-methyl-H3 K4 (07-473), di-methyl-H3 K79 (07-366), and acetylated H3 K14 (07-353) antibodies from upstate.

Results

The expression of stress responsive genes is specific to the SET1 deletion

Deletion of *SET1* increases the DNA repair capacities of the checkpoint mutants by inducing repair genes via Rad53 [25]. However, it is so far unclear which *SET1* deletion is

Table 1
Yeast strains used in this study

Strain	Genotype	References
UCC1001	*MATa*, *ura3-52*, *leu2-Δ1*, *trp1-Δ1*, *his3-Δ200*, *lys2-801*, *ade2-101*, *TELadh4*::*URA3*	[42]
set1Δ	UCC1001, *set1Δ*::*KAN*	[42]
BY4742	*MATα*, ura3*Δ0*, *leu2Δ0*, *his3Δ1*, *lys2Δ0*	Euroscarf
Y11256	BY4742, *set2Δ*::*KanMX4*	Euroscarf
Y13771	BY4742, *bre1Δ*::*KanMX4*	Euroscarf
Y14276	BY4742, *dot1Δ*::*KanMX4*	Euroscarf
Y14425	BY4742, *rad6Δ*::*KanMX4*	Euroscarf
Y14611	BY4742, *rtf1Δ*::*KanMX4*	Euroscarf
Y15727	BY4742, *paf1Δ*::*KanMX4*	Euroscarf
WZY42	*MATa*, *ura3-52*, *leu2Δ1*, *trp1Δ63*, *his3Δ200*, *lys2-801*, *ade2-101*, *hht1-hhf1*::pWZ405-F2F9-*LEU2*, *hht2-hhf2*::pWZ403-F4F10-*HIS3*, YCp50-copyII (*HHT2-HHF2*)	[27]
W3031A	*MATa*, *ura3-1*, *leu2-3*, *112*, *trp1-1*, *his3-11*, *15*, *can1-100*, *ade2*-1	Hanes
YC77	Isogenic to WZY42, pRS314-H3(K79A)-H4	This study
YC79	Isogenic to WZY42, pRS314-H3(K4A/K79A)-H4	This study
YC81	Isogenic to W3031A, *set1Δ*::*KanMX4*	This study
YC83	Isogenic to U960-5C, *set1Δ*::*KanMX4*	This study
YC85	Isogenic to WZY42, pWZ414-F13 (*HHT2-HHF2*)	This study
YC86	Isogenic to WZY42, pRS314-H3(K4A)-H4	This study
YC87	Isogenic to WZY42, pRS314-H3(K36A)-H4	This study
U960-5C	*MATa*, *ura3-1*, *leu2-3*, *112*, *trp1-1*, *his3-11*, *15*, *rad53Δ*::*HIS3*, *sml1-1*	[26]

required for triggering the *RAD53* dependent pathway. Possible explanations for this feature of *SET1* deletion include loss of Set1 protein (regardless of HMT activity), loss of histone H3 K4 methylation, or loss of methylation of an unknown factor(s). As a first step toward clarifying this, the expression of several stress responsive genes was tested in the *SET1* deletion background to identify those downstream of Rad53 (Fig. 1). The wild-type (UCC1001) and isogenic mutant *set1Δ* yeasts were grown to the exponential phase under standard conditions and harvested to prepare mRNAs. The expression of each gene, which is selected based on its inducible activity upon methylmethanesulfonate (MMS), a DNA damaging agent, was monitored by RT-PCR. *DDR48*, *ECM4*, *FLR1*, and *GTT2* were strongly induced to a different level by *set1Δ* even without any external DNA damage (Fig. 1A), whereas expression of *SED1*, which was used as a control, remained unaffected (Compare lanes 1 and 2). Each of the *set1Δ* inducible genes encodes a DNA damage-responsive protein (*DDR48*), a plasma membrane multidrug transporter (*FLR1*), a glutathione transferase (*GTT2*), and a factor involved in cell wall organization and biosynthesis (*ECM4*) [28–31]. However, other genes such as *SSU1* (plasma membrane sulfite pump; [32]) and *CPA2* (carbamoyl phosphate synthase; [33]) were not induced by *set1Δ*, although they were strongly induced by treatment with 0.1% MMS in both backgrounds [34]. This shows that a subset of stress responsive genes, as well as previously identified Rfa2-regulated genes such as *DDR48*, is induced by the loss of *SET1* even without any DNA damage.

In order to determine if induction was also dependent on Rad53, a *rad53Δ*/*set1Δ* double deletion strain was generated and the gene expression pattern was compared with that of *set1Δ*. This strain carries an extra *sml1-1* mutation to suppress lethality because *rad53Δ* is non-viable. Fig. 1B and C show that the induction of *ECM4*, *FLR1*, and *GTT2* was reduced by a *RAD53* deletion (lane 3), indicating that *set1Δ* dependent expression of those genes is in the downstream of Rad53. Therefore, we decided to analyze the *SET1* null phenotype by monitoring the expression of *ECM4*, *FLR1*, and *GTT2*.

The yeast SET domain protein, Set1, methylates histone H3 K4 and plays a role in transcription, heterochromatin maintenance, and DNA repair [5,12,35]. The methylation of H3 on K36 or K79 is also important for the euchromatic and heterochromatic functions, respectively. Like Set1, Dot1 plays a role in telomere silencing [10,11], while Set2 is implicated in the euchromatic transcription elongation by RNA polymerase II [15,36]. To determine if stress responsive gene expression is a common molecular consequence originating from the loss of H3 methylation, RT-PCR was performed with *DOT1* or *SET2* null yeasts (Fig. 1D). Interestingly, neither *dot1Δ* nor *set2Δ* induced gene expression, suggesting that the expression of a subset of stress responsive genes is very specific to the loss of *SET1*.

The histone H3 K4 mutation induces the stress responsive genes

To directly address the causative factor in this pathway that leads to the expression of stress responsive genes, yeast strains containing the wild-type and the K4A, K36A, K79A, and K4A/K79A histone H3 genes were generated as described in experimental procedures. The replacement of lysine with alanine specifically abolished K4 methylation (Fig. 2A). As for the *set1Δ* strain, the K4A allele strongly induced *FLR1*, *GTT2*, and *ECM4* (Fig. 2B, lane 2 and

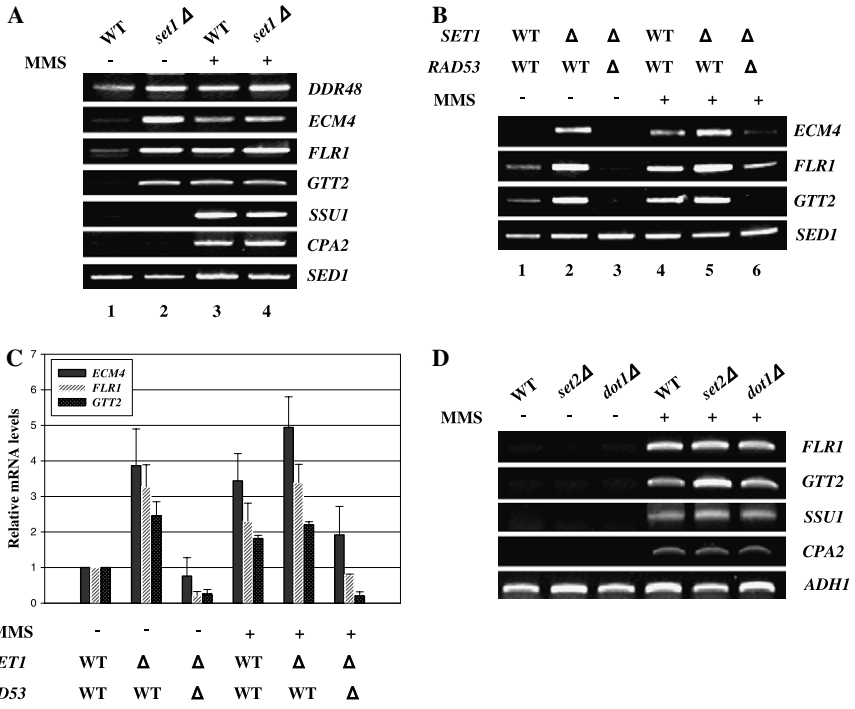

Fig. 1. *SET1* deletion leads to the expression of a subset of stress responsive genes in a Rad53 dependent manner. (A) Some stress responsive genes are induced by the loss of *SET1*. The *set1Δ*-dependent expression of the stress responsive genes in WT (UCC1001) and isogenic *set1Δ* yeasts was monitored by RT-PCR. The cells were grown to an exponential phase (OD 1.0 U of A_{600}), divided into two parts, and further grown in the presence (+) or absence (−) of MMS (final concentration of 0.1%) for 1 h. Total RNA was purified from each sample, and the expression of each gene was analyzed by RT-PCR, as described in experimental procedures. The level of the *SED1* transcript was used as a control. (B) The expression of the stress responsive genes, *ECM4*, *FLR1*, and *GTT2*, is dependent on Rad53. Yeast cells, WT (W3031A), *set1Δ* (YC81), and *rad53Δ*/*set1Δ* (YC83), were grown in the presence or absence of MMS to prepare total RNA as described in (A). The typical gene expression pattern of representative *ECM4*, *FLR1*, and *GTT2* is shown with *SED1* as a control. (C) Quantification of RT-PCR analysis in (B). The assay was repeated at least three times. Each band was quantified, normalized by *SED1* level and compared to the value of the wild-type (lane 1, assigned as 1). (D) The stress responsive gene expression levels in WT (BY4742), *set2Δ* (Y11256), and *dot1Δ* (Y14276) were analyzed by RT-PCR as in (A) *ADH1* is used as a control.

quantitated in 2C). Yeast with a K4A/K79A double mutation induced gene expression as greatly as K4A did, while the single K79A allele did not. This suggests that a H3 K4 substitution is essential for inducing the expression of the stress responsive genes. Since the effect of K4 substitution paralleled *set1Δ*, it is concluded that the lack of H3 K4 methylation by *SET1* removal directly triggers the expression of stress responsive genes via Rad53.

The mono-methylation of H3 K4 is essential to suppress the induction of stress responsive genes

Because histone H3 K4 is observed as mono-, di-, and tri-methylated, and many upstream factors have been identified to regulate the number of methyl groups on K4, we asked whether different states of methylation had unique roles in transcription or chromatin function. To test whether the K4 methylation state affected Rad53 mediated signaling, an attempt was made to remove significant amount of tri-methylation by deleting the RRM domain of Set1. The RRM domain is essential for tri-methylation and TPE, but is not required for mono-/di- methylation of K4 or for the normal growth [37,38]. When the wild-type and RRM-truncated Set1 (ΔRRM) were added back to the *set1Δ* yeasts, immunoblotting analysis clearly showed that ΔRRM failed to recover the tri-methylation of H3 K4 as reported (Fig. 3A). Nonetheless, ΔRRM, as well as wild-type Set1, efficiently suppressed the phenotype of the stress responsive gene induction (Fig. 3A and quantitated data in 3B). This suggests that the loss of the tri-methylation of H3 K4 is not essential to induce the stress responsive genes.

The H3 K4 methylation is regulated by several factors; RNA polymerase II associated Paf1 complex components and mono-ubiquitination of H2B on K123 by Rad6/Bre1. As shown in Fig. 4C, upstream effectors such as *RAD6*, *BRE1*, *PAF1*, and *RTF1* decreased di- and tri-methylation of histone H3 K4, whereas the mono-methylation of H3 K4 is unaffected [19,21,39]. The gene expression pattern in the *RAD6*, *BRE1*, *PAF1*, or the *RTF1* deletion backgrounds was analyzed to determine if *set1Δ* dependent expression of stress responsive genes is attributed to a significant loss of both di- and tri-methylation of K4. Surprisingly, deletion of upstream effectors did not induce the expression of *FLR1* or *GTT2* (Fig. 4). However, MMS still induced their expression, indicating that a lack of gene expression was not due to the compromised transcription activity caused by these genetic modifications. This suggests that the expression of some stress responsive genes

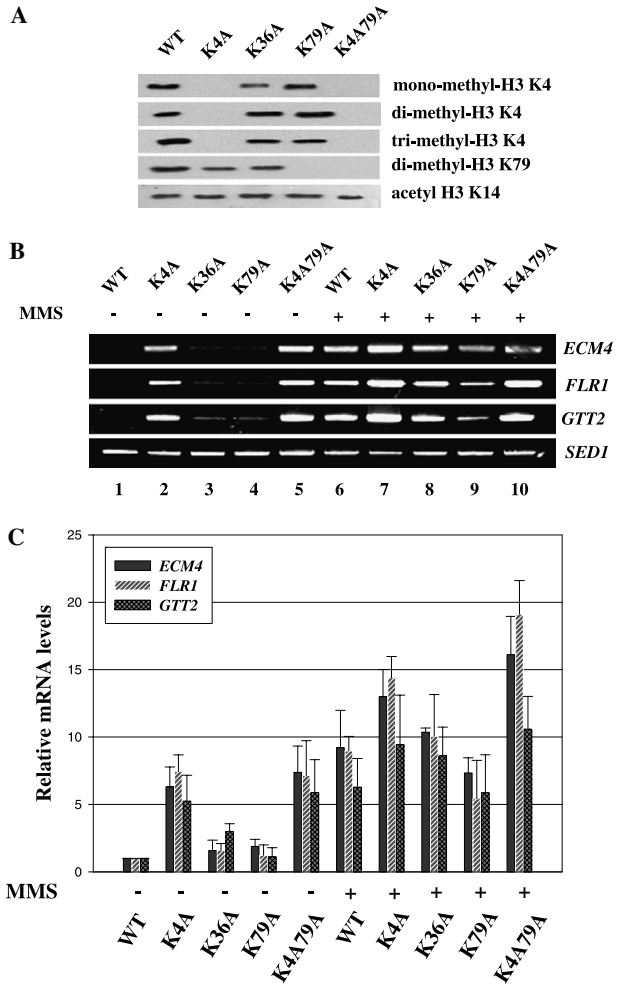

Fig. 2. Histone H3 lysine mutations induce the stress responsive genes. (A) Histone H3 methylation patterns of the various H3 mutant alleles. The yeast strains were generated by shuffling pWZ414-F13 (expressing wild-type histone H3-H4), pRS314-H3(K4A)-H4, pRS314-H3(K36A)-H4, pRS314-H3(K79A)-H4, and pRS314-H3(K4A/K79A)-H4 into WZY42. Total cell extracts were probed with histone H3 methylation specific antibodies. (B) The yeasts, as described in panel (A), were grown at 30 °C in YPD medium. The expression of each gene was monitored by RT-PCR as described. (C) Quantification of RT-PCR analysis in (B) as described in Fig. 1.

is not due to the loss of histone H3 K4 di- or tri-methylation. Given the phenotype of the upstream effector mutants and the result of RRM domain mutant of Set1, it is likely that a loss of the mono-methylation or a loss of total K4 methylation is directly linked to this pathway.

Discussion

This study showed that *set1Δ* resulted in the induction of several stress responsive genes in a *RAD53* dependent manner. Among the methylation targets of Set1, histone H3 K4 might be directly involved in this pathway, because a loss of histone H3 K4 methylation is necessary to induce the target genes. Interestingly, this pathway has differentiated the roles of H3 K4 methylation states. The loss of di- or tri-methylation, which is implicated in the active transcription, did not induce the stress responsive genes, while the total loss of methylation strongly induced them. The presence of mono-methylation of H3 K4 was sufficient to suppress the Rad53 dependent signaling pathway that leads to the induction of stress responsive genes.

Increased survival of the checkpoint mutants in the *set1Δ* background may be due to the elevated repair capacities of the mutants. Rad53 must mediate the signaling pathway that is responsible for increased repair capacities. Our present results support the hypothesis that there is a chromatin surveillance system that is mediated by Rad53, which responds to histone methylation. Loss of H3 K4 di- and tri-methylation is tolerated but further loss of methylation might affect the overall chromatin organization, which is detected by the Rad53 surveillance system. Interestingly, a lack of histone H3 K4 methylation does not induce the phosphorylation of Rad53, which is a marker for checkpoint activation, nor damages the DNA, indicating that it differs from the known Mec1 dependent checkpoint pathway [25,26].

Di- and tri-methylation of H3 K4 is highly correlated to active transcription and is dependent on several upstream factors [40–42]. Our study showed that a loss

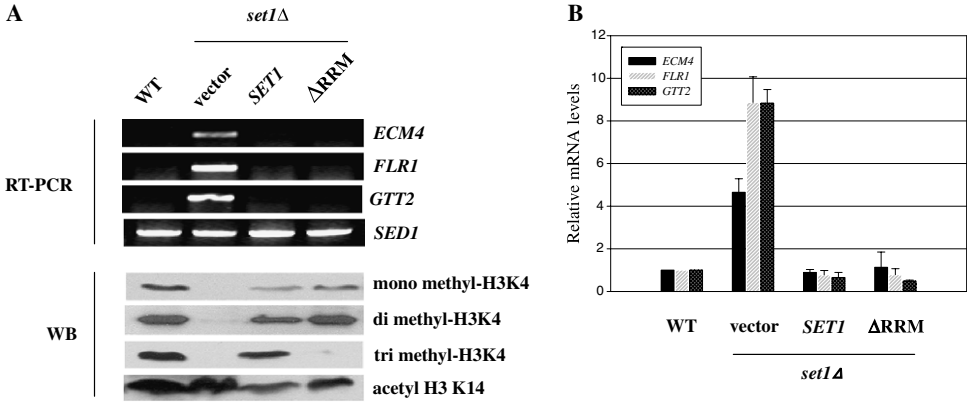

Fig. 3. The suppression of stress responsive gene induction by Set1 ΔRRM. (A) Yeast *set1Δ* (YC81) was transformed with the plasmids expressing no gene, Set1 wild-type, or Set1 ΔRRM. Gene expression was analyzed by RT-PCR (upper panel) and histone methylation pattern was confirmed by Western blot assay with whole cell extracts prepared form each cell (bottom panel). (B) Quantification of RT-PCR analysis in (A) as described in Fig. 1.

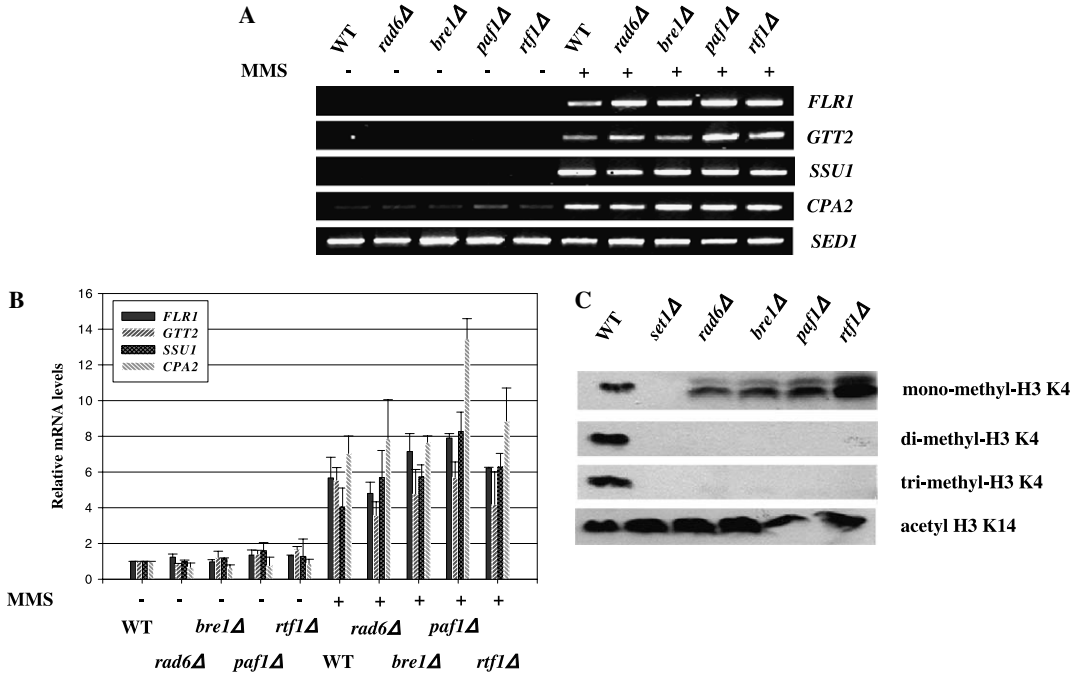

Fig. 4. Induction of the stress responsive genes is not dependent on the upstream effectors of histone H3 K4 methylation. (A) Steady state mRNA levels for *FLR1*, *GTT2*, *SSU1*, *CPA2*, and *SED1* were measured by RT-PCR. WT (BY4742), *rad6Δ* (Y14425), *bre1Δ* (Y13771), *paf1Δ* (Y15727), and *rtf1Δ* (Y14611) strains were grown and treated with or without 0.1% MMS for 1 h before preparing total RNA. (B) Quantification of RT-PCR analysis in (A) as described in Fig. 1. (C) H3 K4 methylation patterns of the upstream effector deletion mutants by immunoblotting analysis.

of upstream factors (Rad6, Bre1, Paf1, and Rtf1) did not mimic the molecular phenotype induced by the loss of *SET1*, even though they play a common role in euchromatic transcription and heterochromatic gene silencing through H3 K4 methylation. This indicates that different states of H3 K4 methylation play different roles. In agreement with the enrichment of H3 K4 tri-methylation in actively transcribed regions, multiple rounds of methylation are regulated by many transcription associated factors, indicating its role in target gene expression. In contrast to the multiple methylation of K4, the role of mono-methylation has not been well characterized. This study showed that mono-methylation was sufficient to suppress the Rad53 dependent stress response pathway, which indicates that minimum chromatin structure can be maintained by H3 K4 mono-methylation. H3 K4 mono-methylation may therefore play a role that is independent of euchromatic transcription and heterochromatic gene silencing mediated by controlled distribution of Sir proteins. The RRM domain of Set1, which is responsible for histone H3 tri-methylation, is essential for the heterochromatic gene silencing [37,38]. In our study, the RRM deleted Set1 mutant suppressed the stress responsive gene induction, suggesting that a loss of tri-methylation or a perturbed heterochromatin structure, which is sufficient to induce a silencing defect, is not the direct cause of induction of the stress responsive genes. Although we know K4 mono-methylation is essential for the suppression of Rad53 mediated stress responsive gene induction, it is not clear yet whether loss of mono-methylation is sufficient to induce this pathway, as we are not able to produce conditions that remove mono-methylation selectively.

A recent report describes that *set1Δ* results in large changes in global gene expression profile [43]. Interestingly, hierarchical clustering analysis of *set1Δ* gene expression profile revealed a close similarity between *set1Δ* and *mad2Δ* [43]. Mad2 contains HORMA domain, which is implicated in the binding to specific chromatin structures [44]. It suggests that the transcriptional defect in the *set1Δ* may be due to changes in global chromatin structure. Because more than 50% of the total histone H3 is methylated on K4 in yeast [45], it might be an important determinant for construction of the global chromatin structure by a proper methylation itself or by an interaction with the K4 binding factors. Clearly, further study will be needed to determine the role of histone H3 K4 methylation to maintain the overall chromatin structure and the mechanism for how the altered chromatin structure is recognized and signaled to Rad53 and further to the stress responsive genes.

Acknowledgments

We thank Drs. S. Buratowski, A. Verreault, T. Kouzarides, S. Roeder, S. Dent-Roth, S.D. Briggs., S. Hanes, and B. Cairns for yeast strains and plasmids and A. Verreault for critical reading of the manuscript. This work was supported by the grant from the Korea Research Foundation Grant (R04-2004-000-10188-0, 2005-0497-000) to E.-J. Cho.

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