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# Methylation of H4 lysines 5, 8 and 12 by yeast Set5 calibrates chromatin stress responses

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## Abstract

Methylation of histones is central to chromatin regulation, and thus novel mechanisms regulating genome function can be revealed through the discovery of new histone methyl marks. Here, we identify Set5 as the first histone H4 methyltransferase in budding yeast, which monomethylates the important H4 lysine residues 5, 8 and 12. Set5's enzymatic activity functions together with the global chromatin-modifying complexes COMPASS and NuA4 to regulate cell growth and stress responses.

A major regulatory mechanism of chromatin function is the dynamic covalent post-translational modification (PTM) of histone N-terminal tails, which controls the structure and accessibility of chromatin to influence diverse processes such as gene expression and DNA repair[1]. One critical histone modification is the methylation of lysine (K) residues, catalyzed by lysine methyltransferases (KMTs), which add a mono-, di- or trimethyl mark. The most extensively characterized *Saccharomyces cerevisiae* KMTs are the evolutionarily conserved enzymes Set1, Set2 and Dot1, which methylate lysines 4, 36 and 79 of histone H3, respectively[2]. Proteomic studies highlight the existence of numerous methylation marks on the other core histones (H4, H2A, and H2B) for which the KMTs remain to be discovered[3,4], suggesting that new mechanisms of genome regulation will be revealed through the identification and analysis of the enzymes that generate these histone marks.

To identify new methylation events at chromatin, we performed a screen for activity of yeast candidate KMTs on histone tails. *In vitro* methylation assays of the uncharacterized KMTs (Supplementary Methods, Supplementary Table 1) revealed robust methylation on the histone H4 tail by the enzyme Set5 (Fig. 1a). Set5 also methylated full-length recombinant H4 and native H4 present in bulk purified histones (Fig. 1b). Set5 showed weak activity on purified nucleosomes *in vitro*, although it was able to methylate H4 in chromatin fractions from yeast (data not shown). The N-terminal tail of H4 contains 6 lysine (K) residues



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**Author Contributions**

E.M.G., G.M. and O.G. conceived of and designed the experiments and wrote the manuscript. G.M. and E.M.G. contributed equally to the work and performed all biochemical and cellular experiments. Mass spectrometry analysis was performed by N.L.Y. and B.A.G.

(Supplementary Fig. 1a), none of which were known targets of methyltransferases in budding yeast. To map the sites of methylation, a library of H4 tail constructs in which all lysines have been mutated to arginine except at one position was generated. Set5 only methylated H4 tails that maintained a lysine at position 5, 8 or 12 (Fig. 1c, Supplementary Fig. 1b). Additionally, Set5 was able to methylate H2A at lysines 4 and 7 (Supplementary Fig. 1c), although to a lesser extent than H4 (H2A represented by * in Fig. 1b).

Mass spectrometry analysis of an H4 peptide methylated by Set5 revealed that Set5 is able to monomethylate lysines 5, 8 and 12 (Supplementary Fig. 1d,e). We next raised antibodies against the H4K5me1 and H4K8me1 epitopes (anti-H4K5me1 and anti-H4K8me1, respectively), which showed high specificity in peptide dot blot assays (Supplementary Fig. 2). Using these antibodies, H4K5me1 and H4K8me1 were detected on recombinant H4 methylated by Set5 (Fig. 1d). Taken together, these data demonstrate that Set5 can monomethylate lysines 5, 8 and 12 of histone H4 *in vitro*.

To determine if the H4 tail is monomethylated by Set5 in cells, we purified histones from wild type and *set5*Δ yeast and probed them with our monomethyl-specific antibodies. *set5*Δ yeast showed a specific decrease in the H4K5me1 and H4K8me1 signal compared to wild type (Fig. 2a). Quantitative mass spectrometry of the purified histones demonstrated a substantial decrease in the amount of monomethyl H4 species in *set5*Δ cells (Fig. 2b). The MS/MS spectrum revealed the existence of all three monomethyl marks, with H4K8me1 as the predominant species (Supplementary Fig. 3a). These data provide evidence that H4K5me1, H4K8me1 and H4K12me1 exist *in vivo*. Furthermore, we show that Set5 is required for H4K5me1 and H4K8me1 generation in cells.

Our data suggest that Set5 might interact with chromatin to methylate H4. Consistent with this hypothesis, immunofluorescence revealed nuclear and cytoplasmic localization of tagged Set5 (Supplementary Fig. 3b). We generated an antibody against recombinant Set5 and confirmed that Set5 is present in both soluble and chromatin-enriched fractions of yeast lysates (Supplementary Fig. 3c), suggesting that Set5 associates with chromatin. Furthermore, co-immunoprecipitation experiments demonstrated an interaction between Set5 and histones H3 and H4 *in vivo* (Fig. 2c). Overall, these results argue that Set5 is a novel chromatin-associated methyltransferase that monomethylates histone H4 in cells.

Genome-scale studies performed with the commercial homozygous diploid Yeast Knockout (YKO) collection have reported defects in cellular stress responses and increased sporulation in the absence of Set5[5–8]. However, we did not observe sensitivity of multiple independently generated *set5*Δ strains to the reported cellular stresses nor an increase in sporulation efficiency (Supplementary Fig. 4a,b; yeast strains listed in Supplementary Table 2). Further phenotypic analysis showed that Set5 does not appear to be regulated by or contribute to the control of the cell cycle (Supplementary Fig. 4c,d). Finally, deletion of *SET5* resulted in only minor changes in global gene expression (data not shown), indicating that loss of Set5 alone does not drastically affect global transcription.

Chromatin function is frequently regulated by crosstalk between different histone-modifying complexes[2]. To determine if Set5 functionally interacts with other SET domain candidate

KMTs, double mutants were generated and subjected to genotoxic and cellular stresses. Intriguingly, of the six SET domain-containing proteins analyzed, only a *set5*Δ *set1*Δ strain showed decreased fitness in the presence of stress compared to either single mutant (Fig. 3a, Supplementary Fig. 5a). Moreover, a *set1*Δ strain harboring an integrated point mutation of a conserved catalytic residue (Y402A) in the *SET5* gene (Fig. 3b and Supplementary Fig. 5b,c) showed similar and even stronger phenotypes to that of *set5*Δ *set1*Δ cells, indicating that the functional genetic interaction between *SET1* and *SET5* is dependent on the catalytic activity of Set5 (Fig. 3c).

Set1 is an integral member of the multi-subunit chromatin-modifying COMPASS complex that mediates methylation of histone H3 at lysine 4 (H3K4)[9,10]. We observed a subtle but reproducible growth defect upon stress in *set5*Δ cells lacking the COMPASS component Sdc1 (Supplementary Fig. 5d), providing additional evidence that *SET5* may functionally interact with COMPASS. Interestingly, H3K4R-expressing yeast, which were unable to grow in the stress conditions tested except cycloheximide, showed slower growth upon deletion of *SET5* in cycloheximide (Supplementary Fig. 5e). Together, these results suggest cooperation between methylation at H3K4 by Set1 and methylation at the H4 tail by Set5 to regulate cell growth under stress conditions.

Set5's target residues on H4 are also subject to acetylation by the NuA4 complex, which plays important roles in cell growth and division[11]. Yeast lacking Yng2—a core component of NuA4—are compromised for global histone H4 acetylation but remain viable with slow growth[12]. Surprisingly, cells lacking both *YNG2* and *SET5*, or *YNG2* and Set5's catalytic activity, were more severely impaired for growth than *yng2*Δ alone. A comparable growth defect was observed in double mutants under both normal and stress conditions (Fig. 3,d,e, Supplementary Fig. 5f). We postulate that methylation and acetylation of H4 either (1) exist in distinct histone subpopulations or (2) are present on different lysines of the same H4 tail, and thereby cooperate to promote cellular fitness. It is also possible that the functional interaction is dependent on other substrates of these enzymes. Regardless, these results demonstrate that Set5 and Yng2 function in parallel to promote optimal cell growth.

Consistent with our genetic interactions between *SET5* and members of COMPASS and NuA4, impaired growth is observed when both COMPASS and NuA4 are mutated[13]. Combined mutation of their target histone residues results in a severe growth defect[14,15] (Supplementary Fig. 5g). This phenotype is likely to be largely dependent on acetylation, but the existence of methylation at H4 lysines 5, 8 and 12 and *SET5's* genetic interactions argue that methylation may contribute to H4 mutant phenotypes as well.

In summary, we demonstrate novel monomethylation events on histone H4 at lysines 5, 8 and 12, which are catalyzed by the first known H4 methyltransferase in budding yeast, Set5. We have implicated Set5's function in cellular fitness and the response to stress together with two global chromatin-modifying complexes, COMPASS and NuA4. Thus, Set5-dependent methylation adds a new layer of regulation to chromatin-based signaling networks. This work expands our current knowledge of the epigenome, increasing the complexity of the array of known histone modifications, and highlights new mechanisms by which histone methylation impacts cellular fitness.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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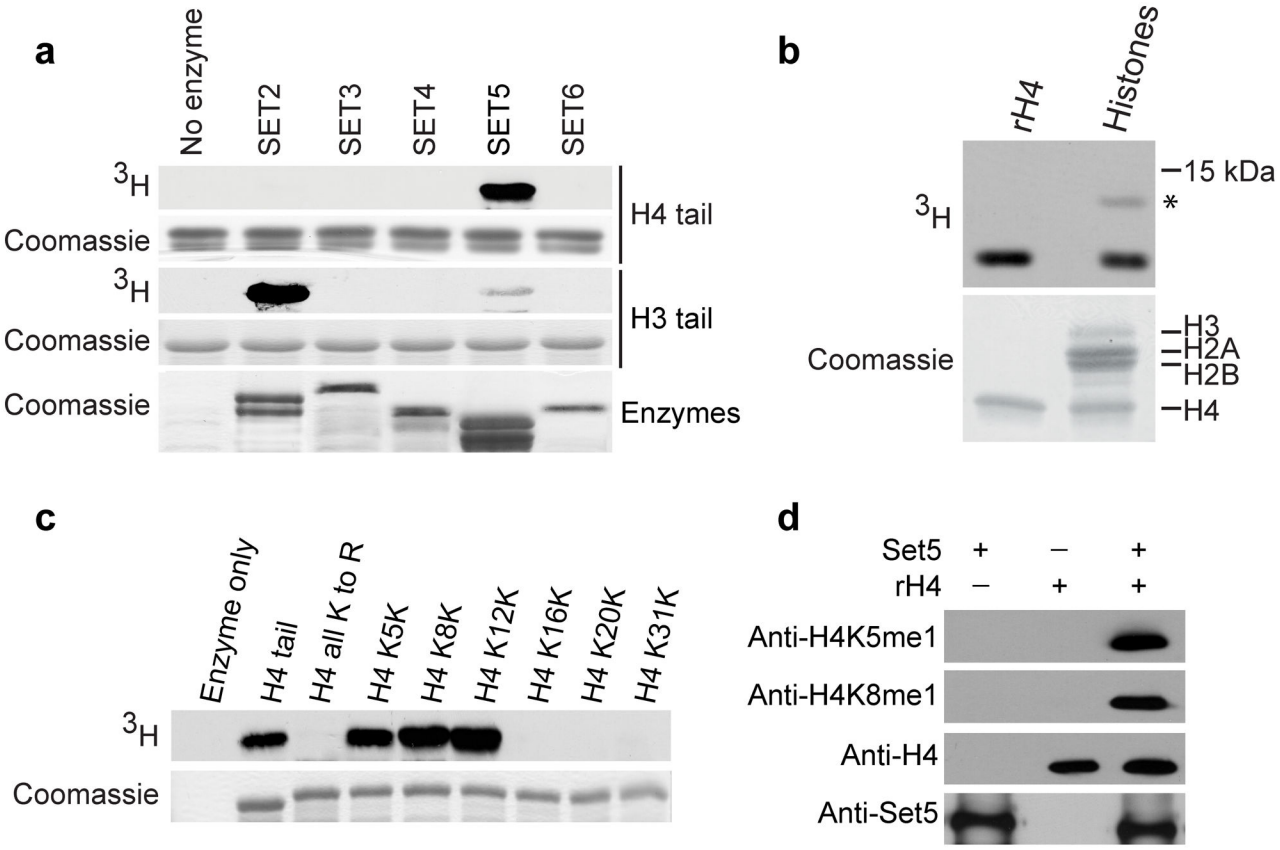


**Figure 1.**

Identification of Set5 as an H4 methyltransferase. (**a**) Methylation assays with recombinant SET domain enzymes, H3 and H4 tails using $^{3}$H-SAM as the methyl donor. Autoradiograph and Coomassie (loading control) are shown. (**b**) Set5 methylates recombinant H4 (rH4) and H4 from native histones purified from calf thymus (* indicates H2A). (**c**) Set5 methylates lysines 5, 8 and 12. Methylation assay on H4 tails with lysines mutated to arginine (all K to R) or single lysines maintained and all other lysines mutated to arginine. (**d**) Monomethylation of H4K5 and H4K8 on rH4. Immunoblots with the indicated antibodies are shown.

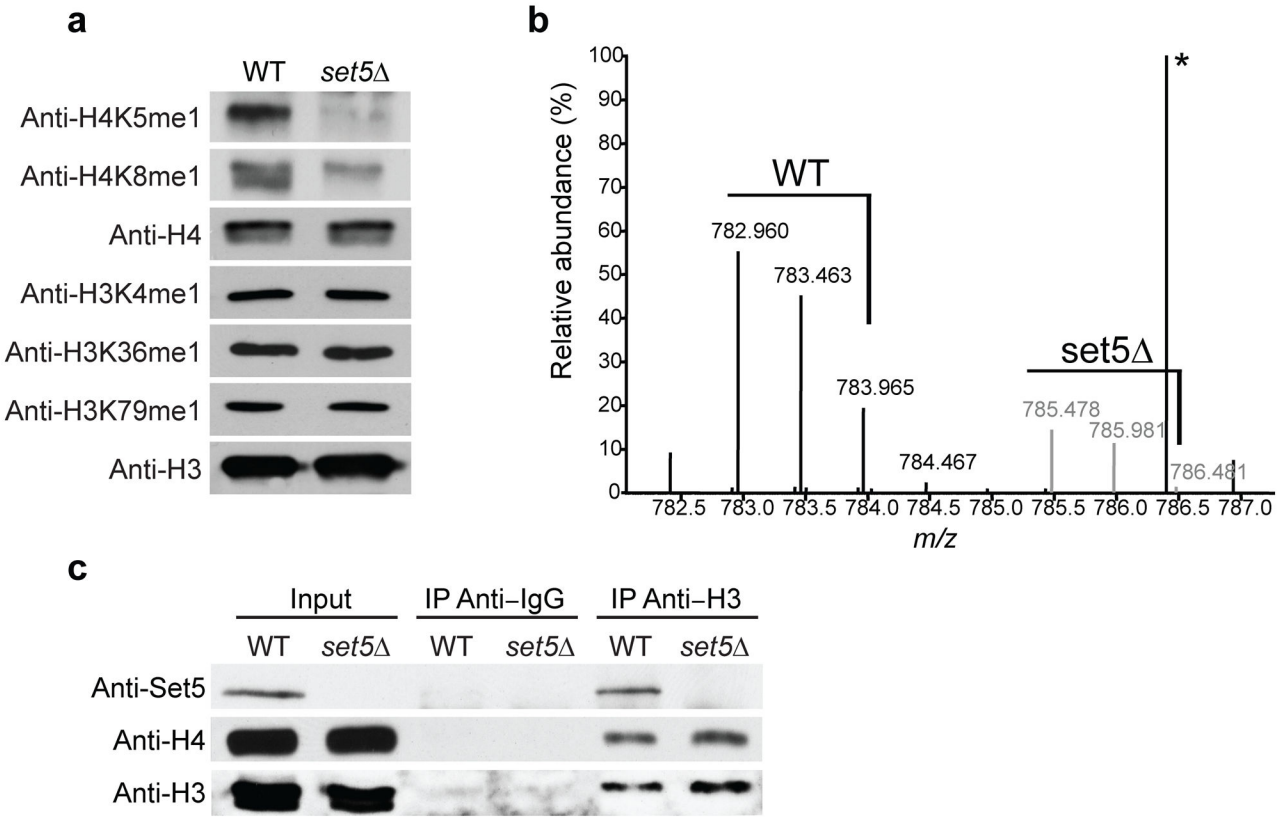


**Figure 2.**

Set5 monomethylates H4 in cells. (**a**) Histones from WT and *set5*Δ yeast were subjected to immunoblotting with the indicated antibodies. (**b**) Quantitative mass spectrometry of purified histone H4 from WT (chemically-labeled with D0-propionyl; black), and from *set5*Δ (D5-propionyl; gray) shows a peptide corresponding to monomethylation of H4, amino acids 4–17. Relative abundance of this peptide decreases in the *set5*Δ cells (black versus gray signal; *non-specific peak). (**c**) Set5 co-precipitates with H3 and H4. Immunoprecipitation using anti-H3 or IgG was performed from WT and *set5*Δ cells; immunoblots are shown.

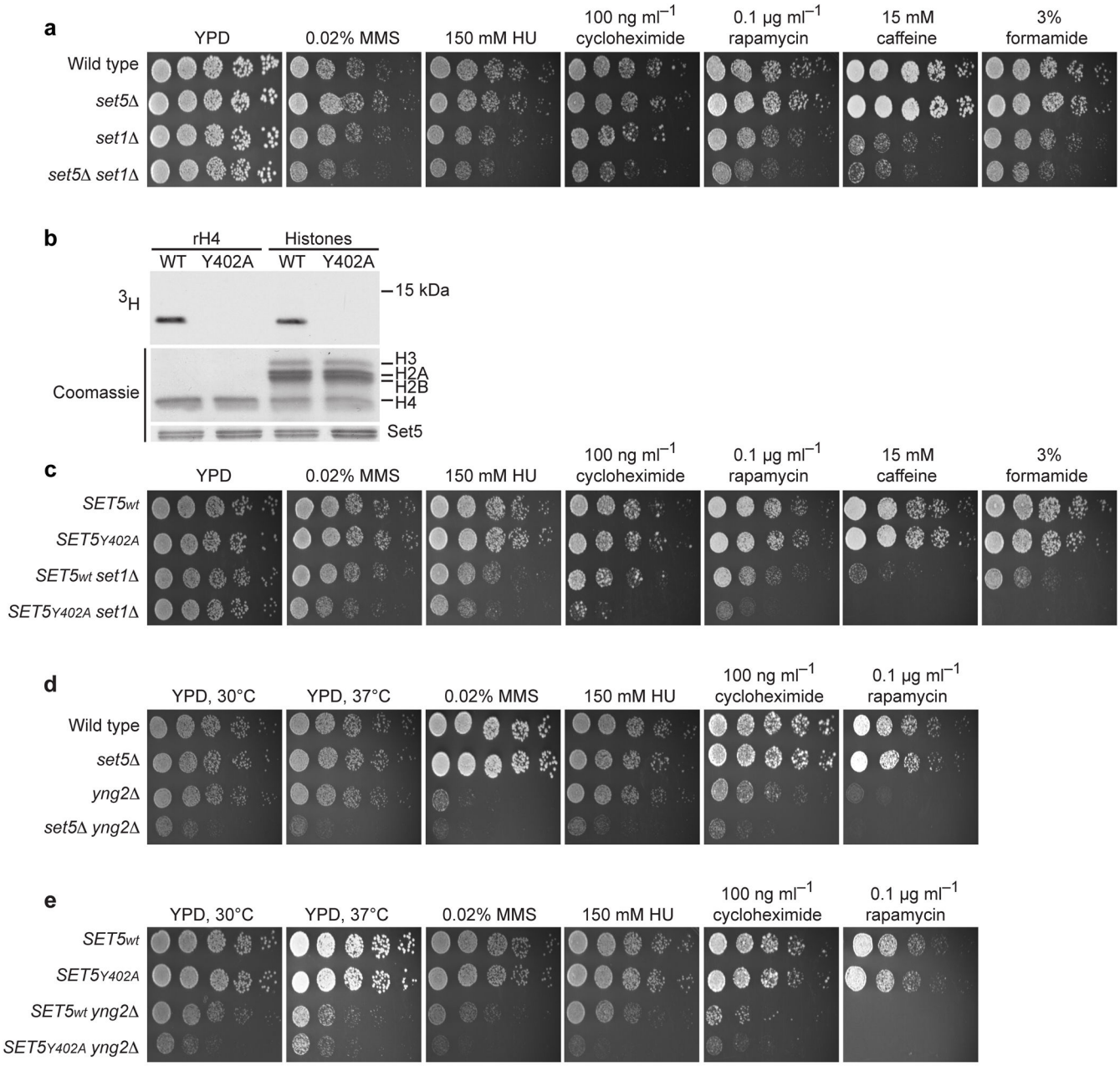


**Figure 3.**

Set5 functions with COMPASS and NuA4 complexes to regulate cell growth and stress responses. (**a**) Decreased fitness of *set5*Δ *set1*Δ upon stress. Four-fold serial dilutions of yeast were spotted and grown for 2–5 days at 30°C. (MMS: methylmethane sulfonate; HU: hydroxyurea) (**b**) Mutation of the conserved Y402 to alanine abolishes Set5's catalytic activity. Methylation assay with wild type or Y402A Set5 on rH4 and native histones. (**c**) The catalytic activity of Set5 is required for normal growth of *set1*Δ cells under stress. Deletion (**d**) or catalytic inactivation (**e**) of *SET5* in *yng2*Δ strains impairs cell growth.