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

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Methylation of histones is central to chromatin regulation, and thus previously unknown 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, which monomethylates the critical H4 lysine residues 5, 8 and 12 in budding yeast. 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 of histone N-terminal tails, which controls the structure and accessibility of chromatin to influence diverse processes such as gene expression and DNA repair. One critical histone modification is the methylation of lysine 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. These studies highlight the existence of numerous methylation marks on other core histones (H4, H2A and H2B), for which the KMTs remain to be discovered, 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 carried out a screen for activity of yeast candidate KMTs on histone tails. In vitro methylation assays of the uncharacterized KMTs (Supplementary Methods and 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 was able to methylate H4 in chromatin fractions from yeast, although it only showed weak activity on purified nucleosomes in vitro (data not shown). The N-terminal tail of H4 contains six lysine residues (Supplementary Fig. 1a), none of which were known targets of methyltransferases in budding yeast. To map the sites of methylation, we generated a library of H4 tail constructs in which all lysines were mutated to arginine, except at one position. Set5 only methylated H4 tails that maintained a lysine at position 5, 8 or 12 (Fig. 1c and 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 is represented by the “*“ in Fig. 1b).

MS 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 to 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 set5Δ yeast and probed them with our monomethyl-specific antibodies. The set5Δ yeast showed a specific decrease in the H4K5me1 and H4K8me1 signal compared to the wild type (Fig. 2a). Quantitative MS of the purified histones demonstrated a substantial decrease in the amount of monomethyl H4 species in set5Δ 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.

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Figure 2  Set5 monomethylates H4 in cells. 
(a) Histones from WT and set5A yeast were subjected to immunoblotting with the indicated antibodies. (b) Quantitative MS of purified histone H4 from WT (chemically labeled with D0-propionyl, black) and from set5A (D5-propionyl, gray) yeast shows a peptide corresponding to monomethylation of H4, amino acids 4–17. The relative abundance of this peptide decreases in the set5A cells (black versus gray signal; *nonspecific peak). (c) Set5 coprecipitates with H3 and H4; Immunoprecipitation (IP) using anti-H3 or IgG was carried out from WT and set5A cells; immunoblots are shown.

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 to 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, communoprecipitation experiments demonstrated an interaction between Set5 and histones H3 and H4 in vivo (Fig. 2c). Overall, these results argue that Set5 is a previously unknown chromatin-associated methyltransferase that monomethylates histone H4 in cells.

Genome-scale studies carried out with the commercial homozygous diploid yeast knockout collection have reported defects in cellular stress responses and increased sporulation in the absence of Set5 (refs. 5–8). We did not observe sensitivity of multiple independently generated set5A strains to the reported cellular stresses nor did we find an increase in sporulation efficiency (Supplementary Fig. 4a,b; yeast strains are 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 cross-talk between different histone-modifying complexes2. To determine if Set5 functionally interacts with other SET domain candidate KMTs, double mutants were generated and subjected to genotoxic and cellular stresses. Notably, of the six SET domain–containing proteins analyzed, only a set5A set1A strain showed decreased fitness in the presence of stress compared to either single mutant (Fig. 3a and Supplementary Fig. 5a). Moreover, a set1A 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 set5A set1A 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 multisubunit 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 set5A cells lacking the COMPASS component Sdc1 (Supplementary Fig. 5d), providing additional evidence that SET5 may functionally interact with COMPASS. Notably, H3K4R-expressing yeast, which were unable to grow in the stress conditions tested except in cycloheximide, showed slower growth upon deletion of SET5 in cycloheximide (Supplementary Fig. 5e). Together, these results suggest there is cooperation between

Figure 3  Set5 functions with COMPASS and NuA4 complexes to regulate cell growth and stress responses. (a) Decreased fitness of set5A set1A upon stress. Four-fold serial dilutions of yeast were spotted and grown for 2–5 d at 30 °C. (YPD, yeast extract peptone dextrose; MMS, methylmethane sulfonate; HU, hydroxyurea). (b) Mutation of the conserved Tyr402 to alanine abolishes Set5’s catalytic activity. Shown are results of the methylation assay with wild-type or Y402A Set5 on H4 and native histones. (c) The catalytic activity of Set5 is required for normal growth of set1A cells under stress. (d,e) Decreased fitness of set5A yng2A cells. Deletion (d) or catalytic inactivation (e) of SET5 in yng2A strains impairs cell growth.
methyltransferase Set5 and Yng2 function in parallel to promote optimal cell growth. A comparable growth defect was observed in double mutants under both normal and stress conditions (Fig. 3d,e and Supplementary Fig. 5f). We postulate that methylation and acetylation of H4 either (i) exist in distinct histone subpopulations or (ii) 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 observations of genetic interactions between SET5 and members of COMPASS and NuA4, impaired growth is observed when both COMPASS and NuA4 are mutated. Combined mutation of their target histone residues results in a severe growth defect (Supplementary Fig. 5g). This phenotype is likely to be mainly dependent on acetylation, but the existence of methylation at H4 lysines 5, 8 and 12 and SET5’s genetic interactions suggest that methylation may contribute to H4 mutant phenotypes as well.

In summary, we demonstrate previously unknown 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 affects cellular fitness.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.