H3K4 methylation dependent and independent chromatin regulation by *JHD2* and *SET1* in budding yeast.

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Abstract

Set1 and Jhd2 regulate the methylation state of histone H3 lysine-4 (H3K4me) through their opposing methyltransferase and demethylase activities in the budding yeast *Saccharomyces cerevisiae*. H3K4me associates with actively transcribed genes and, like both *SET1* and *JHD2* themselves, is known to regulate gene expression diversely. It remains unclear, however, if Set1 and Jhd2 act solely through H3K4me. Relevantly, Set1 methylates lysine residues in the kinetochore protein Dam1 while genetic studies of the *S. pombe* *SET1* ortholog suggest the existence of non-H3K4 Set1 targets relevant to gene regulation. We interrogated genetic interactions of *JHD2* and *SET1* with essential genes involved in varied aspects of the transcription cycle. Our findings implicate *JHD2* in genetic inhibition of the histone chaperone complexes Spt16-Pob3 (FACT) and Spt6-Spn1. This targeted screen also revealed that *JHD2* inhibits the Nrd1-Nab3-Sen1 (NNS) transcription termination complex. We find that while Jhd2’s impact on these transcription regulatory complexes likely acts via H3K4me, Set1 governs the roles of FACT and NNS through opposing H3K4-dependent and -independent functions. We also identify diametrically opposing consequences for mutation of H3K4 to alanine or arginine, illuminating that caution must be taken in interpreting histone mutation studies. Unlike FACT and NNS, detailed genetic studies suggest an H3K4me-centric mode of Spt6-Spn1 regulation by *JHD2* and *SET1*. Chromatin immunoprecipitation and transcript quantification experiments show that Jhd2 opposes the positioning of a Spt6-deposited nucleosome near the transcription start site of *SER3*, a Spt6-Spn1 regulated gene, leading to hyper-induction of *SER3*. In addition to confirming and extending an emerging role for Jhd2 in the control of nucleosome occupancy...
near transcription start sites, our findings suggest some of the chromatin regulatory functions of Set1 are independent of H3K4 methylation.

Introduction

Methylation of histone H3 lysine-4 (H3K4me) represents one of the most comprehensively studied chromatin modifications. Like most histone lysine methylations, H3K4 exhibits mono, di, and tri-methylated states (H3K4me1, H3K4me2, and H3K4me3) that have distinctive regulatory outputs reflecting the recruitment of effector proteins (SANTOS-ROSA et al. 2003; TAVERNA et al. 2006; KIM AND BURATOWSKI 2009). In the budding yeast *Saccharomyces cerevisiae*, H3K4me levels are controlled by the opposing functions of two highly conserved enzymes belonging to the Trithorax/MLL and JARID1 families: the Set1 methyltransferase and the Jhd2 demethylase (KROGAN et al. 2002; INGVARSDOTTIR et al. 2007; LIANG et al. 2007).

Unless otherwise stated, whenever we refer to *SET1* and *JHD2*, we are signifying the budding yeast genes specifically. While Jhd2 appears to act alone, Set1, like Trithorax/MLL, functions within COMPASS, a conserved complex of proteins that function in Set1 targeting and regulation (KROGAN et al. 2002). Surprisingly, despite the crucial role of Trithorax/MLL and JARID during development and disease in animals, neither *SET1* nor *JHD2* are essential for cell viability in budding yeast (BENEVOLENSKAYA et al. 2005; DEY et al. 2008; LOPEZ-BIGAS et al. 2008). Moreover, *JHD2* null mutants (*jhd2Δ*) have surprisingly limited phenotypic impact in otherwise wild-type (WT) mitotic cells grown in the presence of glucose, confounding the study of this important chromatin regulatory protein using yeast (LENSTRA et al. 2011; XU et al. 2012).

Previously, we found that *JHD2* exhibits specific functions during the gametogenesis
phase of the budding yeast life cycle, also known as sporulation (Xu et al. 2012). The formation of robust spores requires that \textit{JHD2} maintain a period of productive transcription in the face of encroaching and developmentally programmed transcriptional quiescence. During this period, \textit{JHD2} globally demethylates H3K4 at intergenic regions upstream of transcription start sites (TSSs) and overlapping with transcription termination sites (TTSs). Associated with these H3K4me defects, \textit{JHD2} also represses the accumulation of noncoding intergenic transcripts genome-wide (Xu et al. 2012). These findings suggest roles for \textit{JHD2} in diverse aspects of transcription, including those related to termination and its associated mRNA processing pathways. Indeed, a recent study found that Jhd2 physically interacts with CPF (cleavage and polyadenylation factor) and causes defects in mRNA 3’ untranslated region length for some genes (Blair et al. 2016). More recently, we determined that H3K4 demethylation by Jhd2 occurs not only during sporulation, but also in mitotic cells in response to non-fermentable carbon sources or to nitrogen manipulations that lead to increased levels of alpha-ketoglutarate, an intermediary metabolite essential for demethylase activity of Jhd2 and the entire Jumonji family of demethylases to which JARIDs belong (Liang et al. 2007; Soloveyчик et al. 2016).

Our previous studies identified developmental/nutritional contexts during which \textit{JHD2} impacts gene expression, but the mechanisms remain opaque. To gain further insight into genetic pathways by which \textit{JHD2} and \textit{SET1} impact gene expression, we used a targeted screening approach. Because genome-wide screens have been unsuccessful in the identification of gene deletions that exhibit interactions with \textit{jhd2Δ} ((Costanzo et al. 2016), our unpublished findings), we selected essential components of the transcriptional machinery and tested whether temperature sensitive (TS) mutants of these components genetically interact with \textit{jhd2Δ}, and in most cases, \textit{set1Δ} as well. Using this approach, we find that \textit{JHD2} genetically inhibits the
essential RNA PolII transcription regulatory complexes Spt6-Spn1, FACT (facilitates chromatin
transcription), and NNS (Nab3-Nrd1-Sen1) (FORMOSA et al. 2001; VASILJEVA AND BURATOWSKI
2006; MCDONALD et al. 2010). More detailed genetic experiments reveal that JHD2’s inhibitory
role can be attributed solely to reversal of H3K4me. Accordingly, genetic interrogation of SET1
confirms a positive regulatory impact of H3K4me, but also illuminates a counterbalancing and
inhibitory role of SET1 that acts independent of H3K4me. Using molecular biological
experiments, we determine that Jhd2 opposes the positioning of a Spt6-Spn1-deposited
nucleosome near the 3’ of the SRG1 non-coding transcript, and accordingly impacts the
induction of the downstream SER3 gene known to be regulated by SRG1, perhaps explaining at
least some of the basis for the genetic interactions. In summary, our findings identify three
transcriptional regulatory complexes subject to regulation by Jhd2 and Set1, and illuminate that
SET1 impinges upon them through H3K4me dependent and independent means.

Results

Genetic interactions of JHD2 with essential transcription regulatory factors

We used a targeted screening approach to identify mutants that exhibit genetic
interactions with jhd2Δ. Because Jhd2 reverses a histone modification associated with active
transcription, we evaluated temperature sensitive (TS) alleles of essential genes related to
transcriptional control as interacting candidates of jhd2Δ. We used strains sourced from a library
created and curated previously (LI et al. 2011), with the only exceptions being nab3-42 and
spt16-319, which we obtained separately (O’DONNELL et al. 2004; DARBY et al. 2012). All
strains described here were engineered using genetic crosses and tetrad dissection. Strain fitness
was assessed using spot assays to compare growth rates at varied temperatures. Deletion of
*JHD2* or *SET1* did not cause growth defects under these conditions (Fig. 1A, B, and C). For all
results shown, we isolated at least 2 independently constructed strain replicates through tetrad
dissection. Though replicates and WT control strains are not always shown in the interest of
space, all results we report here are upheld in these replicates.

Given the physical association of Jhd2 with CPF/CF, we first evaluated *jhd2Δ* genetic
interactions with TS mutations in 10 different subunits of this large complex (*BLAIR et al. 2016*).
Somewhat surprisingly, we failed to identify any reproducible interactions with CPF/CF (Table
1). NNS functions alongside CPF as one of two distinct mRNA processing pathways in budding
yeast. Nab3 and Nrd1 encode RRM domain containing proteins that recognize specific
sequences in nascent transcripts (*STEINMETZ AND BROW 1996; CARROLL et al. 2004; CREAMER
*et al. 2011*). The Sen1 subunit is homologous to the human helicase protein Senataxin and
provides the activity that physically dislodges PolII from the DNA (*PORRUA AND LIBRI 2013;
CHEN et al. 2014*). While the majority of mRNA 3’ end processing of protein coding genes
occurs through CPF, NNS controls the transcriptional termination and processing of noncoding
RNAs. The most widely studied targets of NNS are snRNAs, snoRNAs, and cryptic untranslated
transcripts (CUTS) (*URSIC et al. 1997; STEINMETZ et al. 2001; ARIGO et al. 2006; THIEBAUT et
al. 2006*).

We found that *jhd2Δ* robustly suppressed 2 different alleles of *NAB3, nab3-42* and *nab3-
11* (Table 1, Fig. 1A and S1A). Deletion of *JHD2* also modestly suppressed *sen1-1* (Table 1, Fig.
S1B). As shown, incubation at high temperature was sufficient to eliminate growth of *nab3-42*,
confirming that *jhd2Δ* did not provide bypass suppression and that Jhd2 therefore acted to inhibit
*nab3-42* function at semi-permissive temperatures (Fig. 1A). Though we only show the lack of
bypass suppression for nab3-42 in the interest of space, this was the case with all interactions we report here. NNS termination is coupled with polyadenylation by the TRAMP complex and RNA processing by Exosome (Vasiljeva and Buratowski 2006; Vanacova and Stefl 2007). We observed a similar suppressive interaction of jhd2Δ with the sole essential TRAMP component, MTR4 (Table 1 and data not shown). Interestingly, the only exosome subunit encoding gene we investigated, MTR3, provided the solitary example of a negative interaction with JHD2; jhd2Δ caused a modest but reproducible enhancement of the mtr3-ts growth defect (Table 1 and data not shown).

Among the numerous other essential genes we interrogated, including those contributing to the functions of mediator, RNAPII and its associated C-terminal domain modifications, and TATA binding protein (TBP), only temperature sensitive alleles of genes encoding Spt6-Spn1 and FACT subunits exhibited genetic interactions with jhd2Δ (Table 1, Fig. 1B and 1C). Spt6-Spn1 and FACT are conserved heterodimeric histone chaperone complexes that enable RNA PolII transcription through chromatin, deposit nucleosomes in the wake of elongating polymerase, and suppress spurious intragenic transcription (Kaplan et al. 2003; Duiña 2011; Hainer et al. 2011a). While Spt6-Spn1 and FACT have many common and overlapping functions, they exhibit differences in histone binding specificity and in their abilities to disrupt nucleosomes during transcription (Bortvin and Winston 1996; Orphanides et al. 1999; Belotserkovskaya et al. 2003; McCulloch et al. 2015). FACT can disrupt and reassemble nucleosomes, while Spt6-Spn1 only has the ability to reassemble them (Orphanides et al. 1998; Orphanides et al. 1999; Belotserkovskaya et al. 2003). We also confirmed a previous finding that the temperature sensitive growth defect of ess1-H146R, which encodes a CTD-associated prolyl-isomerase, was suppressed by jhd2Δ (Ma et al. 2012).
H3K4me dependent and independent modulation of NNS and FACT by JHD2 and SET1

As JHD2 reverses H3K4me, we hypothesized that increased H3K4me caused by jhd2Δ accounted for the genetic interactions described above. Indeed, H3K4me3 has been suggested to promote NNS function, with set1Δ enhancing the growth and termination phenotypes caused by alleles of NRD1 that delete portions of its N-terminus (TERZI et al. 2011). Terzi et al. also showed that set1Δ enhanced termination defects caused by nab3-11, though they did not evaluate SET1’s impact on nab3-11 growth properties (TERZI et al. 2011). We therefore tested if jhd2Δ suppression of NNS mutations could occur in the absence of SET1. Unexpectedly, set1Δ suppressed the growth defect of nab3-42 to a degree that exceeded jhd2Δ suppression, with the triple mutants resembling nab3-42 set1Δ (Fig. 1A). Terzi et al. 2011 found that H3K4me3 promotes NNS function using truncation alleles of NRD1 (TERZI et al. 2011), but the suppression of nab3-42 by set1Δ presented here suggested that H3K4me antagonizes NNS function. To rule out some unusual allele specific interaction of nab3-42 with set1Δ, we replicated the set1Δ and jhd2Δ interactions with NAB3 using the nab3-11 allele (Fig. S1A). Furthermore, and consistent with the NAB3 results, sen1-1 was also suppressed by both jhd2Δ and set1Δ, again with the triple mutants resembling sen1-1 set1Δ (Fig. S1B). While none of our experiments overlap with those of Terzi et al, their findings would not have predicted ours nor vice versa. In particular, our finding that set1Δ suppressed the nab3-11 growth defect is somewhat discordant with Terzi et al’s finding of an aggravating consequence of set1Δ on nab3-11 for transcriptional termination. Among the many possible explanations for this discrepancy, a simple view is that the essential functions of NAB3 may not be directly reflected by termination defects described by Terzi et al.
Our targeted screen also identified the FACT subunit encoding alleles \textit{pob3-7} and \textit{spt16-ts} as exhibiting alleviating genetic interactions with \textit{jhd2Δ} (Table 1, Fig. 1B and 1C). Using the same experimental approach as with NNS subunits, we tested if \textit{jhd2Δ} suppression of \textit{pob3-7} and \textit{spt16-ts} required \textit{SET1}. Curiously, and unlike the case with NNS subunits, \textit{pob3-7} and \textit{spt16-ts} exhibited distinct genetic interactions in combination with \textit{set1Δ}, even though both factors function in the FACT complex. We found that \textit{set1Δ} suppressed the growth defect of \textit{pob3-7} mutants to a level comparable to the suppression conferred by \textit{jhd2Δ}, with the triple mutants showing no evidence of synergy (Fig. 1B). In contrast, the \textit{spt16-ts} temperature sensitive growth defect was suppressed by \textit{jhd2Δ} in a \textit{SET1} dependent manner, suggesting that H3K4me promotes Spt16 function (Fig. 1C). We confirmed these genetic interactions with additional alleles of \textit{POB3} and \textit{SPT16}, \textit{pob3-L78R} and \textit{spt16-319} (Fig. S1C and S1D). The differences in \textit{SET1} genetic interaction phenotypes observed for \textit{SPT16} and \textit{POB3} mutants suggest complexities in nucleosome interactions, or that their encoded proteins may exhibit separable functions apart from the FACT complex.

To investigate how both \textit{set1Δ} and \textit{jhd2Δ} can rescue certain temperature sensitive growth defects, we employed strains that express H3K4 amino acid substitution mutants from a chromosomal locus in place of wild type H3 (DAi \textit{et al.} 2008). The most widely utilized amino acid substitutions for this approach are alanine (H3K4A) and arginine (H3K4R). We first confirmed the histone mutations by sequencing and then determined that H3K4A and H3K4R alone or in combination with \textit{set1Δ} did not cause any growth phenotypes under the conditions of our spotting assays (Fig. S2A). Unexpectedly, we found that H3K4A and H3K4R modified \textit{pob3-7} and \textit{nab3-11} mutants in opposing fashion. H3K4A suppressed \textit{pob3-7} and \textit{nab3-11} temperature sensitivity (Fig. 2A and S2B). Suppression by H3K4A and \textit{set1Δ} did not display any
synergy. H3K4R caused the opposite effect of H3K4A, exacerbating the temperature sensitive
growth defect of *nab3-11* and *pob3-7* (Fig. 2A, 2B, 2C, S2B, and S3A). We note that this latter
result is consistent with previous findings suggesting a positive impact for H3K4me3 on NNS
function (*Terzi et al. 2011*).

To shed light on the confounding differences in H3K4A and H3K4R phenotypes, we
performed additional studies exploiting an annotated synthetic lethal interaction between *set1Δ*
and *csf1Δ* (*Costanzo et al. 2016*). *CSF1* (cold sensitive for fermentation) encodes a conserved
yet poorly characterized protein required for growth on glucose at low temperatures and is
involved in secretory protein maturation and amino acid metabolism. (*Toki et al. 2000; Copic
et al. 2009; Costanzo et al. 2016*). In support of the interpretation that *csf1Δ set1Δ* lethality was
caused by loss of H3K4me, we found that *jhd2Δ* suppressed *csf1Δ* and that *JHD2* overexpression
using an integrated galactose-inducible allele enhanced *csf1Δ* (Fig. S2C and S2D). The *csf1Δ*
enhancement by *GAL-JHD2* was abrogated by mutation of histidine-427 to alanine (H427A),
which renders Jhd2 catalytically inactive (*Ingvarsdottir et al. 2007; Liang et al. 2007*) (Fig.
S2C and S2D).

We constructed diploid strains heterozygous for *csf1Δ, set1Δ*, and H3K4A or H3K4R
mutants and interrogated them using tetrad dissections. In addition to confirming *csf1Δ set1Δ*
synthetic lethality, we found that H3K4R exhibited synthetic lethality with *csf1Δ* (Fig. 2D). In
stark contrast, H3K4A had no consequence for *csf1Δ* viability, and remarkably, suppressed the
synthetic lethality of *csf1Δ set1Δ* (Fig. 2D). We dissected dozens of tetrads and confirmed that
this pattern of inheritance was highly stereotypical and not due to random spore inviability
(Table 2). It is noteworthy that while both H3K4A and H3K4R abolish H3K4 methylation,
arginine retains the positively charged characteristic of lysine in contrast to the aliphatic alanine

residue. Accordingly, arginine much more closely resembles the structure of a lysine residue compared with alanine. These results suggest that H3K4R more reliably recapitulates the consequences of set1Δ for chromatin structure and point toward neomorphic characteristics of H3K4A, which seem likely to manifest differently depending on genetic context. We therefore proceeded with our studies using the H3K4R mutant.

To reconcile the H3K4R mutant phenotype with suppression of NNS and FACT subunits by set1Δ, we constructed strains combining H3K4WT or H3K4R with set1Δ and NNS/FACT mutants. We found that set1Δ conferred suppression of the growth phenotypes of pob3-7, nab3-11, and sen1-1 even in the context of H3K4R albeit not to the extent seen in H3K4WT (Fig. 2A, S2B, S3A, and S3B). Thus, the suppression of NNS and FACT subunits by set1Δ in H3K4R mutants suggests that some activity of Set1 acted in opposition to NNS and FACT independent of H3K4me (Fig. 3).

To further evaluate the positive impact of H3K4me on NNS and FACT and the role of JHD2 in this, we performed analogous experiments with jhd2Δ. Suppression of nab3-11, pob3-7, and sen1-1 by jhd2Δ could no longer occur in the context of H3K4R, arguing that Jhd2 opposed NNS and FACT function solely through reversal of H3K4me (Fig. 2B, 2C, and S3C). Based on these results as well as previous findings from Terzi et. al, we propose counterbalancing regulation of NNS and FACT by Set1 and Jhd2, with H3K4me promoting their functions and an unknown inhibitory impact conferred by Set1 in a H3K4 independent manner (Fig. 3).

To gain insight into how Set1 opposed FACT independent of H3K4, we constructed strains that had the endogenous SET1 locus replaced with SET1-G951S, an allele that abolishes Set1 enzymatic activity, but does not disrupt the integrity of COMPASS (NAGY et al. 2002; SCHIBLER et al. 2016). We found that SET1-G951S suppressed pob3-7 to an extent that was
equivalent to set1Δ in strains expressing a WT allele of H3K4 (Fig. S4). As enzymatically dead alleles of SET1 lead to a depletion of Set1 protein levels, this was to be expected (SOARES et al. 2014). Curiously, in strains expressing H3K4R, which do not deplete Set1 levels to nearly the extent seen with enzymatically dead alleles of Set1 (SOARES et al. 2014), SET1-G951S failed to suppress pob3-7 at 30˚ C, but did suppress equivalently to set1Δ at the more stringent temperature of 31.5 ˚C (Fig. S4). Similar results were obtained with nab3-11 (data not shown). These results, while complex, suggest that the H3K4-independent inhibition of pob3-7 by Set1 may involve both enzymatic and non-enzymatic roles of this protein (Fig. 3).

**Spt6-Spn1 was opposingly governed by JHD2 and SET1 through H3K4me3**

The complexity of interactions exhibited by NNS and FACT with SET1 rendered them difficult to further study without a more detailed understanding of what regulatory functions SET1 has independent of H3K4. We therefore extended our genetic analysis to Spt6-Spn1. As mentioned above, we determined that TS alleles of both Spt6-Spn1 subunits, spt6-14 and spn1-K192N, were suppressed by jhd2Δ (Table 1, Fig. 4A and 4B). In contrast to NNS and FACT, the temperature sensitive growth defects of both Spt6-Spn1 subunits were suppressed by jhd2Δ in a SET1 and H3K4 methylation dependent manner (Fig. 4A, B, C, and data not shown). Moreover, and consistent with the interpretation that SET1-mediated H3K4me promoted Spt6-Spn1 function, both set1Δ and H3K4R enhanced the growth defects of spt6-14 and spn1-K192N (Fig. 4A, B, C, and data not shown).

To validate that Jhd2 demethylation of H3K4 opposed Spt6-Spn1, we complemented the jhd2Δ-suppressed strains with previously described JHD2 expressing plasmids (MERSMAN et al. 2009). Cells harboring these plasmids express JHD2 under the control of a constitutive PYK1
promoter, which leads to modestly increased levels of Jhd2 and associated decreased H3K4me3
levels. These effects on H3K4me could be abrogated by the H427A mutation or by deletion of its
conserved PHD domain, which may enable Jhd2 recruitment to chromatin (MERSMAN et al.
2009). As expected, we found that jhd2Δ suppression of spt6-14 and spn1-K192N could be
reverted by JHD2 complementation and that the growth defects of spt6-14 and spn1-K192N were
in fact enhanced by the JHD2 overexpressing plasmids (Fig. S5A and S5B). Importantly, this
suppression was lost in the H427A and PHD domain mutants (which both encode stable proteins
(MERSMAN et al. 2009)) (Fig. S5A and S5B). Our findings support the conclusion that
suppression of Spt6-Spn1 alleles by jhd2Δ was due to the loss of H3K4 demethylation.

To gain insights into which H3K4 methylation species accounted for suppression of Spt6-
Spn1, we genetically interrogated COMPASS complex subunits known to specifically perturb
H3K4me3 or H3K4me3 and -me2. The effects of bre2Δ and spp1Δ on H3K4me have been
extensively described. Deletion of BRE2 results in a complete loss of H3K4me3 and a significant
decrease of H3K4me2 but no change in H3K4me1, while spp1Δ results in a substantial decrease
of H3K4me3 with no detectable consequence on bulk H3K4me2 or -me1 (SCHNEIDER et al.
2005; DEHE et al. 2006; TAKAHASHI et al. 2009; MARGARITIS et al. 2012). As with set1Δ, we
found that both bre2Δ and spp1Δ enhanced the growth defect of spn1-K192N (Fig. 4D).
Moreover, jhd2Δ suppression of spn1-K192N was completely dependent on BRE2 and SPP1
(Fig. 4D). We compared bulk H3K4me3 levels by western blot in spt6-14 and spt6-14 jhd2Δ at a
semi-permissive temperature. Consistent with the genetic data, we observed a modest increase in
bulk H3K4me3 levels in the spt6-14 jhd2Δ double mutant (Figure 4E). We confirmed the
reproducibility of this by quantifying the relative abundance of H3K4me3/H3 as well as of
H3/Pgk1 from three biological replicates of spt6-14 and spt6-14 jhd2Δ (Fig. S6). Collectively,
our data support the conclusion that *JHD2* and *SET1* opposingly governed the function of Spt6-Spn1 through H3K4me3 (Fig. 3).

**JHD2 repressed TSS-associated nucleosome occupancy and transcriptional induction of a Spt6-Spn1 regulated gene**

Reflecting its role in the recycling of nucleosomes in the wake of elongating PolII, Spt6-Spn1 mutation causes reduced histone occupancy genome-wide (IvANOVSka *et al.* 2011). We asked if *jhd2∆* suppression of Spt6-Spn1 involved a reversal of these histone occupancy defects. First, we used western blots to measure bulk histone H3 levels and found that *jhd2∆, set1∆,* and *spt6-14* mutants showed no apparent defects in histone H3 levels at the permissive temperature of 23°C (Figure 5A). At the semi-permissive temperature of 30°C *spt6-14* exhibited a dramatic reduction in H3 levels, consistent with its role as a histone chaperone (Figure 5A). This reduction was not reversed by *jhd2∆* suggesting that suppression did not occur by generically improving Spt6-Spn1 histone recycling or chaperone function (Figure 4E, 5A, and S6).

To gain more detailed insight into the relationship between Jhd2 and Spt6-Spn1, we measured chromatin-associated histone H3 in WT, *jhd2∆, spt6-14,* and *spt6-14 jhd2∆* cells at the *SRG1-SER3* locus using chromatin immunoprecipitation with an anti-H3 antibody followed by real time PCR quantification of the immunoprecipitated DNA (ChIP-qPCR). In order to evaluate nucleosome positions at high resolution across *SRG1-SER3,* we used 33 closely spaced PCR amplicons to measure immunoprecipitated DNA (HAINER *et al.* 2011b). The averaged results of 3 independent biological experiments are shown (Fig. 5B). As previously described, an abundance of H3 was detected throughout the *SRG1* transcript unit extending into the *SER3* gene in WT cells. These H3 levels were dramatically depleted in *spt6-14* mutants grown at the semi-
permissive temperature, confirming that their deposition was Spt6 dependent (Hainer et al. 2011b) (Fig. 5B). Consistent with our western blotting results, spt6-14 jhd2Δ mutants did not exhibit improved H3 deposition at SRG1-SER3 at the semi-permissive temperature we used (Fig. 5B). In WT and jhd2Δ cells with normal SPT6 function, the H3 ChIP profiles were nearly superimposable throughout the entire SRG1-SER3 region with one conspicuous exception: jhd2Δ caused a highly significant increase in H3 abundance at a single distinctive peak near the 3’ end of the SRG1 transcript unit, presumably reflecting a well-positioned nucleosome (Fig. 5B, peak denoted with a red asterisk, p < 0.05). This JHD2-repressed nucleosome was positioned ~75 base pairs upstream of the SER3 TSS, and positioned ~75 base pairs downstream of this TSS we observed an additional H3 ChIP peak in jhd2Δ, though this second peak was not statistically significant (Fig. 5B, denoted with a blue arrow). Spanning the SER3 TSS, H3 ChIP signal was noisy in both WT and jhd2Δ, suggesting a dynamic state of nucleosomal occupancy at this region (Fig. 5B).

We performed our ChIP experiments from cells grown in rich media, a condition in which SER3 transcription is repressed in a manner dependent on Spt6-Spn1-deposited nucleosomes across the SER3 TSS in response to SRG1 transcription (Pruneski and Martens 2011) (Fig. 5B). A simple encapsulation of our findings posits that JHD2 prevented Spt6-Spn1-deposited nucleosomes from accumulating at positions on the flanks of the SER3 TSS, resulting in dynamic and noisy nucleosome occupancy across the SER3 TSS (Fig. 5B). We detected no differences in SER3 abundance from WT and jhd2Δ cells grown in rich media, suggesting that this altered chromatin architecture did not impact SER3 repression (data not shown). To determine if it instead impacted SER3 induction, we subjected WT and jhd2Δ cells to serine starvation and compared their relative SER3 mRNA abundance using quantitative PCR
amplification of reverse transcribed RNA (RT-qPCR). We found that SER3 was maximally expressed 1 hour after serine withdrawal, and that SER3 mRNA accumulated to ~1.7-fold increased levels in jhd2Δ at this timepoint (Fig 5C). Following 2 hours of serine starvation, yeast cells adapt and repress SER3 induction, and jhd2Δ had no consequence for this repression (MARTENS et al. 2005) (Fig. 5C). Thus, the phased nucleosomes flanking the SER3 TSS we observed in jhd2Δ were associated with a hyper-induction of SER3.

Discussion

In this work, we identify novel genetic interactions between the H3K4 demethylase JHD2 and genes encoding subunits of the essential transcription regulatory complexes NNS, FACT, and Spt6-Spn1. Our genetic findings support the conclusion that reversal of H3K4me by Jhd2 opposes the functions of these complexes (Fig. 3). In the case of NNS, our findings are in good agreement with a previous study suggesting that H3K4me3 promotes NNS function (TERZI et al. 2011). A simple prediction from our findings is that set1Δ, which abolishes all H3K4 methylation, should cause the opposite phenotype as, and exhibit epistasis to, jhd2Δ. While this was indeed the case for Spt6-Spn1 and the SPT16 subunit of FACT, TS alleles of SEN1, NAB3, and POB3 were each suppressed by set1Δ to an extent equivalent to, or exceeding that by jhd2Δ. More detailed genetic studies revealed that SET1 opposed the functions of SEN1, NAB3, and POB3 in a manner that was independent of H3K4me. We point out that the Swd2 subunit of COMPASS also functions within the NNS-related termination complex APT, which could potentially explain the different NNS interactions with SET1 deletion versus K4 mutation shown in this study (Nedea et al., 2003; Soares and Buratowski, 2012). Our genetic model posits that
H3K4me positively influences NNS and FACT functions, and that Set1 exhibits an additional
counterbalancing H3K4me-independent activity that negatively impacts these complexes (Fig. 3). For Spt6-Spn1, our genetic data suggested a positive impact by H3K4me3 with no repressive
H3K4-independent role for Set1 (Fig. 3). Our molecular studies showed that Jhd2, and by
inference H3K4me reversal, negatively regulated the accumulation of Spt6-Spn1 deposited
nucleosomes flanking the SER3 TSS, resulting in hyper-induction of SER3 upon serine
starvation.

In contrast to our findings and what has been reported previously (Hainer et al. 2011b),
Ramakrishnan et al found that both jhd2Δ and set1Δ caused dramatic up-regulation of SER3 in
rich serine-replete conditions (Ramakrishnan et al. 2016). Under such conditions SER3 is
normally strongly repressed, and we found that levels were so low as to be nearly undetectable
with no difference between WT and jhd2Δ (Fig. 5C) (Martens et al. 2004; Martens et al.
2005; Hainer et al. 2011b). To reconcile these discrepancies, we point out that all of the
experiments in the Ramakrishnan et al study were performed in a strain background that utilized
plasmid expressed histone H2A-H2B, with the endogenous H2A-H2B genes deleted, a condition
known to reduce histone dosage and alter transcription (Clark-Adams et al. 1988). This is not
to say we reject the findings of Ramakrishnan et al; rather, the findings of Ramakrishnan et al
seem to illuminate a facile way to investigate chromatin biology in yeast, by ‘sensitizing’ the
chromatin through reduced histone dosage. Whatever the case may be, our findings uphold an
emerging role for Jhd2 in the control of nucleosome occupancy at TSS’s and TTS’s. JHD2’s
known developmental role in opposing nucleosome accumulation at these regions may be
attributable to it antagonistic impact on Spt6-Spn1 (Xu et al. 2012). As Spt6-Spn1, Set1, Jhd2,
and H3K4me are conserved throughout eukaryotes, these mechanisms may have broad
significance.

An important feature of our findings relates to functions of Set1 that are independent
H3K4 methylation. The only confirmed non-H3K4 enzymatic target of Set1, or indeed of any
member of this highly conserved family of enzymes, is the essential kinetochore protein Dam1.
Dam1 lysine residue methylation by Set1 regulates the phosphorylation status of neighboring
serine residues by mediating the opposing activities of Ipl1 and Glc7 kinase and phosphatase
(Zhang et al. 2005). Schizosaccharomyces pombe Set1 (spSet1) controls transcription at silent
mating loci, telomeric regions, and retrotransposon (Tf) elements, as well as the clustering of
diverse genomic regions encoding Tf elements. Interestingly, spSet1 accomplishes these sundry
functions in a manner dependent on differing domains of Set1 that do, or do not, have
consequences for H3K4me. The two key regulatory roles of spSet1 appear dependent on spSet1’s
enzymatic activity and on its RNA binding RRM2 domain, a domain found in both fission and
budding yeast Set1 (Tresaugues et al. 2006; Lorenz et al. 2014; Mikheyeva et al. 2014).
Consistent with the RRM2 having important regulatory significance independent of H3K4me, a
recent study identified many mRNA targets bound by the budding yeast Set1 RRM2 domain
(Sayou et al. 2017). Thus, the RRM2 domain may underlie at least some of the H3K4me-
independent regulatory functions of Set1 we identify here. Our genetic results, however, also
suggest that enzymatic activity of Set1 directed towards a non-H3K4 substrate has regulatory
significance, as may be the case for S. pombe Set1 (Lorenz et al. 2014; Mikheyeva et al.
2014). It seems highly plausible that chromatin-associated factors may be targets of regulatory
lysine methylation by Set1 and other histone methyltransferases.
**Materials and Methods**

Yeast Strains and Plasmids

Standard yeast genetic methods were used for construction of all strains. Yeast strains and plasmids used in this study are listed in Supplementary Data Table 1. All strains were constructed through genetic crosses followed by dissections the BY4742 background. Yeast strains were inoculated into several mL of YPD (1% yeast extract, 2% peptone, and 2% glucose) and grown overnight at room temperature (23°C). Each strain was diluted to an OD$_{600}$ = 0.4, serially diluted five times and spotted on synthetic complete media (YNB media (Multicell Wisent) containing 5 g/L of ammonium sulfate and either 2% glucose or 2% galactose.

Western blots

Exponential cultures with OD$_{600}$ between 1-2 were lysed by vortexing with acid washed glass beads in SUMEB buffer as described previously (Fredrickson et al. 2011). Total protein concentration was quantified using an RC/DC assay (BioRad). Equal amounts of protein were electrophoresed on 12% SDS-PAGE gels, and transferred onto Amersham Hybond-P membranes (GE). Immunoblot analysis was performed using standard procedures. All blots were scanned with a ChemiDoc XRS+ Imaging System. Band intensities were quantified using ImageJ 1.51 v software.

Chromatin immunoprecipitation
Strains were grown at 30°C in YPD to OD$_{600}$ 0.8. Crosslinking was performed in 1% formaldehyde for 15 minutes at room temperature (23°C) and then immediately quenched with glycine at a final concentration of 125 mM. Cells were pelleted and washed 2 times with ice cold PBS. Cell pellets were snap frozen in 2 mL screwcap tubes using liquid nitrogen and stored at -80°C. Frozen pellets were resuspended in lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1 mM EDTA pH 8.3, 1% Triton X-100, 0.1% NaDOC) without thawing and topped off with glass beads. Cells were lysed through multiple rounds of beadbeating and then sonicated to shear the chromatin to fragment sizes of approximately 200 to 500 bp. Cross-linked chromatin fragments were immunoprecipitated with antibody overnight. Protein A-Sepharose beads were then added to the samples, and samples were incubated for 90 minutes. The immunoprecipitated complexes were washed with lysis buffer, lysis buffer containing 500 mM NaCl, wash buffer (10 mM Tris-HCl pH 8.0, 0.25M LiCl, 0.5% NP40, 0.5% NaDOC, 1mM EDTA pH 8.3), and TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Next, the immunoprecipitated chromatin was eluted from beads with elution buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS) and then eluted with TE/0.67% SDS (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.3, 0.67% SDS, 0.334 mg/mL proteinase K). The two elutions were combined. Whole cell extract samples were topped off with TE/1% SDS (10 mM Tris-HCl pH 8.0, 1mM EDTA, pH 8.3, 1% SDS, 0.25 mg/mL proteinase K). Formaldehyde cross-linking was reversed by incubating the eluates at 65°C overnight. DNA from the eluates was treated with RNAse A and purified with a QIAquick PCR purification kit (Qiagen). Immunoprecipitated fractions (IP) and whole-cell extracts (Input) containing DNA were analyzed by real-time PCR as described previously (Xu et al. 2012) using the primers listed in Supplementary Data Table 2. SYBR green signal was measured using the BioRad iQ5 Multicolor Real Time PCR Detection System.
SER3 RT-qPCR analysis

5-10 OD equivalents were harvested for RNA extraction. For RT-qPCR, RNA was extracted with acidic phenol at 65°C for 30 min. RNA was then purified, precipitated and resuspended in RNAse free water. cDNA was prepared as described previously using random priming (Xu et al. 2012). Quantitative PCR quantification of cDNA using SYBR green was measured using the BioRad iQ5 Multicolor Real Time PCR Detection System as described previously (Xu et al. 2012). Primer sequences for SER3 and SCR1 are shown in Supplementary Data Table 2. Transcript levels for each primer pair tested were normalized to the reference transcript SCR1.

SER3 induction assays

Yeast strains were inoculated into several mL of YPD and grown overnight at room temperature (23°C). Each strain was diluted to an OD₆₀₀ = 0.4 in synthetic complete media and grown to OD₆₀₀ = 2 at 30°C. At this time, cells were harvested by centrifugation, washed with water, and resuspended in synthetic media lacking serine. Growth of cells was resumed with constant shaking at 30°C. Cell samples were collected at specific intervals and frozen using liquid nitrogen for analysis by RT-qPCR as described previously (Xu et al. 2012). Primer sequences for SER3 and SCR1 are shown in Supplementary Data Table 2. RNA isolation, cDNA synthesis, and qPCR is described above.
References


Dey, B. K., L. Stalker, A. Schnerch, M. Bhatia, J. Taylor-Papidimitriou et al., 2008 The histone demethylase KDM5b/JARID1b plays a role in cell fate decisions by blocking terminal differentiation. Molecular and cellular biology 28: 5312-5327.


Margaritis, T., V. Oreal, N. Brabers, L. Maestroni, A. Vitaliano-Prunier et al., 2012 Two distinct repressive mechanisms for histone 3 lysine 4 methylation through promoting 3'-end antisense transcription. PLoS Genet 8: e1002952.


Santos-Rosa, H., R. Schneider, B. E. Bernstein, N. Karabetsou, A. Morillon et al., 2003
Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin.
Molecular cell 12: 1325-1332.
Takahashi, Y. H., J. S. Lee, S. K. Swanson, A. Saraf, L. Florens et al., 2009 Regulation of H3K4 trimethylation via Cps40 (Spp1) of COMPASS is monoubiquitination independent: implication for a Phe/Tyr switch by the catalytic domain of Set1. Mol Cell Biol 29: 3478-3486.
Taverna, S. D., S. Ilin, R. S. Rogers, J. C. Tanny, H. Lavender et al., 2006 Yng1 PHD finger binding to H3 trimethylated at K4 promotes NuA3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. Molecular cell 24: 785-796.


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Figure 1. TS alleles of NNS and FACT subunits exhibit genetic interactions with H3K4 modifying enzymes.

Yeast strains with the indicated genotypes were serially diluted ten-fold, spotted onto agar plates containing synthetic complete media, and grown at indicated temperatures. Genetic interactions of jhd2Δ and set1Δ with different temperature sensitive alleles of NNS and FACT subunits are shown: (A) nab3-42 (B) pob3-7 (C) spt16-ts.

Figure 2. Genetic suppression of NNS and FACT by set1Δ and jhd2Δ dependent and
independent of H3K4me.

Plate spot assays (as described in Figure 1) were used to compare the growth of the indicated strains. Isogenic strains were constructed through genetic crosses with H3K4A and H3K4R substitution mutants from the Dharmacon Non Essential Histone H3 & H4 Mutant Collection.

(A) Genetic interactions of set1Δ, H3K4A, and H3K4R with pob3-7. (B) Genetic interactions of jhd2Δ and H3K4R with nab3-11. Growth of two independent isolates of each genotype is shown.

(C) Genetic interactions of jhd2Δ and H3K4R with pob3-7. (D) Diploid strains containing csf1Δ, set1Δ, and either H3K4A or H3K4R were sporulated and dissected onto YPD medium. A representative field of the haploid ascospores recovered and their respective genotypes are shown.

**Figure 3.** Genetic model of FACT and NNS regulation by Set1 and Jhd2.

Methylation of H3K4 promoted FACT and NNS function while Set1 exhibited an inhibitory function independent of H3K4. Spt6-Spn1 is positively regulated by H3K4me3.

**Figure 4.** Spt6-Spn1 complex temperature sensitive mutants are rescued by an increase in H3K4me3 through jhd2Δ.

Plate spot assays (as described in Figure 1) were used to compare the growth of the indicated strains. Genetic interactions of jhd2Δ and set1Δ with Spt6-Spn1 are shown: (A) spt6-14 (B) spn1-K192N. (C) Isogenic Strains were constructed through genetic crosses with the H3K4R substitution mutant from the Dharmacon Non Essential Histone H3 & H4 Mutant Collection.

Genetic interactions of jhd2Δ and H3K4R with spn1-K192N. (D) Genetic interactions of jhd2Δ, nonessential COMPASS subunits spp1Δ and bre2Δ, and spn1-K192N. (E) Western blot analysis...
of H3K4me3, Histone H3, and Pgk1 were performed on biological duplicates of *spt6-14* and
*spt6-14 jhd2Δ*. Pgk1 serves as a loading control.

**Figure 5.** *JHD2* deletion does not rescue Spt6-Spn1 mutants by compensating for defects in chromatin structure.

(A) Western blot detection of H3K4me3, pan-H3, and Pgk1 are shown from extracts of wildtype and mutant cells grown at 23°C (permissive) and 30°C (semi-permissive temperature). Pgk1 serves as a loading control. (B) Histone H3 ChIP was performed on strains grown at 30°C (semi-permissive temperature) across the *SRG1-SER3* locus (schematic of locus shown above graph). H3 ChIP signal was normalized to signal from whole cell lysate (WCL) DNA. The transcription start site of *SER3* is positioned at 0 and for *SRG1* at -480. Error bars represent standard error of the mean. Significance as calculated by a two-tailed student's t-test is shown where *p* < 0.05. A blue arrow denotes an additional H3 ChIP peak in *jhd2Δ* that was not statistically significant. (C) *SER3* transcript level was measured by RT-qPCR at specific intervals after serine withdrawal in wildtype and *jhd2Δ* mutants. *SER3* signal was normalized to *SCR1*.

**Supp. Fig. 1.** Alternative NNS and FACT subunit TS alleles exhibit the same genetic interaction profile as *NAB3*.

Yeast strains with the indicated genotypes were serially diluted ten-fold, spotted onto agar plates containing synthetic complete media, and grown at indicated temperatures. Genetic interactions of *jhd2Δ* and *set1Δ* with different temperature sensitive alleles of NNS and FACT subunits are shown: (A) *nab3-11* (B) *sen1-1* (C) *pob3-L78R* (D) *spt16-319*. 
Supp. Fig. 2. Arginine substitution of H3K4 most reliably mimics set1Δ.

Plate spot assays (as described in previous figures) were used to compare the growth of the indicated strains. (A) Isogenic Strains were constructed through genetic crosses with H3K4A and H3K4R substitution mutants from the Dharmacon Non Essential Histone H3 & H4 Mutant Collection. Genetic interactions of H3K4A and H3K4R with set1Δ. (B) Genetic interactions of set1Δ and H3K4A with nab3-11. (C) Genetic interaction of jhd2Δ and csf1Δ is shown. (D) Yeast strains with the indicated genotypes were serially diluted ten-fold, spotted onto agar plates containing synthetic complete media containing glucose or galactose to compare the growth of the indicated strains. JHD2 is expressed under the control of a galactose inducible promoter. The H427A mutation disrupts the histone demethylase activity of Jhd2.

Supp Fig. 3. Genetic suppression of NNS by set1Δ and jhd2Δ dependent and independent of H3K4me.

Plate spot assays (as described in previous figures) were used to compare the growth of the indicated strains. Isogenic Strains were constructed through genetic crosses with H3K4A and H3K4R substitution mutants from the Dharmacon Non Essential Histone H3 & H4 Mutant Collection. (A) Genetic interactions of set1Δ and H3K4R with nab3-11. Growth of two independent isolates of each genotype is shown. (B) Genetic interactions of set1Δ and H3K4R with sen1-1. (C) Genetic interactions of jhd2Δ and H3K4R with sen1-1.

Supp Fig. 4. H3K4-independent suppression of pob3-7 by Set1 may involve both enzymatic and non-enzymatic activities.

Plate spot assays (as described in previous figures) were used to compare the growth of the
indicated strains. Analyzed is a G951S mutation that disrupts histone methyltransferase activity of Set1.

**Supp Fig. 5.** The enzymatic activity and the PHD domain of Jhd2 opposes Spt6-Spn1. *JHD2, JHD2(H427A),* and *JHD2(PHDΔ)* expression was controlled by the constitutive *PYK1* promoter and delivered to the indicated yeast strains on a low copy CEN-ARS plasmid marked by *LEU2*. Each yeast strain was grown overnight in YNB-leu to maintain the plasmid. Cells were serially diluted, plated on YNB-leu plates, and grown at various temperatures. (A) *spn1-K192N* (B) *spt6-14*.

**Supp Fig. 6.** Western blot quantification. Western blot quantification for *spt6-14* and *spt6-14 jhd2Δ* at the semi-permissive temperature (30°C) for n = 3 normalized to WT. Error bars depict 1 standard deviation. (A) H3K4me3/H3 (B) H3/Pgk1.

**Table 1.** Genetic interactions of *JHD2* with essential transcription regulatory factors. Specific TS allele is listed in the left column the associated protein complex of each factor shown in the corresponding middle column. In the right column, any observed genetic interactions with *jhd2Δ* are documented. N.I. no interaction, + positive interaction (+ weakest, ++ medium, +++ strong suppression), - weak negative interaction.

**Table 2.** Observed and expected genetic frequencies of *csf1Δ* with histone H3K4A and H3K4R amino acid substitution mutants.
Diploid strains mmy7658 (H3K4R), mmy7659 (H3K4WT), and mmy7660 (H3K4A) were sporulated and dissected onto YPD medium. These strains are heterozygous for set1Δ, csf1Δ, and the corresponding histone amino acid substitution. The observed frequency of each genotype was calculated by dividing to the total number of spores counted and is shown in the parentheses.
Fig. 1

A.

WT
jhd2Δ
set1Δ
jhd2Δ set1Δ
nab3-42
nab3-42 jhd2Δ
nab3-42 set1Δ
nab3-42 jhd2Δ set1Δ

B.

WT
jhd2Δ
set1Δ
jhd2Δ set1Δ
pob3-7
pob3-7 jhd2Δ
pob3-7 set1Δ
pob3-7 jhd2Δ set1Δ

C.

WT
jhd2Δ
set1Δ
jhd2Δ set1Δ
spt16-ts
spt16-ts jhd2Δ
spt16-ts set1Δ
spt16-ts jhd2Δ set1Δ
Fig. 2

A.  
- pob3-7 H3K4WT
- pob3-7 H3K4WT set1Δ
- pob3-7 H3K4A
- pob3-7 H3K4A set1Δ
- pob3-7 H3K4R
- pob3-7 H3K4R set1Δ

B.  
- nab3-11 H3K4WT
- nab3-11 H3K4WT
- nab3-11 H3K4R
- nab3-11 H3K4R
- nab3-11 H3K4WT jhd2Δ
- nab3-11 H3K4WT jhd2Δ
- nab3-11 H3K4R jhd2Δ
- nab3-11 H3K4R jhd2Δ

C.  
- pob3-7 H3K4WT
- pob3-7 jhd2Δ H3K4WT
- pob3-7 H3K4R
- pob3-7 jhd2Δ H3K4R

D.  
- csf1Δ H3K4A
- csf1Δ
- csf1Δ set1Δ
- csf1Δ set1Δ H3K4A
- csf1Δ H3K4R
- csf1Δ set1Δ H3K4A
- csf1Δ set1Δ H3K4A
Fig. 5

A. 

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B. 

AIM9 — SRG1 — SER3

C. 

SER3 mRNA levels

0h 0.25h 1h 2h

Serine Starvation

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