Insights into Transcriptional Regulation by Individual H3K4 Methylation Marks in S. Cerevisiae

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Abstract

Set1 is a lysine methyltransferase in *S. cerevisiae* that catalyzes the mono, di and tri methylation of the fourth lysine on the amino terminal tail of histone H3 (H3K4). Set1-like methyltransferases are evolutionarily conserved, and research has linked their function to developmental gene regulation and several cancers in higher eukaryotes. Set1 is a member of the multiprotein COMPASS complex in *S. cerevisiae*. The H3K4 methylation activity of COMPASS regulates gene expression and chromosome segregation *in vivo*. The three distinct methyl marks on histone H3K4 act in discrete ways to regulate transcription. Trimethylation of H3K4 is usually associated with active transcription whereas dimethylation of H3K4 is associated with gene repression. In this study, amino acid substitution mutants of *SET1* that encode partial function Set1 proteins capable of H3K4me1, H3K4me1 and H3K4me2, or H3K4me1and H3K4me3 were analyzed to learn more about the roles of individual H3K4 methyl marks in transcription. The findings reveal a previously unappreciated role for H3K4me1 in activation of transcription of the *HIS3* gene in *S. cerevisiae* cultures grown under histidine-starvation conditions. Surprisingly, induction of the *HIS3* gene in cultures grown under histidine starvation is not accompanied by significant changes in the profiles of H3K4-methylated nucleosomes at the *HIS3* gene in *SET1* wild-type strains and *set1* partial-function mutants. The data show that H3K4me1 supports induction of *HIS3* mRNA to wild-type levels under histidine-starvation conditions and that higher-order H3K4 methylation (H3K4me2 and H3K4me3) is not required.

Introduction

The structural organization of eukaryotic DNA into chromatin regulates transcription by RNA polymerases (Han and Grunstein 1988, Izban and Luse 1992, Kornberg and Thonmas 1974, Wasylyk and Chambon 1979, Workman and Kingston 1998). Nucleosomes, the fundamental structural subunits of eukaryotic chromatin, consist of 147 bp of double-stranded DNA wound around a histone core octamer (Lawrence, et al. 2016, Luger, et al. 1997, Venkatesh and Workman 2015). The histone core octamer has two copies of each of the histones, H2A, H2B, H3 and H4. To overcome the physical barrier imposed by chromatin, RNA polymerases rely on the action of *trans*-acting proteins and protein complexes, including chromatin remodelers, transcription factors, co-activators and histone-modifying enzymes make chromatin accessible to RNA polymerase II to access DNA sequences (Castillo, et al. 2017, Chatterjee, et al. 2011, Côté, et al. 1998, Lee, et al. 1993, Lee, et al. 2007, Santos-Rosa, et al. 2003).

Post-translationally modified histones regulate chromatin characteristics and functions, including accessibility, compaction, nucleosome dynamics, replication, and transcription (Chatterjee, et al. 2011, Lawrence, et al. 2016, Santos-Rosa, et al. 2003, Soriano, et al. 2014, Zhang, et al. 2015). Histones are targets for many covalent modifications including methylation, acetylation, phosphorylation and ubiquitylation (Allfrey, et al. 1964, Zhang, et al. 2015). Covalently modified histones in chromatin may recruit or facilitate the interaction of effectors, such as chromatin-remodeling complexes and transcription factors, with chromatin (Castillo, et al. 2017, Lawrence, et al. 2016). For example, acetylated lysines are recognized by bromodomain-containing proteins ((Dhalluin, et al. 1999); reviewed

in (Filippakopoulos and Knapp 2014)), like the Swi2/Snf2 subunit in the SWI/SNF chromatin-remodeling complex, which aids in the generation of open and accessible chromatin ((Tamkun, et al. 1992), reviewed in (Swygert and Peterson 2014)). The dynamic exchange of acetyl groups onto and off of histones acts as a switch between active and repressive chromatin, respectively (reviewed in (Eberharter and Becker 2002, Kuo and Allis 1998)). In contrast to histone acetylation, the outcome of histone methylation is context-dependent; methylated histones have the capacity to activate or repress transcription (Hyun, et al. 2017).

Methylation of histones is catalyzed by histone methyltransferases (HMTases). Set1 is an HMTase that catalyzes the mono-, di- and trimethylation of the fourth lysine on the amino terminal tail of histone H3 (Briggs, et al. 2001, Qu, et al. 2018, Shilatifard 2012). *S. cerevisiae* Set1 is a member of the COMPASS complex (Complex Associated with Set1, Figure 1) (Bae, et al. 2020, Briggs, et al. 2001, Miller, et al. 2001, Morillon, et al. 2005, Mueller, et al. 2006, Roguev, et al. 2001). The function of members of the SET family of H3K4 HMTases are conserved in eukaryotes (Miller, et al. 2001, Takahashi, et al. 2011). Mutations in Set1-like H3K4 HMTases alter segmentation in *Drosophila melanogaster* and floral development in *Arabidopsis thaliana* (Breen 1999, Jiang, et al. 2011, Shilatifard 2012). The human homologs of Set1, including MLL1 and its translocation alleles, are implicated in several hematological malignancies, such as mixed lineage leukemia, acute myeloid leukemia and acute lymphoblastic leukemia (Kandoth, et al. 2013, Roguev, et al. 2001, Ruault, et al. 2002, Shilatifard 2012, Slany 2009). The conservation of Set1-like proteins from yeast to humans underscores their importance in biological processes.

The effect of H3K4 methylation on chromatin accessibility depends on the chromatin context, consistent with data showing that H3K4 mono-, di-, and trimethylation may have different effects on gene transcription (Kusch 2012, Pokholok, et al. 2005). Chromatin immunoprecipitation (ChIP) and ChIP-seq experiments revealed that the distribution of K4-monomethylated (H3K4me1), K4-dimethylated (H3K4me2) and K4-trimethylated (H3K4me3) histone H3 across open reading frames (ORFs) is not identical (Bernstein, et al. 2005, Soares, et al. 2017). For the most highly expressed genes in *S. cerevisiae*, nucleosomes with H3K4me3 peak at the promoter and up to ~200 bp beyond the transcription start site, nucleosomes with H3K4me2 are enriched in the middle of the ORF, and nucleosomes with H3K4me1 are found predominantly at the 3' end of an ORF (Berger 2007, Pokholok, et al. 2005). Methylation of histones is not known to change the structure of chromatin on its own, instead methylated histones act by recruiting effector proteins to chromatin (Musselman, et al. 2012, Pray-Grant, et al. 2005, Taverna, et al. 2006).

H3K4-methylated histones may be recognized by chromodomain-containing proteins (Eissenberg 2012) and plant homeodomain (PHD) finger domains, including the COMPASS member Spp1 (He, et al. 2019). H3K4me3 is usually associated with active transcription (Kusch 2012, Ng, et al. 2003, Schneider, et al. 2005). H3K4me3 is recognized by the chromodomain-containing protein, Chd1, a member of the SAGA transcription coactivator complex (Pray-Grant, et al. 2005). The transcription factor TAF3 also interacts with trimethylated H3K4 to recruit $TF_{II}D$ to gene promoters (Vermeulen, et al. 2007). In yeast, the

chromatin remodeler Isw1 interacts with methylated H3K4 to generate open, accessible chromatin at the 5' end of the *MET16* gene (Santos-Rosa, et al. 2003). H3K4me3 is also recognized by other protein complexes, some of which are negative effectors of transcription (Musselman, et al. 2012, Taverna, et al. 2006). In response to DNA damage, the PHD domain of ING2 (INhibitor of Growth 2), a subunit of the mSin3a−HDAC1 histone deacetylase complex, binds to H3K4me3, stabilizing mSin3a−HDAC1 at the promoter of cyclin D1 gene and other proliferation genes, leading to repression of transcription (Shi, et al. 2006). H3K4me2 is associated with repression of transcription and has been shown to interact with HDACs that reduce acetylated histones at the 5' ends of some highly expressed genes (Kim and Buratowski 2009, Pinskaya and Morillon 2009).

Progress is being made toward understanding the roles H3K4me1 plays in gene regulation. In yeast, H3K4me1 regulates chromatin remodeling at osmostress-induced genes (Nadal-Ribelles, et al. 2015). In mammalian cells, there is compelling evidence that H3K4me1 promotes interactions between enhancers and promoters by facilitating the binding of chromatin remodelers (Local, et al. 2018, Yan, et al. 2018). However, a catalytically defective H3K4 HMTase also facilitates enhancer-promoter interactions meaning that an additional mechanism that doesn't require H3K4me1 is likely to promote enhancer-mediated effects as well (Dorighi, et al. 2017, Rickels, et al. 2017). Recent work has shown that the patterns of H3K4me1 with H3K4me3 and H3K27me3 at promoters in human and mouse germ cells and ESCs may predict the transcriptional state of a promoter (Bae and Lesch 2020).

Set1 is the only H3K4 HMTase in *S. cerevisiae* making it an excellent system to study the effect of the three H3K4 methyl marks on transcription. In a previous study, mutants of *SET1* were made that encode proteins with amino acid substitutions in the SET domain of Set1 (Figure 1) (Williamson, et al. 2013). Wild-type Set1 generates H3K4me1, H3K4me2 and H3K4me3. Amino acid substitution mutants were constructed to alter residues near and in the active site of Set1 to generate mutants with different H3K4 methylation capabilities. The *set1-Y967A* alleles is null mutant with no detectable H3K4 methylation that is indistinguishable from a *set1Δ* mutant. The *set1-G951A* mutant produces H3K4me1 and very low levels of H3K4me2. Although G951 is not in the active site, it is highly conserved in Set1 homologs and has been shown to be important for Set1 function (Dillon, et al. 2005, Lee, et al. 2018, Nislow, et al. 1997, Sollier, et al. 2004). Two additional partial function mutants were generated: *set1-Y967F* that generates H3K4me1 and low levels of H3K4me3 and *set1-R1013H* that generates H3K4me1 and H3K4me2 (Figure 1).

In this study, the *set1* amino-acid substitution mutants and the *HIS3* gene were studied to learn about the roles of individual H3K4 methyl marks in RNA polymerase II transcription in *S. cerevisiae*. *HIS3* codes for imidazole glycerol phosphate dehydratase (Fink 1964), the enzyme that catalyzes the sixth step in the biosynthesis of histidine in *S. cerevisiae*. The herbicide 3-Amino-1,2,4-triazole (3AT) is a competitive inhibitor of the *HIS3* gene product and is used to induce histidine-starvation leading to the production of the general amino acid control regulator, Gcn4 (Brennan and Struhl 1980, Hope and Struhl 1985). Gcn4 activates transcription of the *HIS3* gene, increasing the level of *HIS3* mRNA and biosynthesis of histidine (Hill, et al. 1986).

Our work shows that in the absence of H3K4me2 and H3K4me3, H3K4me1 promotes transcription of the *HIS3* gene under histidine-starvation conditions in *S. cerevisiae*. A role of H3K4me1 in activation of transcription has not been reported previously. In addition, we also demonstrate that one or more of the genes required for biosynthesis of isoleucine and valine (Falco and Dumas 1985, Falco, et al. 1985) is activated by H3K4me1 when H3K4me3 and H3K4me2 are absent. The results indicate a previously unrecognized role for H3K4me1 in the activation of transcription of the *HIS3* gene and one or more of the *ILV* genes by RNA polymerase II.

Materials And Methods

Media

Standard media preparation protocols were used (Rose, et al. 1990). YPADTU is YPD media supplemented with 40 mg/L adenine hemisulfate, 80 mg/L L-tryptophan and 20 mg/L uracil. Where indicated, 1 M 3-Amino-1,2,4-triazole (3AT) made in sterile H_2O was added to media to a final concentration of 10 mM. Sulfometuron Methyl (SMM), made in dimethyl sulfoxide (DMSO), was added to media to a final concentration of 1 µg/mL. SC Complete is defined synthetic medium containing all nutrients required for yeast cell growth. SC-His, SC-Ile Val and SC-Trp are synthetic complete yeast growth media lacking histidine, isoleucine and valine, or tryptophan, respectively.

Yeast Strains

Strains of *S. cerevisiae* used in this study are listed in Supplementary Table 1. Yeast strains were made by standard genetic crosses and genetic transformation. The generation and initial characterization of mutant alleles of *SET1* were described previously (Williamson, et al. 2013). Plasmids containing *HHT2-HHF2* and *hht2*-K4R-*HHF2* have been described previously (Briggs, et al. 2001). For this study, the genes encoding *HHT2* and *HHF2* or *hht2*-K4R-*HHF2* were cloned into pRS414 plasmids (Sikorski and Hieter 1989) that carry a *TRP1* selectable marker. The endogenous *S. cerevisiae HHT1-HHF1* and *HHT2-HHF2* genes encoding the histones H3 and H4 were deleted and replaced with selectable marker genes. At least one copy of the genes encoding H3 and H4 is required for viability. Therefore, the cells expressed either the wild-type *HHT2-HHF2* genes or a mutant version of *HHT2 (hht2-H3K4R)* with a wild-type *HHF2* gene from a plasmid. The mutant *hht2-K4R* gene encodes histone H3-K4R, an amino acid substitution variant of histone H3 that cannot be methylated by Set1 because the lysine at position 4 is replaced with an arginine residue. Plasmids were transformed into cells from 1 ml saturated culture, as previously described except that 40 µg of denatured salmon sperm carrier DNA was used (Chen, et al. 1992). Cells were plated on SC-Trp solid agar and incubated at 30°C to obtain transformants.

Molecular Rendering of COMPASS

The structure of the COMPASS complex from PDB: 6BX3 (Qu, et al. 2018) was modified using PyMOL (v.1.7.4.5 Schrodinger (2015)) to highlight modified amino acid residues in the Set1 protein (Figure 1).

Growth Assays

Cultures were grown to saturation at 30°C in SC-His or YPADTU liquid medium. Eight, five-fold serial dilutions were made in sterile water. The last six dilutions were plated (5 µl) on each of four types of solid agar plates: SC-His, SC-His + 10 mM 3AT, SC-complete, and SC-complete + 10 mM 3AT. Plates were imaged after 24 and 42-44 hours of incubation at 30°C. For experiments with yeast strains expressing histone H3K4 or H3K4R from plasmids, six 10-fold serial dilutions were made in sterile water and dilutions were plated, as described above. Plates were incubated at 30°C and imaged after 42 hours. For Ile Val starvation growth assays, eight, five-fold serial dilutions were made in sterile water using overnight cultures grown in YPADTU. The last six dilutions were plated (5 µl) on solid agar plates: SC-Ile Val + DMSO, SC-Ile Val + 1 µg/mL SMM, SC-complete + DMSO, and SC-complete + 1 µg/mL SMM. Plates were imaged after 72-96 hours of incubation at 30°C.

RNA Isolation and Northern Blotting

Saturated cultures of yeast strains grown in SC-His liquid medium were diluted into 50 mL of fresh SC-His medium at ~$4x10^6$ cells/mL and grown to a density of 1-$2x10^7$ cells/mL at 30° C in a shaker incubator. After 4 hours, 500 µL of 1 M 3AT (final concentration 10 mM) or sterile distilled water were added to the cultures, which were incubated at 30° C in a shaker incubator for one hour. Total RNA was extracted from yeast cultures, as previously described (Schmitt, et al. 1990) with the following modifications. Cells were resuspended in 450 µl AE buffer (50 mM $C_2H_3NaO_2$ pH 5.3, 10 mM EDTA), and then transferred to a microfuge tube to which 50 µl 10% SDS was added. Extraction with an equal volume of chloroform:isoamyl alcohol (24:1) was performed before the addition of 50 µl 3M sodium acetate pH 5.3 and 2.5 volumes 100% ethanol. After precipitation, RNA was resuspended in sterile milliQ H_2O and stored at -70°C. RNA (15 µg) was analyzed by Northern blotting, as described previously (Swanson, et al. 1991). The steady-state level of *HIS3* transcript was detected by hybridization with a strand-specific ^{32}P-labeled riboprobe. To normalize loading of RNA samples, a ^{32}P-labelled *ACT1* DNA probe synthesized by random priming was used to detect the *ACT1* mRNA level. For the northern blots analyzing the *set1-G951A* strain, a ^{32}P-labelled *HIS3* DNA probe synthesized by random priming was used to detect the *HIS3* mRNA level. To analyze the Northern blots, the ratio of *HIS3/ACT1* mRNA was calculated for each strain grown in SC-His and SC-His + 10 mM 3AT. To calculate the fold change of *HIS3* transcript in presence of 3AT, the ratio of *HIS3/ACT1* mRNA for each strain grown in SC-His+10mM 3AT was normalized to the corresponding *HIS3/ACT1* transcript levels in cultures grown in SC-His.

The optimal induction of *HIS3* transcript after addition of 3AT was determined using a time course experiment (Supplementary Figure 2). The steady-state level of *HIS3* transcript was detected as described above. To normalize loading of RNA samples, a ^{32}P-labelled 18S ribosomal RNA riboprobe was used to detect the rRNA level. To analyze the Northern blots, the ratio of *HIS3* mRNA/18S rRNA was calculated for each strain at each time point. Then, the ratio of *HIS3* mRNA/18S rRNA at 5, 10, 30, 60, and 120 min post addition of 10 mM 3AT was normalized to the corresponding ratio at 0 min (prior to

adding 3AT). All blots were imaged using a G.E Typhoon FLA 7000 and quantified using G.E Imagequant TL 8.1 software.

Whole Cell Protein Extracts and Western Blotting

Yeast whole cell protein extracts were prepared as described (Mueller, et al. 2006). Proteins from clarified whole-cell extracts (8, 20 or 40 µg) were separated on 10% SDS−polyacrylamide gels, transferred to PVDF membrane, and probed with α-histone H3 (ab1791, Abcam; 1:1000), α-K4-monomethyl H3 (13-0040, Epicypher, 1:2000), α-K4-dimethyl H3 (710796, Invitrogen; 1:1000), or α-K4-trimethyl H3 (13-0041, Epicypher; 1:2000). Antibody binding was detected with HRP-conjugated α-rabbit secondary antibodies (1706715, Biorad; 1:2000) and Clarity Western ECL substrate (Bio-Rad, Hercules, CA). Western blots were imaged on an Amersham Imager 600 and quantified using Imagequant TL 8.1 software.

Chromatin Immunoprecipitation

Saturated cultures of yeast strains grown in SC-His liquid medium were diluted into 200 mL of fresh SC-His medium at ~$4x10^6$ cells/mL and grown to a density of $1-2x10^7$ cells/mL at 30° C in a shaker incubator. After 4 hours, 500 µL of 1 M 3AT (final concentration 10 mM) or sterile distilled water were added to the cultures, which were incubated at 30° C in a shaker incubator for 60 min. Lysates were prepared as previously described (Strahl-Bolsinger, et al. 1997) with the following modifications. Breakage of cells was performed in 500 µl lysis buffer (50 mM HEPES/KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Sodium deoxycholate, 1 mM PMSF, 1 mM benzamidine, 1 µg/ml each leupeptin pepstatin, and bestatin) using a Mini-BeadBeater 16 (Biospec) at 4°C, 1 min beating followed by 2 min of rest, repeated four times. Chromatin in 1 ml lysis buffer was sonicated in a 4°C waterbath (Bioruptor Water Cooler, Diagenode) using a Bioruptor 300 Sonication System (Diagenode) for 300 cycles of 30 sec on, 45 sec off, power setting high, to shear chromatin to a length <1,000 bp. Sonicated chromatin was clarified by centrifugation at 13K rpm, 4°C, 30 min. Sonicated chromatin was incubated with antibody in a total volume of 500 µl for 16-18 hrs with rocking at 4°C. The following antibodies were used; α-H3, abcam ab1791 (4 µg/IP, lot #s: GR3297884-1,GR3297878-1,GR3356864-1 and GR3366670-1), α-H3K4me3, Epicypher 13-0041 (2 µg/IP, lot #s: 20083002-42 and 20218003-49), α-H3K4me2, Epicypher 13-0027 (4 µg/IP lot #: 20252002-04), α-H3K4me1, Epicypher 13-0040 (2 µg/IP lot #s: 19338001-42 and 20178005-44). IPs were processed as described previously (Bryk, et al. 2002), with the following exceptions. Pierce protein A/G agarose beads were used to pull down crosslinked protein-DNA complexes (Pierce Biotechnology, Thermofisher, IL, USA). ChIP eluates were purified using the ChIP DNA clean and concentrator kit, as recommended by the manufacturer (Zymo Research Corp, CA, USA). Purified extracts eluted in 100 µl elution buffer were stored at -70°C.

Analysis of ChIPs

Quantitative Polymerase Chain Reactions (qPCR) were performed to analyze the distribution of H3K4me1, H3K4me2, H3K4me3 and total histone H3 at *HIS3* promoter, 5' and 3' regions of the *HIS3* ORF,

the promoter of the *ACT1* gene, and a 284-bp intergenic region on chromosome *VIII* from 384624 to 384908. Oligonucleotides are listed in Supplementary Table 2.

To analyze the H3K4me marks in ChIP eluates, duplicate reactions using 2.5 µl input DNA (1:10) and 2.5 µl immunoprecipitated DNA were amplified in 10 µl reactions containing 0.5 µM each oligonucleotide and 1X homemade master mix (1X New England Biolabs (NEB) PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1X Evagreen (Biotium), and 1 unit NEB Taq). Reactions were performed in a BioRad CFX96 Real-Time System C1000 Thermal 71 Cycler. The PCR parameters were 1 cycle of 95^oC, 3 minutes; 30 cycles of 95^oC for 30 seconds, 58^oC for 30 seconds, 72^oC for 30 seconds; 1 cycle of 95^oC for 30 seconds followed by melt curve analysis from 65^oC to 95^oC in 0.5^oC increments for 5 seconds each. For analysis of *HIS3* 3' ORF, the annealing temperature was 54^oC. The threshold cycle reading was taken after each 72^oC elongation step. Percentage of DNA immunoprecipitated (%IP) was calculated by dividing the signal from IP DNA by that of input DNA for all H3K4me ChIPs and total histone H3 ChIPs from each location. %IP for each H3K4me mark was normalized to the %IP of total H3 at each location.

Statistical Analysis

 Statistical analysis for the Northern blots and ChIP experiments was performed using the Mann Whitney U test (Allaire 2012) on R studio.

Results

Histone H3K4 methylation by Set1 is required for robust growth of yeast cultures when grown under nutrient-stress conditions

The positions of the amino acids substitutions in the *set1* mutants used in this study are shown in a reproduction of the cryo-EM structure of COMPASS (Qu, et al. 2018) (Figure 1A). We analyzed *set1* mutants with the indicated cellular levels of H3K4me1/2/3 based on Western blotting (Figure 1B and C): two null mutants, *set1D* and *set1-Y967A*, with no detectable H3K4me1/2/3; *set1-G951A* with H3K4me1 at 58% of *SET1*^{+} (WT), H3K4me2 at <5% of WT and no detectable H3K4me3; *set1-Y967F* with H3K4me1 at 36% of WT, H3K4me2 at <5% of WT and H3K4me3 at 12% of WT; and *set1-R1013H* with H3K4me1 at 56% of WT, H3K4me2 at 49% of WT, and no detectable H3K4me3.

The effects of alterations in the levels of H3K4me1/2/3 on the growth of yeast cultures under histidine starvation conditions were evaluated with saturated yeast cultures expressing either wild-type or amino-acid substitution variants of Set1 on four different solid agar media (Figure 2). After 42 hours of incubation, the six cultures grew similarly on SC Complete medium with the herbicide 3-Amino-1,2,4-triazole (3AT), SC Complete medium without 3AT, and synthetic medium lacking histidine, SC-His (Figure 2A and 2B right panel). In contrast, all cultures grew less well on SC-His+3AT (Figure 2B, left panel). This result was expected because 3AT is a competitive inhibitor of the enzyme encoded by the *HIS3* gene and causes histidine starvation in medium lacking histidine.

Reduced growth of yeast strains expressing the null mutants (*set1D* and *set1-Y967A*) was observed on SC-His+3AT (Figure 2B, left panel). The extent of growth of yeast strains expressing partially or fully functional Set1 proteins (*set1-G951A, set1-Y967F, set1-R1013H, or SET1⁺*) was similar, and all grew better than the null mutants. Given that the *set1-G951A* mutant catalyzes H3K4me1 mainly with very low H3K4me2 and undetectable H3K4me3 (Figure 1B and C), the results suggest that H3K4me2 and H3K4me3 are not required for wild-type growth under histidine-starvation conditions.

A literature search was performed to identify other genes regulated by the GAAC pathway that might be also regulated by H3K4 methylation. The *ILV* genes, *ILV1*, *ILV2*, *ILV3*, *ILV5* and *ILV6*, encode enzymes that catalyze the biosynthesis of isoleucine and valine in *S. cerevisiae*. Sulfometuron methyl (SMM) is a competitive inhibitor of the *ILV2* gene, acetolactate synthase (Falco and Dumas 1985). SMM was used in plate growth assays to evaluate the role of H3K4 methylation on growth of cultures during starvation for Ile and Val. Saturated cultures of yeast strains expressing wild-type Set1 or *set1* amino-acid substitution variants were analyzed for growth on medium with or without SMM. All strains tested grew equally well on SC Complete media + DMSO (Figure 3A, right). Likewise, differences in growth were not observed on SC Complete containing SMM, indicating that SMM in defined complete medium does not cause a growth defect (Figure 3A, left). Equal growth of strains was also observed on synthetic media lacking isoleucine and valine (SC-Ile Val + DMSO, Figure 3B, right). However, the *set1* null mutants grew poorly on medium lacking isoleucine and valine in presence of SMM (SC-Ile Val + 1 μg/mL SMM) when compared to strains expressing wild-type *SET1⁺* or the partial function alleles of *set1* (Figure 3B, left). Similar to the results of growth assays performed under histidine-starvation conditions (Figure 2), the partial function mutant *set1-G951A,* which performs mainly H3K4me1 was sufficient to rescue the growth defect exhibited by the *set1* null mutants. These results suggest that H3K4me2 and H3K4me3 are not required for wild-type growth under conditions of isoleucine and valine starvation.

In addition to methylation of histone H3, Set1 methylates the Dam1 protein that functions in chromosome segregation in *S. cerevisiae* (Latham, et al. 2011, Zhang, et al. 2005)*.* To verify that the growth phenotypes observed in Figure 2 were due to changes in methylation of histone H3 and not another target of Set1, *SET1⁺* and *set1* mutant alleles were transformed into yeast strains that express either wild-type histone H3 or a mutant version of H3 (*hht2-K4R*) that cannot be methylated by Set1 due to the substitution of lysine at position 4 with arginine (Figure S2). The *set1* null mutants that express wild-type histone H3 (pH3K4) grow less well than the other yeast strains on SC-His+3AT (Figure S2C). In contrast, growth of cultures expressing *SET1⁺* or the partial function alleles and the unmethylatable form of histone H3 (pH3K4R) was indistinguishable from that of the null mutants on SC-His+3AT agar (Figure S2B). These results indicate that methylation of histone H3 by Set1 is required for robust growth of yeast cultures during histidine starvation. We next explored the role of H3K4 methylation in expression of *HIS3* by measuring steady-state levels of *HIS3* mRNA and the presence of H3K4me methyl marks at the *HIS3* gene in wild-type *SET1* strains, *set1* null mutants and *set1* partial function mutants.

Histone H3K4 methylation by Set1 is required for wild-type expression of the *HIS3* gene in cultures grown in nutrient-stress conditions

The level of *HIS3* mRNA in cultures expressing wild-type *SET1⁺* or the *set1* amino-acid substitution variants was measured by Northern hybridization (Figure 4). Histidine starvation was induced by adding 10 mM 3AT to log-phase cultures for 1 hr prior to isolation of RNA. The *HIS3:ACT1* level was increased 13.7-fold in *SET1⁺* cultures when histidine starvation was induced. The *HIS3:ACT1* levels were increased in the null mutants (*set1Δ* and *set1-Y967A*), yet the levels were significantly lower than those in the *SET1⁺* cultures. *HIS3:ACT1* levels were upregulated in the *set1-G951A*, *set1-Y967F* and *set1-R1013H* mutants and were not significantly different from those in the *SET1⁺* cultures. From these results, we conclude that *set1-G951A*, an allele mainly capable of generating H3K4me1, supports the activation of transcription of the *HIS3* gene under histidine-starvation conditions. Therefore, the growth defects observed for the *set1* null mutants (*set1Δ* and *set1-Y967A*, Figure 2B) may be explained by the fact that these mutants make less *HIS3* mRNA than the *SET1⁺* strain and the partial function *set1* mutants under histidine-starvation conditions. However, we note that even though the average *HIS3:ACT1* level in the *set1-R1013H* mutant under histidine-starvation conditions was higher than that in the *set1* null mutants, the level was not statistically different from the *SET1⁺* or *set1* null cultures. This observation will be addressed further in the discussion.

Histone H3K4 methylation patterns at the *HIS3* gene do not change significantly during histidine starvation

To evaluate the presence of H3K4 methyl marks at the *HIS3* promoter during histidine starvation, ChIPs were performed to measure H3K4me1, H3K4me2 and H3K4me3 at the *HIS3* promoter and two positions in the *HIS3* ORF (Figure 5A and B). H3K4me1/2/3 marks were detected at the *HIS3* gene in yeast strains expressing wild-type *SET1* and the partial function mutants, *set1-G951A*, *set1-Y967F* and *set1-R1013H* in the absence and presence of 3AT (Figure 5C and D). The distribution of H3K4 methyl marks was also evaluated at the promoter of the *ACT1* gene and an intergenic region on chromosome *VIII* (Supplemental Figure 3). One surprising result was that for the most part the levels of H3K4me1, H3K4me2 and H3K4me3 associated with the promoter and 5' ORF of the *HIS3* gene in wild-type *SET1⁺* cultures did not change in cultures grown in presence of 3AT, conditions that we show in Figure 4 cause an increase in steady-state *HIS3* mRNA.

H3K4me3: Wild-type Set1 is able to convert lower-level H3K4 methyl marks to H3K4me3. Consistent with this, H3K4me3 signals at the *HIS3* gene and other genomic regions analyzed by ChIP were significantly higher in the *SET1⁺* cultures compared to the partial function *set1* mutant cultures (Figures 5C, 5D, and S3, blue bars). H3K4me3 was detected above background (*set1D* mutant) at the *HIS3* gene and other genomic regions in the *set1-Y967F* mutant, although the levels were significantly lower than those in the *SET1* cultures. Consistent with previously published data (Pokholok, et al. 2005), the average level of H3K4me3 was higher at the *HIS3* promoter and 5' ORF region than the *HIS3* 3' ORF, in the *SET1⁺* and *set1-Y967F* samples. H3K4me3 was detected at background levels at the three regions of *HIS3* gene, the *ACT1* promoter or the intergenic region in the *set1-G951A* and *set1-R1013H* mutant samples with or without 3AT-treatment.

H3K4me2: The levels of H3K4me2 at the *HIS3* gene were low in the *SET1*, *set1-G951A*, *set1-Y967F* and the *set1-R1013H* cultures when compared to each strain's most prominent H3K4 methyl mark. With the exception of the *SET1* and *set1-R1013H* cultures, the levels of H3K4me2 measured at the *HIS3* promoter, 5' ORF and 3' ORF were at or near background levels in the *set1-G951A* and the *set1-Y967F* cultures grown without 3AT. A slight but significant increase in the level of H3K4me2 relative to background was observed at the *HIS3* promoter in the *set1-G951A* culture grown in the presence of 3AT. At other positions, the level of H3K4me2 was at background levels in the *set1-G951A* mutant.

The *set1-R1013H* mutant can generate detectable levels of H3K4me2 (Figure 1B), and in the ChIP data, the levels of H3K4me2 in the *set1-R1013H* mutant were similar to those in the *SET1* cultures. In contrast, at all positions of the *HIS3* gene that were evaluated with or without 3AT treatment, the levels of H3K4me2 in the *set1-Y967F* mutant remained at background levels. The *set1-Y967F* mutant is unusual in that it generates H3K4me1 and H3K4me3, but H3K4me2 is not detected above background in whole cell extracts or at positions in *HIS3*, *ACT1* or the intergenic region (Figures 1, 5 and S3). One possibility to explain the phenotype of the *set1-Y967F* mutant is that the substitution of phenylalanine for tyrosine at position 967 of Set1 alters the active site in a manner that allows the H3 tail to be monomethylated at K4 and possibly released, but when H3K4me1 is the substrate of COMPASS, two additional methylation events occur without the release of H3K4me2 resulting in the production of H3K4me3.

H3K4me1: In the *SET1*^{+} cultures, the level of H3K4me1 was lower than that of H3K4me3 at the three positions analyzed in the *HIS3* gene in the absence and presence of 3AT (Figure 5). In contrast, in the *set1-G951A* strain, H3K4me1 was the predominant form of K4-methylated H3 (Figure 5). This is not a surprise because the *set1-G951A* mutant is not proficient in generating H3K4me2 and H3K4me3. The *set1-R1013H* mutant is poor at generating H3K4me3. As such, in the *set1-R1013H* mutant, H3K4me1 and H3K4me2 are more abundant than H3K4me3 at the three positions analyzed in the *HIS3* gene in untreated cultures (Figure 5A). However, the H3K4 methylation profile for the *set1-R1013H* mutant is different at the *HIS3* promoter and 5' ORF in 3AT-treated cultures, where the levels of the three forms of H3K4me were similar (Figure 5B). The profiles at the 3' ORF and *ACT1* promoter are more like those in the untreated samples (Figures 5 and S3). In the *set1-Y967F* mutant, we observed slightly higher average levels of H3K4me1 than H3K4me3 at the three positions analyzed in the *HIS3* gene in untreated cultures (Figure 5A). This profile switched when the *set1-Y967F* mutant was treated with 3AT such that the average level of H3K4me3 was higher than H3K4me1 at the *HIS3* gene (Figure 5B). A switch in the levels of H3K4me1 and H3K4me3 was not observed at the *ACT1* promoter upon treatment with 3AT (Figure S3).

The results show that H3K4me3 is present at background levels at the *HIS3* gene in the *set1-G951A* mutant, yet robust growth and induced transcription of the *HIS3* gene occurs during treatment of the *set1-G951A* mutant with 3AT. The phenotypes of the *set1Δ* mutant indicate that H3K4 methylation is required for robust growth and induction of *HIS3* transcription during histidine starvation. Therefore, we conclude that H3K4me1 supports transcription of the *HIS3* gene under histidine-starvation conditions in the absence of H3K4me2 and H3K4me3. The implications of these findings with respect to transcription and the mechanisms of gene regulation are discussed below.

Discussion

Analysis of partial function variants of Set1 identified a role for H3K4 monomethylation in transcription by RNA polymerase II. Much of the existing literature on H3K4 methylation focuses on the role of H3K4me2 and H3K4me3 on transcription (Kusch 2012, Musselman, et al. 2012, Pray-Grant, et al. 2005, Schneider, et al. 2005, Taverna, et al. 2006). The effect of H3K4 monomethylation on transcription is not well characterized.

Previous studies in *S. cerevisiae* showed that H3K4me1 inhibits RSC-independent chromatin remodeling thereby preventing induction of osmostress genes (Nadal-Ribelles, et al. 2015).   H3K4me1 has also been associated with transcriptional silencing in skeletal muscle cells, embryonic fibroblasts, macrophages, and human embryonic stem cells (Cheng, et al. 2014). In contrast to a repressive role in transcription, the findings here show that H3K4me1 supports the activation of transcription by Pol II when *S. cerevisiae* cultures are subjected to nutrient-starvation conditions.

The three states of methylation at lysine 4 of histone H3 play different roles in the regulation of gene expression (Kusch 2012, Pokholok, et al. 2005). Here, mutants of *SET1* were used to characterize the effects of different states of H3K4-methylation on expression of the *HIS3* gene in *S. cerevisiae*. Growth assays, Northern blotting experiments and chromatin IPs (Figures 2-5) show that a partial function mutant of Set1, *set1-G951A*, which catalyzes H3K4me1 mainly, supports increase in the steady-state level of *HIS3* mRNA and wild-type growth under histidine-starvation and isoleucine-valine starvation conditions. Wild-type growth under histidine-starvation and isoleucine-valine starvation conditions is also seen with a second partial function allele, *set1-R1013H*, that is capable of H3K4 mono- and di-methylation but not H3K4me3 (Figure 1).   H3K4me3 is not required for induction of *HIS3* transcript in *set1-R1013H* cultures grown under histidine-starvation condition. Both *set1-G951A* and *set1-R1013H* are incapable of accumulating H3K4me3 at the *HIS3* gene (Figure 5), therefore activation of transcription cannot be attributed to the accumulation of H3K4me3 at the *HIS3* promoter.   Also, because H3K4me2 is less than 5% of the wild-type level in the *set1-G951A* mutant and does not accumulate at the *HIS3* gene under histidine-starvation condition, we conclude that H3K4me1, in the absence of H3K4me2 and H3K4me3, supports the increase in steady-state levels of *HIS3* transcript under histidine-starvation conditions and promotes wild-type growth.

The growth defects exhibited by yeast strains expressing either of the two *set1* null mutants, *set1Δ* or *set1-Y967A*, under histidine-starvation conditions were due to the lack of Set1 histone methylation activity. Comparison of data from null mutants to those from the *set1-G951A* mutant shows that Set1 monomethylation activity is required for the upregulation of *HIS3* mRNA to wild-type levels under histidine-starvation conditions. It is possible that in addition to the *HIS3* gene, one or more other genes in the histidine biosynthetic pathway is also upregulated in the presence of H3K4me1. Robust growth under isoleucine-valine starvation also occurred in the *set1-G951A* mutant but not the null mutants (Figure 3). Therefore, the data suggest that induction of one or more of the *ILV* genes that catalyze the biosynthesis

of Ile and Val (*ILV1, ILV2, ILV3, ILV5, ILV6*) occurs the presence of H3K4me1 in cells lacking H3K4me3. The specific role of H3K4 methylation in upregulation of the *ILV* genes will be determined in the future.

ChIP analysis showed that the three forms of K4-methylated H3 are present at the *HIS3* promoter and the 5' and 3' regions of the *HIS3* ORF in wild-type *SET1⁺* cultures. In *SET1⁺* cultures, H3K4me3 was detected across the gene, with the highest signals at the promoter and 5' ORF (Figure 5). This profile was expected based on previous studies showing a similar H3K4me3 profile at many genes in yeast (Berger 2007, Pokholok, et al. 2005). H3K4me2 levels at the positions analyzed in the *HIS3* gene were lower than those of H3K4me3 and H3K4me1 (Figure 5). In general, H3K4me1 levels at these regions were lower than those of H3K4me3 and slightly higher than those of H3K4me2.

Histidine-starvation caused a 13.7-fold increase in the level of *HIS3* gene transcript (Figure 4) in *SET1⁺* yeast cultures, yet increases in the levels of H3K4me3, H3K4me2 and H3K4me1 were not detected at the *HIS3* promoter in cultures grown under histidine-starvation conditions (Figure 5A and B). In fact, significant changes in H3K4me signals in the presence and absence of 3AT were not detected (except for H3K4me1 in the 5' ORF in *set1-Y967F* and *set1-R1013H*). These results indicate that while histidine starvation causes an increase in *HIS3* mRNA expression (Hill, et al. 1986), it does not affect the H3K4 methylation at the positions of the *HIS3* gene that were analyzed in this study. The data show that *S. cerevisiae* cells require Set1 and H3K4 methylation to elicit a wild-type transcriptional response to histidine starvation. However, higher-level H3K4 methylation is not required; H3K4me1 supports this transcriptional response even when present at levels similar to those observed under non-starvation conditions.

These results suggest that H3K4 methylation alone is unlikely to determine the transcriptional response to histidine starvation at the *HIS3* gene. Changes in the levels of other histone marks, such as acetylation, in combination with H3K4 methylation, may be required to recruit transcription effectors responsible for induction of the *HIS3* gene under histidine-starvation conditions. The *HIS3* gene is regulated by the General Amino Acid Control (GAAC) pathway and requires Gcn4 for initiation of transcription. Growth of yeast cultures in 3AT activates Gcn4, which recruits the histone acetyltransferase (HAT) Gcn5 to many promoters, including the *HIS3* promoter, leading to activation of transcription (Kuo, et al. 2000, Kuo, et al. 1998). In the future, we will determine if H3K4me1-mediated activation of transcription is accompanied by an increase in acetylation of histones at the promoters of target genes. Genome-wide studies using the partial function *set1* mutants under normal and nutrient-stress conditions will provide high-resolution data, potentially identify more genes regulated by H3K4me1, and may reveal interesting patterns not observed in single gene analysis.

A related goal is to identify transcription effectors that read H3K4me1 and contribute to the activation of transcription at *HIS3* and other genes. Initiation of transcription requires the assembly of many transcription factors at gene promoters. Gcn4 recruits the SAGA complex (Spt-Ada-Gcn5 acetyltransferase) to gene promoters (Kuo, et al. (2000). Gcn4 also interacts with Gcn5, SWI/SNF, the SRB/Mediator complex, RNA polymerase II, TFIID, and Nua4 (Ginsburg, et al. 2009, Natarajan, et al. 1999,

Swanson, et al. 2003).   Identification of a protein that reads the H3K4me1 will help elucidate an H3K4me1-dependent mechanism of transcription activation.

H3K4 methyltransferases are conserved across species from yeast to humans and have important roles in regulation of gene expression (reviewed in Shilatifard (2012)). The involvement of trimethylated H3K4 in gene activation (Pray-Grant, et al. (2005)) and gene repression (Shi, et al. (2006)) provide an illustration of the complexity of Set1-like methyltransferase-mediated regulation. The identification and interpretation of H3K4me1-mediated mechanisms will contribute to our understanding of transcriptional regulation and their impact on complex processes, like development and differentiation, in higher organisms. Moreover, evaluating the effects of altered H3K4 methylation patterns in the partial function *set1* mutants on other transcription-related processes, such as splicing and elongation, will provide deeper insight into the mechanisms that regulation transcription.

Abbreviations

H3K4, histone H3 lysine 4; H3K4me1, K4-monomethylated histone H3; H3K4me2, K4-dimethylated histone H3; H3K4me3, K4-trimethylated histone H3; HAT, histone acetyltransferase; ORFs, open reading frames; 3AT, 3-Amino-1,2,4-triazole.

Declarations

Conflicts of interest/Competing interests: Not applicable

Availability of data and material: The authors will make all data and unique research materials and data freely available to other investigators.

Code availability: Not applicable

Authors' contributions: SHP, RJ, ND and MB conceived the experiments. Preliminary data were generated by RJ and ND. Data in figures were generated by ND. The manuscript was written by ND and MB. After review and comments, the manuscript was approved by all authors.

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

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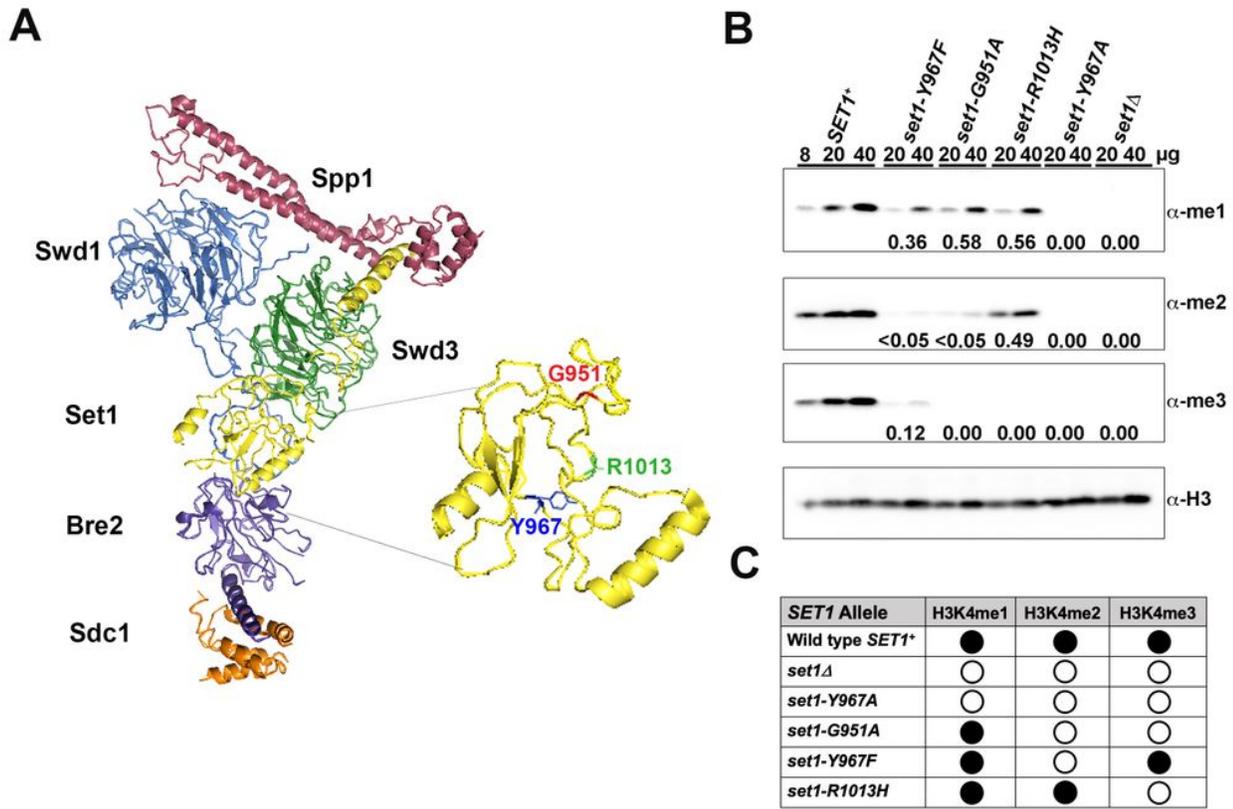
Figures

Figure 1

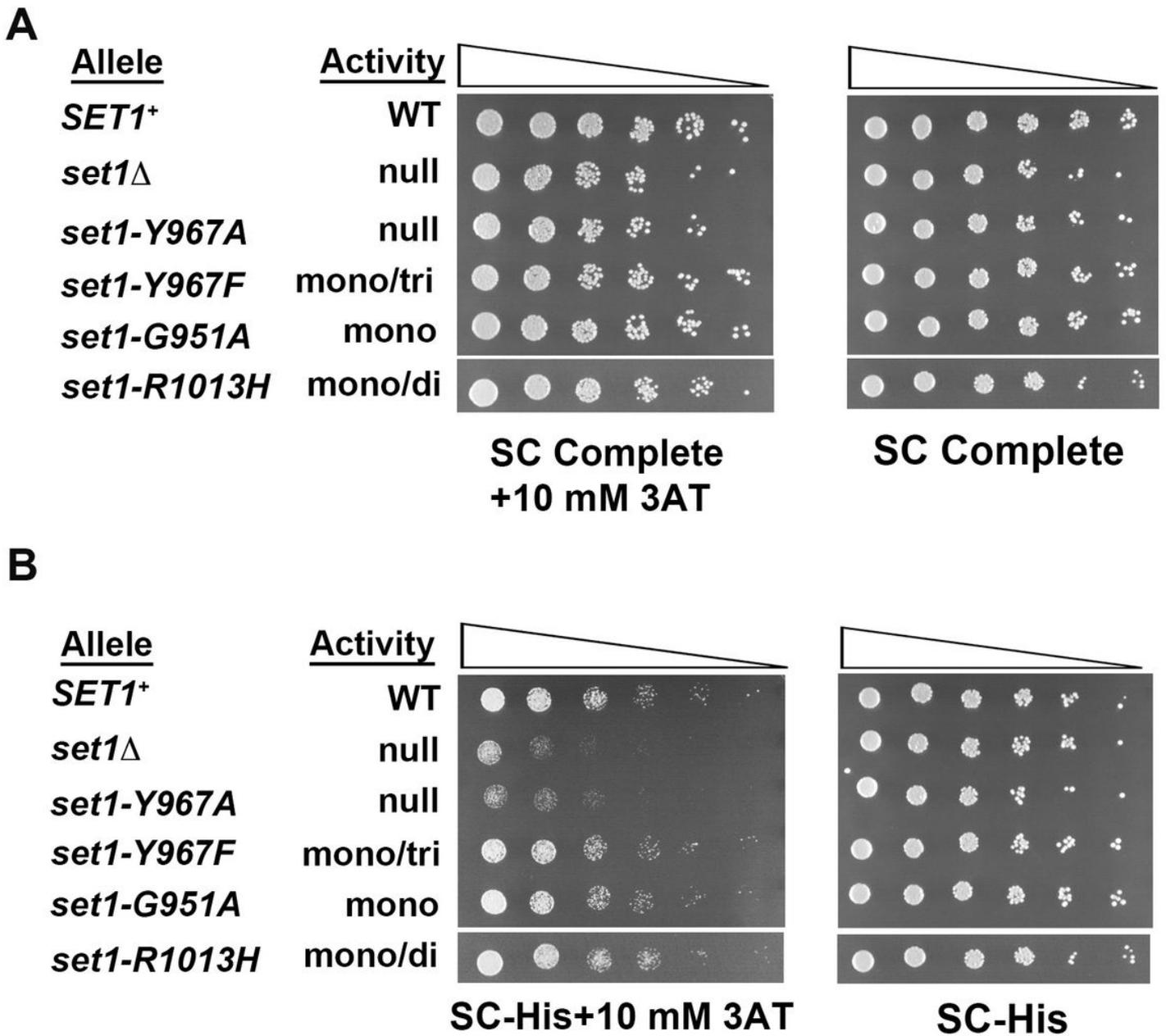please see the manuscript file for the full caption

Figure 2

Growth of mutants lacking Set1 methylation activity is sensitive to histidine starvation in the presence of 3AT. Five-fold serial dilutions of yeast cultures expressing wild type SET1+ or set1 mutant alleles were spotted onto SC Complete agar with or without 10 mM 3AT (A) and SC-His agar with or without 10 mM 3AT (B). Allele, relevant genotype, and Activity, H3K4 methylation capacity based on Figure 1B, are indicated on the left of the panels. Null, no detectable H3K4me1/2/3; mono, H3K4me1 with low or no H3K4me2/3; mono/di, detectable H3K4me1 and H3K4me2; mono/tri, detectable H3K4me1 and H3K4me3 (Williamson, et al. 2013) (Figure 1B). Agar plates were incubated at 30oC for 42 hrs prior to imaging. (n=3)
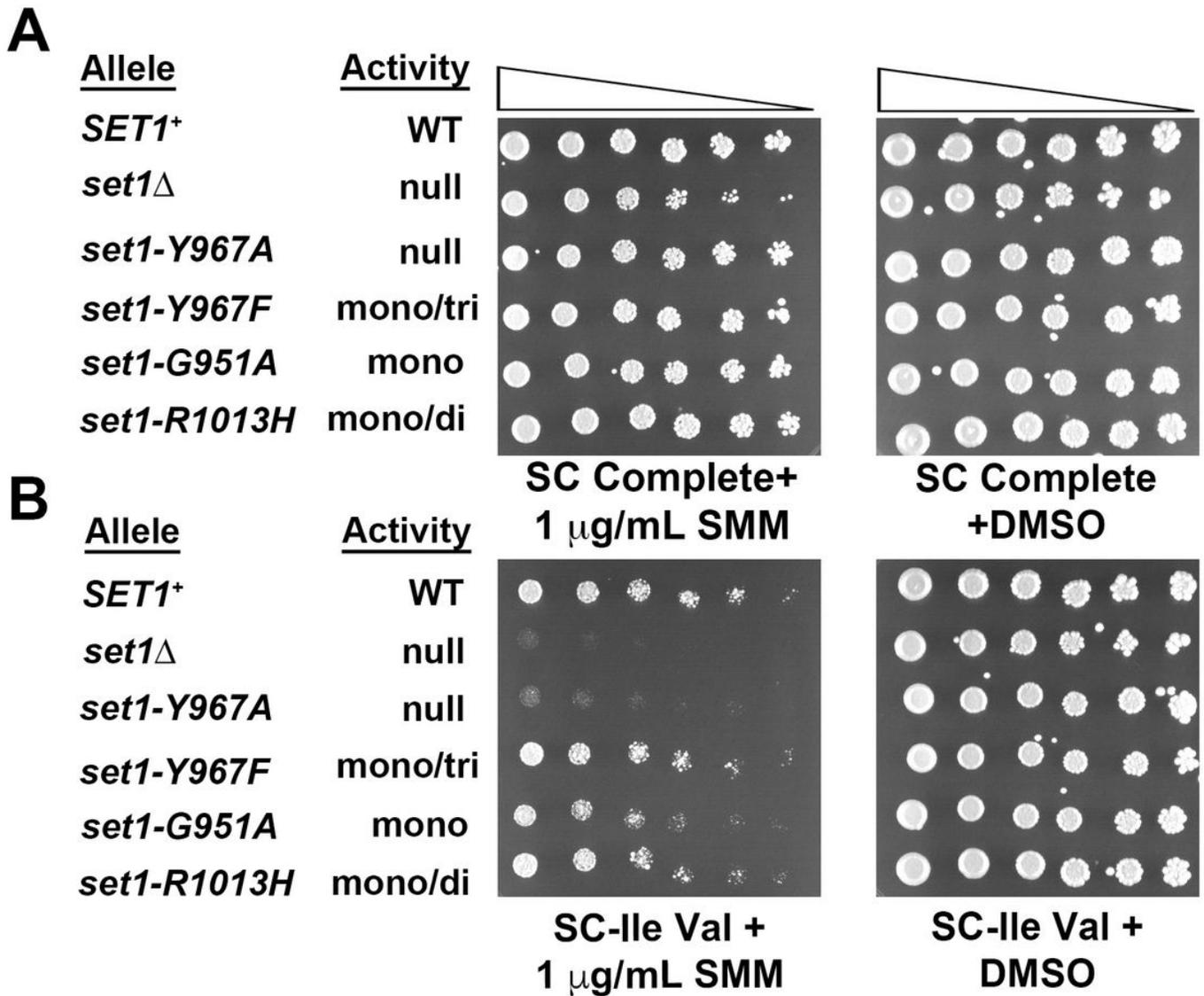
Figure 3

Growth of mutants lacking Set1 methylation activity is sensitive to Ile and Val starvation in the presence of SMM. Five-fold serial dilutions of yeast strains expressing wild type SET1 or mutant set1 alleles were spotted onto SC Complete and SC-Ile Val solid media with 1 μg/mL SMM or DMSO. A. SC Complete plates without or with 1 μg/mL SMM. B. SC-Ile Val plates without or with 1 μg/mL SMM. Plates were incubated at 30oC for 4-5 days prior to imaging. Other labels, as in Figure 2.
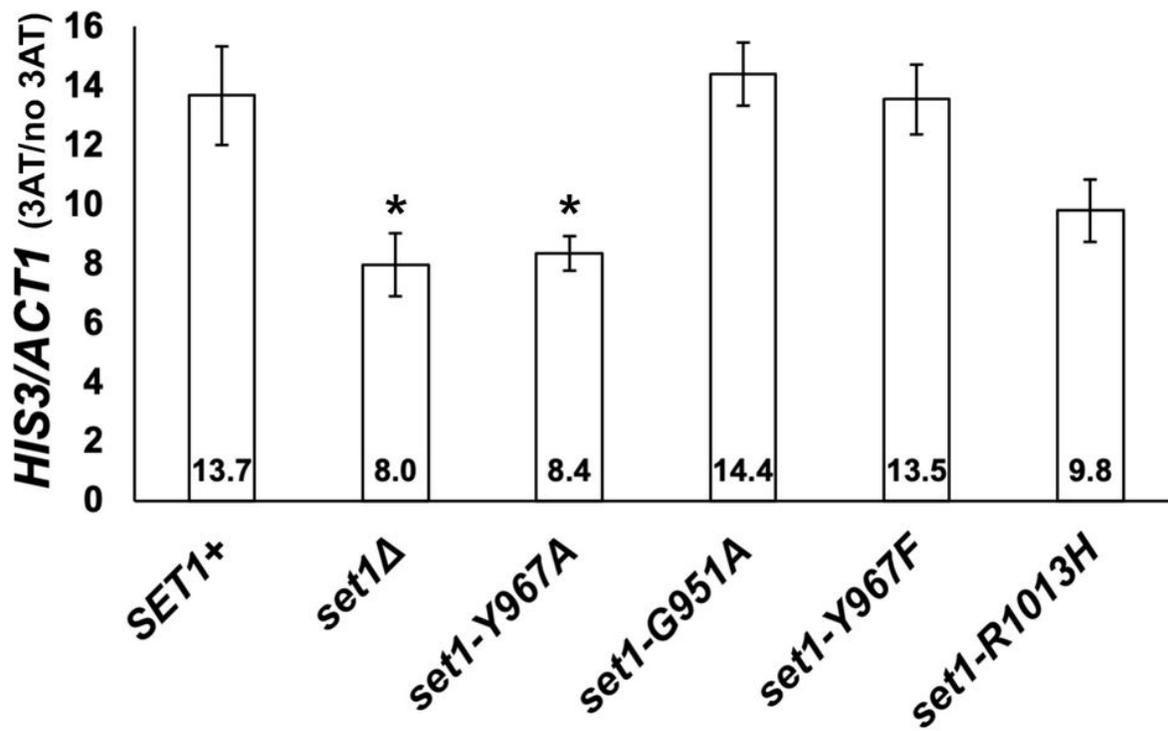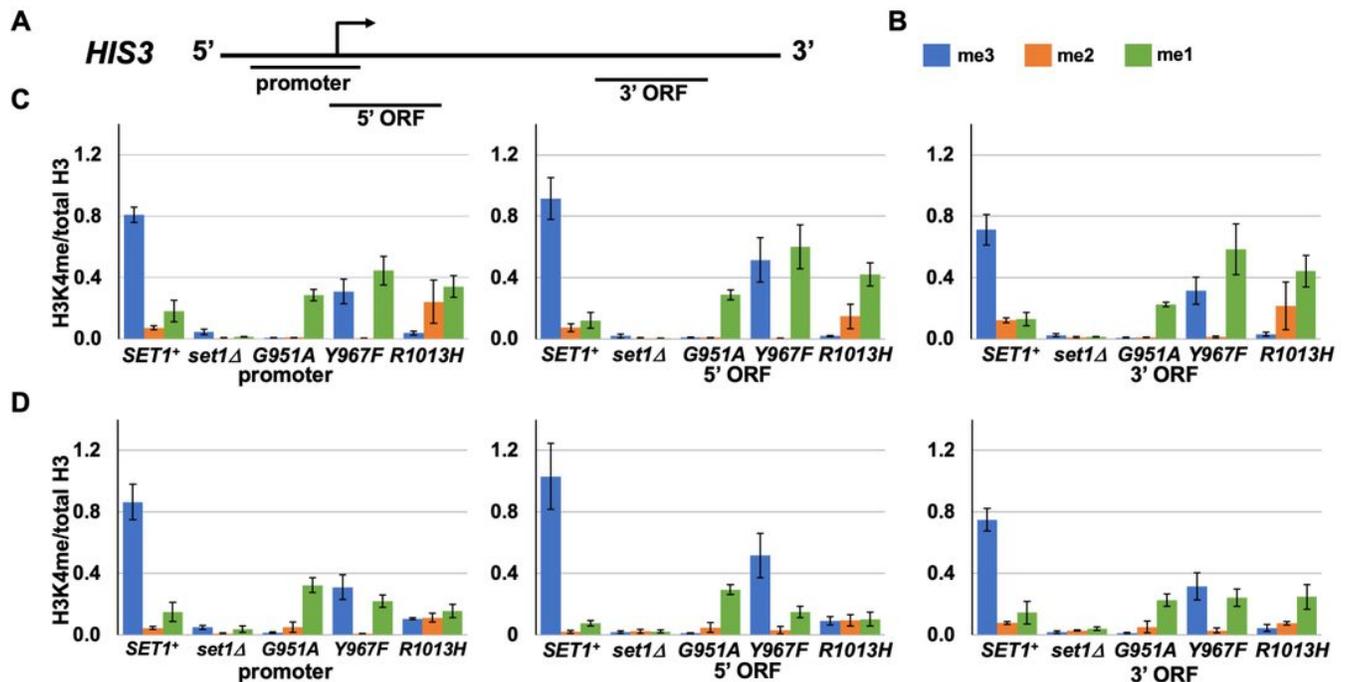
Figure 4

please see the manuscript file for the full caption

Figure 5

Distribution of H3K4me marks at three positions of the HIS3 gene. A. Schematic showing the regions of HIS3 gene (promoter, 5' ORF and 3' ORF) evaluated by qPCR after ChIP for H3K4me1, H3K4me2 and H3K4me3. B. Key to graph. C. Levels of H3K4me measured at the HIS3 promoter, 5' ORF and 3' ORF in yeast cultures grown in SC-His. D. Levels of H3K4me measured at the HIS3 promoter, 5' ORF and 3' ORF in yeast cultures grown in SC-HIS+10 mM 3AT. Error bars, (+/- SEM, n=4).

Supplementary Files

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