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Regulation of Set1-mediated methylation of Dam1

John A. Latham

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by

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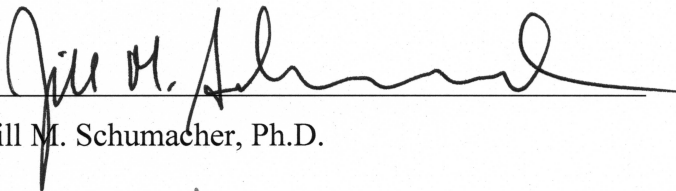
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REGULATION OF SET1-MEDIATED METHYLATION OF DAM1

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Publication No._____

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Eukaryotic genomes exist within a dynamic structure named chromatin in which DNA is wrapped around an octamer of histones forming the nucleosome. Histones are modified by a range of posttranslational modifications including methylation, phosphorylation, and ubiquitination, which are integral to a range of DNA-templated processes including transcriptional regulation. A hallmark for transcriptional activity is methylation of histone H3 on lysine (K) 4 within active gene promoters. In *S. cerevisiae*, H3K4 methylation is mediated by Set1 within the COMPASS complex. Methylation requires prior ubiquitination of histone H2BK123 by the E2-E3 ligases Rad6 and Bre1, as well as the Paf1 transcriptional elongation complex. This regulatory pathway exemplifies cross-talk *in trans* between posttranslational modifications on distinct histone molecules. Set1 has an additional substrate in the kinetochore protein Dam1, which is methylated on K233. This methylation antagonizes phosphorylation of adjacent serines by the Ipl1 Aurora kinase. The discovery of a second Set1 substrate raised the question of how Set1 function is regulated at the kinetochore. I hypothesized that transcriptional regulatory factors essential for H3K4 methylation at gene promoters might also regulate Set1-

mediated methylation of Dam1K233. Here I show that the regulatory factors essential for

COMPASS activity at gene promoters is also indispensable for the methylation of

Dam1K233. Deletion of members of the COMPASS complex leads to loss of

Dam1K233 methylation. In addition, deletion of Rad6, Bre1, or members of the Paf1

complex abolishes Dam1 methylation. The role of Rad6 and Bre1 in Dam1 methylation

is dependent on H2BK123 ubiquitination, as mutation of K123 within H2B results in

complete loss of Dam1 methylation. Importantly, methylation of Dam1K233 is

independent of transcription and occurs at the kinetochore. My results demonstrate that

Set1-mediated methylation is regulated by a general pathway regardless of substrate that

is composed of transcriptional regulatory factors functioning independently of

transcription at the kinetochore. My data provide the first example of cross-talk *in trans*

between modifications on a histone and a non-histone protein. Additionally, my results

indicate that several factors previously thought to be required for Set1 function at gene

promoters are more generally required for the catalytic activity of the COMPASS

complex regardless of substrate or cellular process.

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Chapter 1

Introduction

Chromatin structure and function

Eukaryotic genomes do not exist as naked DNA within the nucleus. Rather, genomes exist as a dynamic structure of DNA and proteins, which was named chromatin by Walther Flemming in 1879. The term chromatin is derived from the Greek word for color *khroma* due to its pronounced staining with basophilic dyes (Paweletz, 2001). Soon after the discovery of chromatin, Albrecht Kossel in 1884 isolated from goose erythrocytes the highly basic proteins from chromatin naming them histones (Hnilica et al., 1989). Originally, chromatin and histones were thought to mainly serve a structural role as a DNA scaffold allowing for its compaction into the nucleus. It was not until the midpoint of the 20th century that it was hypothesized that histones may serve another function as gene repressors (Stedman, 1950). Eventually, it was shown through the work of a large body of researchers that chromatin dynamically regulates a number of processes essential for normal cellular functions (Groth et al., 2007; Li et al., 2007).

The fundamental unit of chromatin is the nucleosome (Kornberg, 1974). The nucleosome consists of an octamer of histone proteins comprised of two heterodimers of the core histones H2A and H2B and a heterotetramer of the core histones H3 and H4. Around this octamer of proteins is wrapped 146 base-pairs of DNA (Luger et al., 1997). Histones contain a central globular domain that forms the major architecture of the nucleosome and a flexible, unstructured N-terminal tail that protrudes outside of the nucleosome

(Luger et al., 1997). Binding to the DNA as it enters and exits the nucleosome are linker histones such as H1 (Happel and Doenecke, 2009). The roles of the linker histones are to stabilize the nucleosome and help in compaction and formation of higher order chromatin structures. The structure of uncondensed nucleosomes on DNA is called "beads on a string" due to its resemblance to that structure (Olins and Olins, 1974). The first order of chromatin compaction is into a solenoid structure called the 30-nanometer fiber (Tremethick, 2007). Chromatin can be further compacted through mechanisms that are not yet clear into the highly condensed mitotic chromosomes (Woodcock and Ghosh, 2010).

Chromatin is divided into two main categories, heterochromatin and euchromatin (Lamond and Earnshaw, 1998). Heterochromatin is tightly compacted resulting in restricted access to the underlying DNA by RNA polymerases and other DNA binding proteins (Bühler and Gasser, 2009). A well-characterized example of heterochromatin regulating DNA-templated activity is X-chromosome inactivation (Payer and Lee, 2008). In *S. cerevisiae*, heterochromatin inhibits transcription at the silent loci of centromeres, telomeres, and mating loci (Bühler and Gasser, 2009). In contrast, within euchromatin nucleosomes are less tightly compacted. Euchromatin is the chromatin environment at actively transcribed genes, genes poised for transcription, and genomic loci undergoing DNA repair (Groth et al., 2007; Li et al., 2007). In order to establish or maintain euchromatin and heterochromatin, cells utilize different histone variants, ATP-dependent chromatin remodeling complexes, and different combinations of posttranslational modifications of the histones (Bassett et al., 2009).

Histone posttranslational modifications

Dynamic chromatin structures are governed in part by posttranslational modifications of the histones (Kouzarides, 2007). Using the example of gene regulation, posttranslational modifications actively determine whether a particular locus is silenced, repressed, or transcribed. These modifications were first discovered in the 1960s (Allfrey et al., 1964), but it was not until the end of the 20th century that a link between gene regulation and histone modifications were found, when a transcriptional activator was determined to acetylate histones (Brownell et al., 1996). Since then, it has it become clear that covalent histone modifications play critical regulatory roles in gene regulation and other DNA-templated processes (Kouzarides, 2007).

A large number of residues within the histones are modified by posttranslational modifications (Figure 1) (Latham and Dent, 2007). Lysines are subject to acetylation. In addition, lysines within histones can be monoubiquitinated, serving a signaling role rather than a role in protein degradation. Serines and tyrosines are modified by phosphorylation. Both lysines and arginines are modified by methylation. Arginines can be modified by up to two different methyl groups while lysines can be methylated up to three times. Furthermore, the degree of methylation can result in different functional outcomes (Latham and Dent, 2007).

Covalent histone modifications are associated with specific regulatory functions. For instance, acetylation is associated with active gene transcription while lysine methylation

Figure 1. Common histone posttranslational modifications

Several different histone residues undergo posttranslational modification. Shown are the

types of amino acids and the modifications covalently linked to them. Adapted from

(Latham and Dent, 2007).
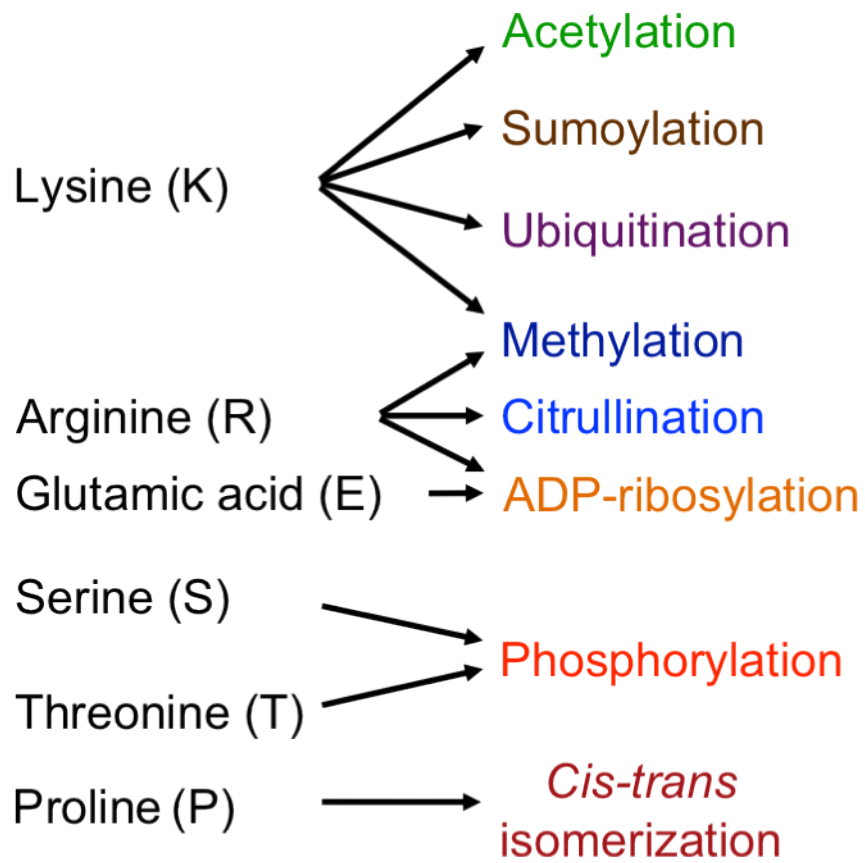
Figure 1

is associated with both gene transcription and repression depending on which lysine is modified and whether it is mono-, di-, or trimethylated (Kouzarides, 2007). The most well characterized modifications occur on the flexible N-terminal tails of histones, especially H3 and H4. However, modifications also occur within the globular regions and the C-terminal regions of histones (Hyland et al., 2005). It is thought that modifications within the central-fold domain of histones mediate contact with the DNA and other histones, whereas modification of the N-terminal tails serve as docking sites for histone modification "readers".

Covalent histone modifications serve as binding sites for effector molecules (Taverna et al., 2007). These "readers" bind to posttranslational modifications through modification specific binding domains such as PHD domains for lysine methylation and bromo domains for acetylated lysines (Taverna et al., 2007). Additionally, modification(s) can serve as inhibitors that prevent the binding of specific proteins. These effects serve as the basis of the histone code hypothesis, which posits that patterns of histone modifications and their binding by effector molecules lead to specific downstream functions (Strahl and Allis, 2000; Turner, 2000).

Modification of histones occurs in distinct patterns that can predict a specific DNA-templated activity at a given locus. These patterns are established by "cross-talk", in which modification on one residue can influence the modification of the same residue or another residue (Latham and Dent, 2007). Cross-talk between modifications on the same residue is called cross-talk *in situ* (Latham and Dent, 2007). An example of *in situ*

regulation is acetylation or methylation of H3K9. Methylation of H3K9 is a

transcriptionally repressive mark, and it prevents acetylation of this residue, which is a

mark of transcriptional activation. Conversely, when H3K9 is acetylated, methylation is

prevented (Latham and Dent, 2007).

Cross-talk between posttranslational modifications on the same histone molecule is called

cross-talk *in cis*. This type of cross-talk between modifications is exemplified by

phosphorylation of H3S10 blocking methylation of H3K9 and vice-versa, methylation of

H3K9 inhibiting phosphorylation of H3S10 (Latham and Dent, 2007). Examples of

cross-talk *in cis* are found not only on histones, but have been found on non-histone

proteins such as p53 (Latham and Dent, 2007).

Regulation of H3K4 methylation by H2B ubiquitination

The canonical example of cross-talk between histone modifications *in trans* is regulation

of H3K4 methylation by H2B ubiquitination at active gene promoters (Figure 2) (Dover

et al., 2002; Sun and Allis, 2002). This pathway is unidirectional, as deletion of the

H3K4 methyltransferase Set1 or mutation of H3K4 has no effect on H2B ubiquitination

(Sun and Allis, 2002). This "trans-tail" pathway of histone modifications is conserved

from budding yeast all the way to humans. In yeast, H2BK123 ubiquitination is required

for H3K4 methylation; while in humans, ubiquitination of H2BK120 is required (Dover

et al., 2002; Sun and Allis, 2002; Zhu et al., 2005). Not all levels of H3K4 methylation

Figure 2. Regulation of H3K4 methylation at promoters

Set1-mediated methylation of H3K4 is highly regulated at active promoters. Rad6 is

phosphorylated by Bur1-Bur2. Rad6 and Bre1, together with the Paf1 complex,

ubiquitinate H2BK123. H2B ubiquitination is negatively regulated by Ubp8 or Ubp10.

Rpt4 and Rpt6 link H2B ubiquitination to H3K4 methylation mediated by the COMPASS

complex.

Transcriptional Regulation
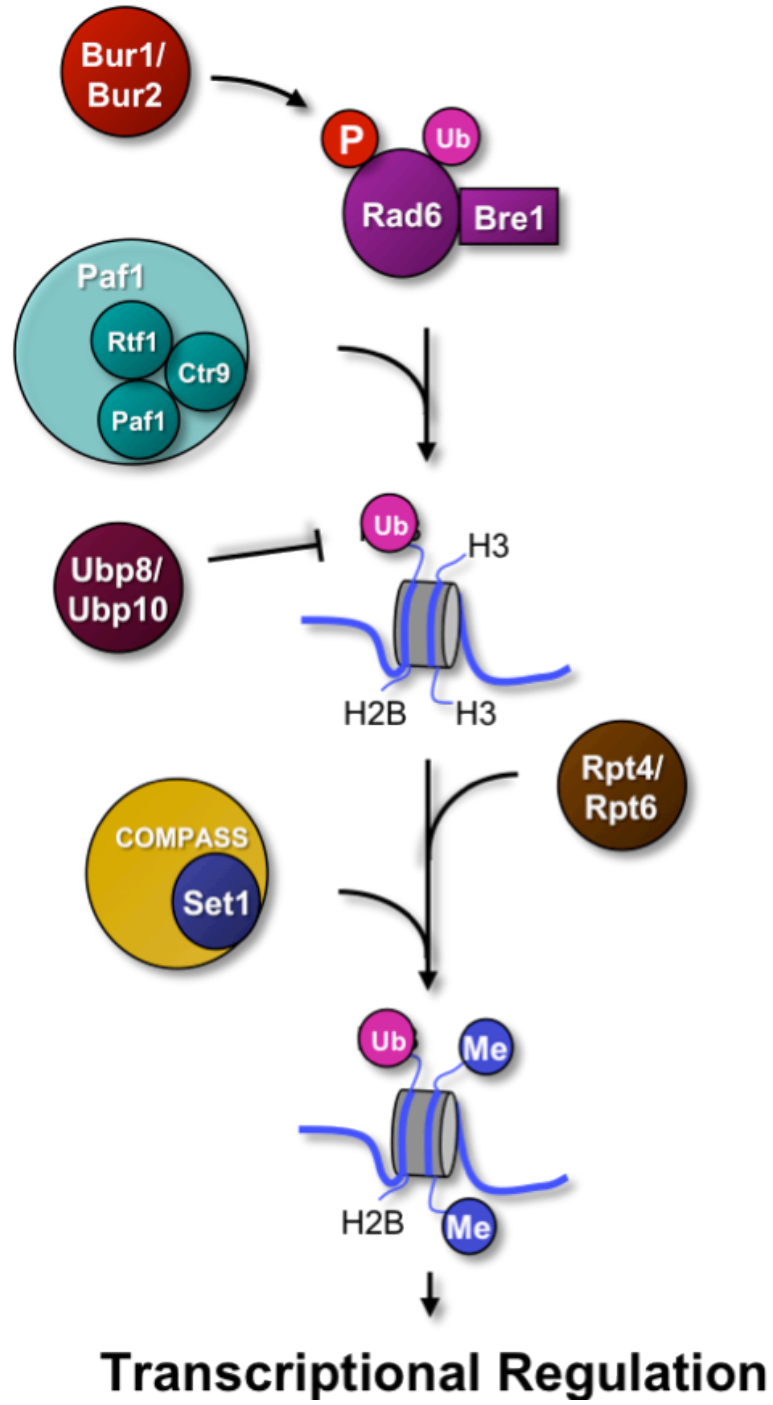
Figure 2

require H2B ubiquitination as it is not required for H3K4 monomethylation, but only for H3K4 di- and trimethylation (Dehe et al., 2005; Shahbazian et al., 2005). Interestingly, Dot1-mediated methylation of H3K79 within the H3 globular domain also requires H2BK123 to be ubiquitinated (Ng et al., 2002b). The conservation of the "trans-tail" pathway across evolution allows for use of the *S. cerevisiae* model organism to study a pathway directly applicable to human biology. Over the last several years, regulation of this pathway has been extensively studied and determined to be regulated by a number of factors, as illustrated in Figure 2 and described in detail below.

H2B ubiquitination is mediated by Rad6-Bre1 and the Paf1 complex

H2B ubiquitination at K123 is catalyzed by the E2 ubiquitin conjugating enzyme Rad6 and the E3 ubiquitin ligase Bre1 (Hwang et al., 2003; Robzyk et al., 2000; Wood et al., 2003a). These enzymes are conserved, as the human orthologues of Rad6 are HR6A and HR6B and the Bre1 orthologue is RNF20 (Table 1) (Kim et al., 2005; Koken et al., 1991; Roest et al., 2004). Deletion of Rad6, Bre1, or mutation of H2BK123 to a residue that is unable to be ubiquitinated results in loss of H3K4 di- and trimethylation (Dover et al., 2002; Sun and Allis, 2002).

Bre1 is recruited to promoters independently of Rad6, likely through interaction of transcriptional co-activators (Hwang et al., 2003). However, Rad6 recruitment to promoters is dependent on Bre1, reflecting a two-step process for H2BK123 ubiquitination (Kao et al., 2004). Rad6 is phosphorylated on S120 by the Bur1/Bur2 cyclin dependent kinases (Wood et al., 2005). Phosphorylation on S120 is not required

Table 1: Human orthologs of H3K4 methylation regulatory proteins

Protein complex	*S. cerevisiae*	Human
COMPASS	Set1	MLL
	Bre2 Spp1	ASH2L
	Swd1 Swd2 Swd3	RBBP5 WDR5
Rad6-Bre1c	Rad6	HR6A, HR6B
	Bre1	RNF20
Paf1c	Paf1	hPaf1
	Ctr9	hCtr9
	Rtf1	hRtf1
SAGA	Ubp8	USP22

for recruitment of Rad6 to promoters (Wood et al., 2005). However, the kinases are

required for the catalytic activity of Rad6 towards H2BK123 (Laribee et al., 2005; Wood

et al., 2005). Despite the role that Bur1/Bur2 play in H2BK123 ubiquitination, they only

regulate H3K4 trimethylation through a mechanism that is not yet understood (Laribee et

al., 2005).

Bur1/Bur2 are also essential for recruitment of the Paf1 complex to promoters, which is

necessary for H2BK123 ubiquitination (Laribee et al., 2005). The conserved Paf1

transcriptional elongation complex includes Ctr9, Rtf1, and the Paf1 protein itself (Table

1) (Krogan et al., 2003b; Ng et al., 2003a; Wood et al., 2003b). While the Paf1 complex

is not necessary for Rad6 or Bre1 recruitment to promoters, it is required for their

catalytic activity (Wood et al., 2003b). Additionally, the Paf1 complex mediates

association of Rad6-Bre1 with elongating RNA polymerase II (Wood et al., 2003b).

Deletion of the Paf1 complex member *RTF1* results in Rad6 retention at the promoter and

failure to associate with the elongating RNA polymerase II (Xiao et al., 2005). In *in vitro*

studies, Bre1 binds directly to the Paf1 complex, and this interaction is required for the *in*

vitro ubiquitination of H2BK123 (Kim and Roeder, 2009). This mechanism for H2B

ubiquitination also holds true in human cells as active transcription is required for

H2BK120 ubiquitination (Kim et al., 2009). Since the Paf1 complex is required for

H2BK123 ubiquitination, it is also required for H3K4 di- and trimethylation at promoters

(Krogan et al., 2003b). Additionally, in *in vitro* methyltransferase assays, ubiquitinated

H2B directly stimulates H3K4 methylation (Kim et al., 2009).   A direct role for

transcription in regulating H2B ubiquitination has been observed, as a temperature

sensitive allele of the RNA polymerase II C-terminal domain (CTD) serine 5 kinase

KIN28 results in a global loss of H2B ubiquitination (Xiao et al., 2005). However,

mutation of RNA polymerase II serine 5 to an unphosphorylatable residue results in only

modest decreases in H2BK123 ubiquitination suggesting that the role of Kin28 in H2B

ubiquitination in not solely through CTD serine 5 phosphorylation (Xiao et al., 2005).

H2BK123 is also regulated by deubiquitination. Deubiquitination of H2B is carried out

by two different ubiquitin specific proteases, Ubp8 and Ubp10. Ubp8 is part of the

SAGA complex (Table 1) and requires association with the SAGA complex for its

catalytic activity (Henry et al., 2003; Lee et al., 2005). Ubp8-mediated H2B

deubiquitination occurs at active gene promoters, where H2B undergoes rounds of

ubiquitination and deubiquitination (Henry et al., 2003). Deletion of Ubp8 results in an

increase in H3K4 methylation in a gene specific manner (Shukla et al., 2006).

In contrast, Ubp10 regulates H2BK123 ubiquitin levels at silenced genomic loci such as

telomeric and rDNA (Emre et al., 2005; Gardner et al., 2005). The role of Ubp10 in gene

silencing is to target the histone deacetylase Sir2 to silenced regions of the genome and to

maintain low levels of H3K4 methylation through H2BK123 deubiquitination (Calzari et

al., 2006; Emre et al., 2005; Gardner et al., 2005).

Set1-mediated methylation of H3K4

Set1 is a 1080 amino acid protein and contains a C-terminal SET (*Su(var)3–9, Enhancer*

of zeste, trithorax) domain for which it is named. Set1 is orthologous to the MLL

proteins and hSET1 in humans (Table 1). Set1 was first discovered as a transcriptional repressor (Nislow et al., 1997). Later it was determined that Set1 is a lysine (K) methyltransferase that modified histone H3 N-terminal tails on K4 (Briggs et al., 2001).

Set1 resides within an eight-member complex named COMPASS (COMPlex of Proteins ASociated with Set1) (Table 1) (Miller et al., 2001; Nagy et al., 2002; Roguev et al., 2001). Set1 is the only catalytic member of this complex. However, an intact complex is required for the catalytic activity of Set1. Interestingly, specific subunits are required for different degrees of H3K4 methylation. Swd1, Swd2, and Swd3 are required for all degrees of H3K4 methylation. Sdc1 and Bre2 form a heterodimer and are required for H3K4 di- and trimethylation. Spp1 is only required for trimethylation of H3K4. Deletion of *SHG1* does not affect the levels of H3K4 methylation and its function within the complex is unclear (Dehe et al., 2006; Schneider et al., 2005). Set1-mediated methylation of H3K4 at promoters is also dependent on association with elongating RNA polymerase II via the Paf1 complex (Shilatifard, 2006).

How H2B ubiquitination is required for H3K4 methylation is not yet fully understood. However, recent evidence suggests that H2BK123 ubiquitination is a prerequisite for association of the essential Swd2 subunit with the rest of the COMPASS complex. Loss of H2B ubiquitination does not affect recruitment of the rest of COMPASS to active promoters, but is required for Swd2 association and therefore Set1 catalytic activity (Lee et al., 2007). Swd2 itself is ubiquitinated by Rad6 and Bre1. Loss of Swd2 ubiquitination results in loss of H3K4 trimethylation (Vitaliano-Prunier et al., 2008).

Additionally, it has been suggested that the proteosomal ATPases Rpt4 and Rpt6 are required for H3K4 di- and trimethylation. Rpt4 and Rpt6 recruitment to active promoters is dependent upon H2B ubiquitination. However, hypomorphic mutations of these ATPases have no effect on H2B ubiquitination (Ezhkova and Tansey, 2004). The mechanism by which these ATPases are required for H3K4 methylation is still not clear.

Recently, additional factors that regulate H3K4 methylation but not H2B ubiquitination have been uncovered. Deletion of CCR4/NOT mRNA processing complex members results in increased H3K4 trimethylation, but does not affect H2B ubiquitination (Laribee et al., 2007). However, another group found that the CCR4/NOT complex affects H3K4 trimethylation through recruitment of the Paf1 complex and therefore H2B ubiquitination (Mulder et al., 2007). Deletion of members of the Ctk cyclin-dependent protein complex that phosphorylates RNA polymerase II at serine 2 during transcriptional elongation results in a decrease in H3K4 monomethylation and an increase in H3K4 trimethylation (Wood et al., 2007). Together, the role of these complexes may help in regulating the transition from H3K4 trimethylation at promoters to H3K4 dimethylation within the coding regions of active genes.

Set1 and the Ipl1 Aurora kinase are functionally connected

Another modification to the histone H3 C-terminal tail is phosphorylation of serine 10 by Ipl1, which peaks during mitosis and therefore serves as a mitotic marker (Wei et al., 1999). Ipl1 is the only member of the Aurora kinases in budding yeast. In mammals, there are three Aurora kinases, Aurora A, Aurora B, and Aurora C. While Aurora A has

a primary role in centrosome function and Aurora C functions within germ cells, Aurora B most closely resembles Ipl1 (Vader and Lens, 2008). Aurora B, like Ipl1 in yeast, phosphorylates H3S10 in humans and nematodes during mitosis (Hsu et al., 2000).

In higher eukaryotes, H3S10 phosphorylation undergoes cross-talk *in cis* inhibiting methylation of the adjacent lysine 9 on the histone tail. *S. cerevisiae* lack methylation of H3K9 but histone H3 is methylated at K4 by Set1. Deletion of *SET1* suppresses the temperature-sensitive phenotype of the well-characterized *ipl1-2* allele containing a hypomorphic kinase domain (Zhang et al., 2005). Surprisingly, this genetic interaction is not through posttranslational modification of histone H3 but is mediated through modification of another shared substrate, the Dam1 kinetochore protein (Zhang et al., 2005).

The Dam1 complex

The Dam1 complex is a 10-member stoichiometric complex in which every member is present at a 1:1 ratio (Table 2) (Cheeseman et al., 2001b; Janke et al., 2002). In addition, every member of the Dam1 complex is essential for viability. The Dam1 complex resides in the outer kinetochore and is anchored to the rest of the kinetochore by the Ndc80 complex, specifically through physical interaction with the Ndc80 and Nuf2 proteins (Shang et al., 2003). The Dam1 complex binds to kinetochore microtubules initially at the lateral side then transitions to form plus-end attachments to the microtubules (Shimogawa et al., 2006; Tanaka et al., 2007). This binding tethers the rest of the kinetochore and the centromere to the microtubules. To do this, approximately

Table 2: Dam1 complex members

Dam1 complex
Ask1
Dam1
Duo1
Dad1
Dad2
Dad3
Dad4
Spc19
Spc34
Hsk3

sixteen Dam1 complexes oligomerize to form a ring around a microtubule (Miranda et al., 2005; Westermann et al., 2005). Formation of this ring structure around microtubules is important for chromosome segregation during mitosis as the ring harnesses the power of depolymerizing microtubules to pull the chromosomes pole-ward (Asbury et al., 2006; Westermann et al., 2006). Before chromosome segregation begins, proper bipolar kinetochore-microtubule attachments must occur in order to ensure accurate segregation of chromosomes. This checkpoint is mediated by Ipl1 (Biggins et al., 1999; Tanaka et al., 2002). While several members of the Dam1 complex are phosphorylated by Ipl1, phosphorylation of the Dam1 protein itself on the C-terminus seems to be the most important modification for mitotic progression (Cheeseman et al., 2002). When a proper bipolar spindle forms, there is tension between the sister chromosomes. However, if there is an improper setup, Ipl1 senses the lack of tension between sister chromosomes and phosphorylates members of the Nuf2 and Dam1 complexes including Dam1 itself (Akiyoshi et al., 2010; Cheeseman et al., 2002; Pinsky et al., 2006). This phosphorylation causes disruption of the protein-protein interactions between the two complexes, separating the microtubules from the kinetochore (Cheeseman et al., 2002). The Glc7 PP1 phosphatase then removes the phosphate groups, allowing the protein-protein interactions to be reformed. If a proper mitotic spindle is formed, then mitosis proceeds (Cheeseman et al., 2002). If improper attachment occurs again, another round of phosphorylation and dephosphorylation occurs until a proper bipolar spindle is set up (Cheeseman et al., 2002).

Dam1 is methylated by Set1

Our lab discovered that Set1 and Ipl1 share a second substrate in Dam1, and that Dam1 methylation inhibits its phosphorylation (Zhang et al., 2005). Mutagenesis of conserved lysines in Dam1, along with mass spectroscopy analysis, identified K233 in Dam1 as the primary site of Set1 methylation (Zhang et al., 2005). To confirm that Dam1K233 is methylated by Set1 *in vivo*, we raised an antibody specific to Dam1K233me2. Our lab showed that Dam1K233 is dimethylated and this methylation was abolished in *set1Δ* cells indicating that Set1 does indeed methylate Dam1 on K233 (Zhang et al., 2005).

Hypothesis and original goals

Our previous results raise the question: what regulates Set1-mediated H3K4 methylation versus Dam1K233 methylation? My hypothesis was that transcriptional regulatory factors essential for H3K4 methylation at gene promoters might also regulate Set1-mediated methylation of Dam1K233. In this dissertation, I investigate the regulation of Dam1 methylation using the regulation of H3K4 methylation as a model. I demonstrate that Set1 requires a functional COMPASS complex regardless of substrate, suggesting that the catalytic activity of Set1 requires association with its complex. I determine that the E2 and E3 ubiquitin ligases Rad6 and Bre1 as well as the Paf1 transcription elongation complex are essential for Dam1 methylation. I demonstrate that methylation of Dam1 is not dependent on the Kin28 RNA polymerase II kinase indicating that methylation of Dam1 is independent of transcription. In addition, I discover that the ubiquitin specific protease Ubp8 regulates the level of Dam1 methylation. I also make

the surprising discovery that Dam1K233 methylation is dependent on lysine 123 within H2B, indicating that ubiquitination of H2BK123 is a prerequisite for Dam1 methylation. I demonstrate a close physical association between Dam1 and H2B that is dependent on H2BK123 illustrating for the first time cross-talk between modifications on histones and a non-histone protein. In addition, I show that regulation of Set1-mediated methylation is substrate independent and not specific to H3K4 methylation at promoters. Finally, my results demonstrate previously unknown functions in mitosis for transcription regulatory proteins.

Chapter 2

Materials and Methods

Yeast Strain Construction

All *S. cerevisiae* strains used in this study are listed in Table 3. All yeast growth conditions and microbial techniques were performed as previously described (Amberg et al., 2005). All yeast were grown in rich media YPD or the appropriate selective media.

Unless otherwise noted, all gene replacement was performed with the *KanMX* cassette by transforming yeast with a PCR product containing *KanMX* along with an arm of homology to the gene of interest as previously described (Güldener et al., 1996). The primers for amplifying the *KanMX* cassette for each gene deletion are listed in Table 4. The PCR was performed at 94°C for 5 minutes, then 35 cycles of 94°C for 1 minute, 54°C for 30 seconds, and 72°C for 2 minutes followed by 72°C for 7 minutes. All *KanMX* replacements were genotyped by PCR with either a primer specific to 5' or 3' of the coding region and a primer specific to the *KanMX* insert using primers listed in Table 4. Prior to genotyping by PCR, yeast colonies were spheroplasted by incubating for 10-15 minutes at 37°C in 10μL of Zymolyase solution (2.5mg/mL Zymolyase, 1.2M sorbitol, 0.1M NaPO$_4$ pH 7.4). PCR was then performed on the spheroplasted cells for 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes followed by 72°C for 7 minutes. All gene deletion strains affecting H3K4me2 levels were further confirmed by immunoblotting for H3K4me2 from whole cell extracts

Table 3: Yeast strain used in this study

Name	Genotype	Source
CCY 1076-28B	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP*	(Zhang et al., 2005)
CCY 1076-28B *set1Δ*	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set1Δ::KanMX*	(Zhang et al., 2005)
CCY 1076-28B *paf1Δ*	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, paf1Δ::KanMX*	(Zhang et al., 2005)
yJL46	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, swd1Δ::KanMX*	This study
yJL47	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, sdc1Δ::KanMX*	This study
yJL48	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, spp1Δ::KanMX*	This study
yJL1	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, bre1Δ::KanMX*	This study
yJL2	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ctr9Δ::KanMX*	This study
yJL3	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, rtf1Δ::KanMX*	This study
yJL4	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set2Δ::KanMX*	This study
yJL5	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set3Δ::KanMX*	This study
yJL6	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set4Δ::KanMX*	This study
yJL7	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set5Δ::KanMX*	This study
yJL8	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set6Δ::KanMX*	This study
yJL9	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, set7Δ::KanMX*	This study
yJL10	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, dot1Δ::KanMX*	This study
yJL11	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ubr1Δ::KanMX*	This study
yJL12	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, rad18Δ::KanMX*	This study
CC 1077-6C	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP ipl1-2*	(Zhang et al., 2005)

CC 1077-6C *set1Δ*	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set1Δ::KanMX*	(Zhang et al., 2005)
CC 1077-6C *paf1Δ*	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, paf1Δ::KanMX*	(Zhang et al., 2005)
yJL49	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, swd1Δ::KanMX*	This study
yJL50	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, sdc1Δ::KanMX*	This study
yJL51	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, spp1Δ::KanMX*	This study
yJL13	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, bre1Δ::KanMX*	This study
yJL14	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, ctr9Δ::KanMX*	This study
yJL15	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, rtf1Δ::KanMX*	This study
yJL16	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set2Δ::KanMX*	This study
yJL17	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set3Δ::KanMX*	This study
yJL18	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set4Δ::KanMX*	This study
yJL19	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set5Δ::KanMX*	This study
yJL20	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set6Δ::KanMX*	This study
yJL21	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, set7Δ::KanMX*	This study
yJL22	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, dot1Δ::KanMX*	This study
yJL23	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, ubr1Δ::KanMX*	This study
yJL24	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, rad18Δ::KanMX*	This study

ZK1	MATa, ade2, his3-Δ200, ura3-52, leu2-Δ101, lys2-Δ101::HIS3::lys2-Δ102	(Zhang et al., 2005)
yJL25	MATa, ade2, his3-Δ200, ura3-52, leu2-Δ101, lys2-Δ101::HIS3::lys2-Δ102, rad6Δ::hisG-URA3-hisG	This study
ZK2	MATa, ade2, his3-Δ200, ura3-52, leu2-Δ101, lys2-Δ101::HIS3::lys2-Δ102 ipl1-2	(Zhang et al., 2005)
yJL26	MATa, ade2, his3-Δ200, ura3-52, leu2-Δ101, lys2-Δ101::HIS3::lys2-Δ102 ipl1-2, rad6Δ::hisG-URA3-hisG	This study
ZK3	MATα, lys2-801, his3-d200, ura3-52	(Zhang et al., 2005)
yJL27	MATα, lys2-801, his3-d200, ura3-52, htb1Δ::HphMX3, htb2Δ::KanMX, <pJL2 [URA3, htb1K123R]>	This study
ZK4	MATα, lys2-801, his3-d200, ura3-52, ipl1-2	(Zhang et al., 2005)
yJL28	MATα, lys2-801, his3-d200, ura3-52, ipl1-2, htb1Δ::HphMX3, htb2Δ::KanMX, <pJL2 [URA3, htb1K123R]>	This study
KT1112	MATa, leu2, ura3-52, his3	(Stuart et al., 1994)
ZK5	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3	(Zhang et al., 2005)
ZK5 set1Δ	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, set1Δ::KanMX	(Zhang et al., 2005)
ZK5 dam1K233R	MATa, leu2, ura3-52, his3, dam1::dam1K233R-HA3	(Zhang et al., 2005)
yJL29	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, swd1Δ::KanMX	This study
yJL30	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, sdc1Δ::KanMX	This study
yJL31	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, spp1Δ::KanMX	This study
yJL32	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, bre2Δ::KanMX	This study
yJL33	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, rad6Δ::KanMX	This study
yJL34	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, bre1Δ::KanMX	This study
yJL35	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, ubp8Δ::KanMX	This study
yJL36	MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, ubp10Δ::KanMX	This study

yJL37	*MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, ctr9Δ::KanMX*	This study
yJL38	*MATa, leu2, ura3-52, his3, dam1::DAM1-HA3, rtf1Δ::KanMX*	This study
Y131	*MATa, ade2-1, his3-11,-15, ura3-1, leu2-3,-112, trp1,hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, HTA1-HTB1]>*	(Robzyk et al., 2000)
Y133	*MATa, ade2-1, his3-11,-15, ura3-1, leu2-3,-112, trp1,hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, HTA1-htb1K123R]>*	(Robzyk et al., 2000)
yJL39	*MATα, ade2-1, leu2, ura3, his3, leu2-3,-112, hta1-htb1Δ::LEU2, hta2-htb2Δ, dam1::DAM1-HA3, <pRS413 [HIS3, HTA1-htb1K123R]>*	This study
yAV394	*MATa, trp1, leu2, ura3, his4, cup1::LEU2PM, SKI7-HA3::KanMX4*	van Hoof lab
ZK3 h2bK123R	*MATα, ade2-1, his3, ura3, leu2-3,-112, trp1, lys2-801, hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, HTA1-htb1K123R]>*	(Zhang et al., 2005)
ZK4 h2bK123R	*MATα, ade2-1, his3, ura3, leu2-3,-112, trp1, lys2-801, ipl1-2, hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, HTA1-htb1K123R]>*	(Zhang et al., 2005)
yJL40	*MATα, ade2-1, his3, ura3, leu2-3,-112, trp1, lys2-801, hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, HTA1-htb1K123R]>*	This study
yJL41	*MATα, ade2-1, his3, ura3, leu2-3,-112, trp1, lys2-801, ipl1-2, hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, HTA1-htb1K123R]>*	This study
yJL42	*MATα, ade2-1, his3, ura3, leu2, trp1, dam1::DAM1-HA3, hta1-htb1Δ::LEU2, hta2-htb2Δ, <pRS426 [URA3, hta1-4K/R-htb1K123R]>*	This study
yJL43	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, chd1Δ::KanMX*	This study
yJL44	*MATα, lys2, his3, ura3, leu2, trp-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP, ipl1-2, chd1Δ::KanMX*	This study
Sc657	*MATa, can1-100, ade2-1, his3-11, -15, leu2-3, -112, trp1-1, ura3-1, sug1-3*	(Ezhkova and Tansey, 2004)
Sc660	*MATa, can1-100, ade2-1, his3-11, -15, leu2-3, -112, trp1-1, ura3-1, sug1-25*	(Ezhkova and Tansey, 2004)
Sc671	*MATa, can1-100, ade2-1, his3-11, -15, leu2-3, -112, trp1-1, ura3-1, sug2-1*	(Ezhkova and Tansey, 2004)
yJL45	*MATα, can1-100, ade2-1, lys2, his3, ura3, leu2, trp1-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP ipl1-2, sug1-3*	This study

yJL46	*MATα, can1-100, ade2-1, lys2, his3, ura3, leu2, trp1-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP ipl1-2, sug1-25*	This study
yJL47	*MATα, can1-100, ade2-1, lys2, his3, ura3, leu2, trp1-1, ura3::URA3-tetO112, leu2::LEU-tetR-GFP ipl1-2, sug2-1*	This study
BY4741	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0*	(Brachmann et al., 1998)
yJL48	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, <pRS426 [URA3, Ubiquitin-3XHA]>*	This study
TAP-Spc24	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, SPC24-TAP::HIS3*	Open Biosystems
TAP-Nuf2	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, NUF2-TAP::HIS3*	Open Biosystems
TAP-Duo1	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, DUO1-TAP::HIS3*	Open Biosystems
yJL49	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, SPC24-TAP::HIS3, <pRS426 [URA3, Ubiquitin-3XHA]>*	This study
yJL50	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, NUF2-TAP::HIS3, <pRS426 [URA3, Ubiquitin-3XHA]>*	This study
yJL51	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, DUO1-TAP::HIS3, <pRS426 [URA3, Ubiquitin-3XHA]>*	This study
yJL52	*MATα, leu2, ura3-52,his3, dam1::DAM1-HA3, ubp8Δ::KanMX , <pRS426-pCUP1 [URA3, His7-ubiquitin-G76A]>*	This study
yJL53	*MATα, leu2, ura3-52,his3, dam1::DAM1-HA3, bre1Δ::KanMX, <pRS426-pCUP1 [URA3, His7-ubiquitin-G76A]>*	This study
yJL54	*MATα, leu2, ura3-52,his3, dam1::DAM1-HA3, <pRS426-pCUP1 [URA3, His7-ubiquitin-G76A]>*	This study
YTX031	*MATa, hta1-htb1Δ::LEU2, hta2-htb2Δ, kin28Δ::KanMX, leu2-3,-112, his3-11,-15, trp1-1, ura3-1, ade2-1, can1-100, <pZS145 HTA1-Flag-HTB1 CEN HIS3>, <pGK13-KIN28-HA>*	(Xiao et al., 2005)
YTX032	*MATa, hta1-htb1Δ::LEU2, hta2-htb2Δ, kin28Δ::KanMX, leu2-3,-112, his3-11,-15, trp1-1, ura3-1, ade2-1, can1-100, <pZS145 HTA1-Flag-HTB1 CEN HIS3>, <pGK33-kin28-ts16-HA>*	(Xiao et al., 2005)
yJL39	*MATa, hta1-htb1Δ::LEU2, hta2-htb2Δ, kin28Δ::KanMX, leu2-3,-112, his3-11,-15, trp1-1, ura3-1, ade2-1, can1-100, <pZS145 HTA1-Flag-HTB1 CEN HIS3>, <pGK13-KIN28-HA>, <pRS416-DAM1-HA3>*	This study

yJL40	*MATa, hta1-htb1Δ::LEU2, hta2-htb2Δ, kin28Δ::KanMX, leu2-3,-112, his3-11,-15, trp1-1, ura3-1, ade2-1, can1-100, <pZS145 HTA1-Flag-HTB1 CEN HIS3>, <pGK33-kin28-ts16-HA>, <pRS416-DAM1-HA3>*	This study
SBY3	*MATa, ura3-1, leu2-3,112, his3-11, trp1-1, can1-100, ade2-1, bar1*	(Biggins et al., 1999)
SBY164	*MATa, ura3-1, leu2-3,112, his3-11, trp1-1, can1-100, ade2-1, bar1+, LYS2+, ndc10-1*	(Biggins et al., 1999)
SBY1117	*MATa, ura3-, leu2-, his3-, trp1-, bar1+, ndc80-1*	(Furuyama and Henikoff, 2009)
yJL41	*MATa, ura3-1, leu2-3,112, his3-11, trp1-1, can1-100, ade2-1, bar1, <pRS414-DAM1-HA3>*	This study
yJL42	*MATa, ura3-1, leu2-3,112, his3-11, trp1-1, can1-100, ade2-1, bar1+, LYS2+, ndc10-1, <pRS414-DAM1-HA3>*	This study
yJL43	*MATa, ura3-1, leu2-3,112, his3-11, trp1-1, can1-100, ade2-1, bar1, <pRS414-DAM1-HA3>*	This study
yJL44	*MATa, leu2, ura3-52, his3, CSE4-myc12:URA3*	This study
yJL45	*MATα, leu2, ura3-52, his3, dam1::DAM1-HA3, CSE4-myc12:URA3*	This study

Table 4: List of primers used in this study

Name	Sequence	Gene and purpose
JL1	GACCGAGTCCTTTTAGTTTGTGTATATCAGCTGGTTCTTTTCGTTCAGCTGAAGCTTCGTACGC	*SWD1* forward KanR replacement
JL2	ATGACTGCATCTTAATTATCCTCGTATAATCTGTAGGGATTGTGAGCATAGGCCACTAGTGGATCTG	*SWD1* reverse KanR replacement
JL3	TGTATGTACACATTTCTGCTGATG	*SWD1* KO genotyping 5' forward
JL4	TAATGGGTCGAACATGTGCT	*SWD1* KO genotyping middle of gene reverse
JL5	CGTGCTCCTATCAAAGTGAGAT	*SWD1* KO genotyping 3' reverse
JL6	CCTTCATAAGCAGGTTCTCTTAAGAGAATTAGGGAAACCTATACACAGCTGAAGCTTCGTACGC	*SDC1* forward KanR replacement
JL7	GTAAGGAAAAATAAATACACATGTATATTTATATAATTATGGTTAGCATAGGCCACTAGTGGATCTG	*SDC1* reverse KanR replacement
JL8	ATGTTCTGTGTTGTGTGCTTCG	*SDC1* KO genotyping 5' forward
JL9	TCAGAGTTATTATTGTGATTGCCA	*SDC1* KO genotyping middle of gene reverse
JL10	CAACATGGAACAAGAGAAGTTCTG	*SDC1* KO genotyping 3' reverse
JL11	GAAAAGGCTACTTCGACCTCAATAATTTCTCAGCCTATCTTTCTACAGCTGAAGCTTCGTACGC	*SPP1* forward KanR replacement
JL12	TATATATATATGTAGAAACTGATATTTGATTAGGCTCCAACGCCGGCATAGGCCACTAGTGGATCTG	*SPP1* reverse KanR replacement
JL13	TAACCCAAGGCGGGTAAAAT	*SPP1* KO genotyping 5' forward
JL14	CATAAGGTTTTTGGCAAGCTCC	*SPP1* KO genotyping middle of gene reverse

JL15	CTGAGGAGTGGTGCTTTTTCAT	*SPP1* KO genotyping 3' reverse
JL16	AGGGCTTTCACCGTTTTTATGCTAATCGTGCTAGCTGATAATAATCAGATCAGCTGAAGCTTCGTACGC	*BRE1* forward KanR replacement
JL17	TATGTGGAGGATATAACACAAACAGTGGAAAAGTGGTAGAATAATTAGTAGCATAGGCCACTAGTGGATCTG	*BRE1* reverse KanR replacement
JL18	GATCGTACTTTGATCAAACCATCG	*BRE1* KO genotyping 5' forward
JL19	CAAGGCTTCAAAGTCGACTCTT	*BRE1* KO genotyping middle of gene reverse
JL20	TCTTTGGAAACATGCGACAG	*BRE1* KO genotyping 3' reverse
JL21	ATCCGCAAGTGAGCATCACAGCTACTAAGAAAAGGCCATTTTTACTACTCCAGCTGAAGCTTCGTACGC	*RAD18* forward KanR replacement
JL22	TTAACAAATGTGCACAAGCTAACAAACAGGCCTGATTACATATACACACCGCATAGGCCACTAGTGGATCTG	*RAD18* reverse KanR replacement
JL23	ACTAAGTACCACTTGAGCAATGCC	*RAD18* KO genotyping 5' forward
JL24	GCTTCGGTATCCTTAGTGCATCTA	*RAD18* KO genotyping middle of gene reverse
JL25	AATACTCCTGTTTCCCGAATGC	*RAD18* KO genotyping 3' reverse
JL26	GGTCACCAGTAATTGTGCGCTTTGGTTACATTTTGTTGTACAGTACAGCTGAAGCTTCGTACGC	*DOT1* forward KanR replacement
JL27	CTACTTAGTTATTCATACTCATCGTTAAAAGCCGTTCAAAGTGCCGCATAGGCCACTAGTGGATCTG	*DOT1* reverse KanR replacement
JL28	TGTCAGCTGCTTCGCTACAT	*DOT1* KO genotyping 5' forward
JL29	CTTTTGCAGCTTTTGGCTTC	*DOT1* KO genotyping middle of gene reverse

JL30	TTCTCTGCCTCCTCCTTCAA	*DOT1* KO genotyping 3' reverse
JL31	TCCCTAATCTTTACAGGTCACACAAATTAC ATAGAACATTCCAAT CAGCTGAAGCTTCGTACGC	*UBR1* forward KanR replacement
JL32	TATATACAAATATGTCAACTATAAAACATA GTAGAGGGCTTGAATGCATAGGCCACTAGT GGATCTG	*UBR1* reverse KanR replacement
JL33	CCTTTTTCTTCGCATCTTCG	*UBR1* KO genotyping 5' forward
JL34	GCAACCACACTCATGACACC	*UBR1* KO genotyping middle of gene reverse
JL35	GCTCATCGGTGGGAAACTAA	*UBR1* KO genotyping 3' reverse
JL41	CTTCTGTGCGGACAAACTCA	*BRE2* KO genotyping middle of gene reverse
JL42	AAGTTTGTAATTGTATTGCACTAATTTGTTG AGAGCACTATAGAACAGCTGAAGCTTCGTA CGC	*RTF1* forward KanR replacement
JL43	ATAAATATAAATATATTTTTACAAACACTG AAATTGTCCTGCCTAGCATAGGCCACTAGT GGATCTG	*RTF1* reverse KanR replacement
JL44	CGCTGCCTTGAATCATGTAA	*RTF1* KO genotyping 5' forward
JL45	AGGTGCTCTCTGTCGCTCTC	*RTF1* KO genotyping middle of gene reverse
JL46	ACTATGCATGGCCTTGTTCC	*RTF1* KO genotyping 3' reverse
JL57	CCAAAGATTATTTTTAGGCAGACAGAGACT AAAAGATAAAGCGTCCAGCTGAAGCTTCGT ACGC	*RAD6* forward KanR replacement
JL58	ATAATATCGGCTCGGCATTCATCATTAAGA TTCTTTTGATTTTTCGCATAGGCCACTAGTG GATCTG	*RAD6* reverse KanR replacement
JL59	GGGGTAGCCGGAGTAGAAAG	*RAD6* KO genotyping 5' forward

JL60	TTGACATGCGGTGGCTTAT	*RAD6* KO genotyping middle of gene reverse
JL61	TGGAGGAATAGAAAGCAAACG	*RAD6* KO genotyping 3' reverse
JL67	TTCTAATTGTCTGGTCCATTTGTGTTGAGAGCAAGAAAAAAAAACCAGCTGAAGCTTCGTACGC	*CTR9* forward KanR replacement
JL68	TCTTTAAAAGTCTTGATTCTAACCCTCGCCTCTTCTTAGAACAATGCATAGGCCACTAGTGGATCTG	*CTR9* reverse KanR replacement
JL69	GGCAATCACCAAATTTTCCA	*CTR9* KO genotyping 5' forward
JL70	CAGACCTGTGGTCTTCAGCA	*CTR9* KO genotyping middle of gene reverse
JL71	AGGACACGAAAAGGTGGATG	*CTR9* KO genotyping 3' reverse
JL72	CCTTGAGCTAAGGCCAACTG	*IPL1* forward
JL73	TTCACGCAAGTCTGTTCGAC	*IPL1* reverse
JL74	ATAGATGCATGGGCTCTTGG	*ipl1-2* sequencing forward
JL75	CGACAAAGGCGAAAGAAAAG	*ipl1-2* sequencing reverse
JL76	GAGACGAGCTTTGGAAATGG	*SUG1* forward
JL77	AACAGCTTGGCGACAGAAAT	*SUG1* reverse
JL78	CTGGAAGCTCAAAGGAATGC	*sug1-3* and *sug1-25* sequencing forward
JL79	CGACACTTGGAGGTGGAAAT	*sug1-3* and *sug1-25* sequencing reverse
JL80	ATCAACGGTGGGAACTTTTG	*SUG2* forward
JL81	CAGCCTCGAAGTCAAATTCC	*SUG2* reverse
JL82	CACCCAACAGTCACACGAAC	*sug2-1* (A56V) sequencing forward
JL83	CCGAATTCCTAACTCCGACA	*sug2-1* (A56V) sequencing reverse
JL85	TCTAGTCTACCGGGCCTCAA	*sug2-1* (E300K) sequencing forward
JL93	TAGTATCTGTAAATCCGTCCTATTGTCATATCACAATCACAGACTCAGCTGAAGCTTCGTACGC	*UBP10* forward KanR replacement

JL94	GCTAATAAAAATGCCTATGAAAAGAGGAA AATCCAGGAATATCGAGTTTTTGCATAGGC CACTAGTGGATCTG	*UBP10* reverse KanR replacement
JL95	CTAAAAACGGCAAACCCGTA	*UBP10* KO genotyping 5' forward
JL96	AAGGAGGCTCCGTAGAAAGC	*UBP10* KO genotyping middle of gene reverse
JL97	CAACCCGTGCTTTAGGAGAG	*UBP10* KO genotyping 3' reverse
JL98	CTACTTGAAACCCTGCTTTTTTATTTGTTA TTAATAATTCAGCTGAAGCTTCGTACGC	*UBP8* forward KanR replacement
JL99	GCTTTTTCTTCTTTTTTGTTTTATTATTATTG TTGAATGCTATTTGCTGAAGCATAGGCCAC TAGTGGATCTG	*UBP8* reverse KanR replacement
JL100	GCAAGCCCATGTAACATCAA	*UBP8* KO genotyping 5' forward
JL101	AGGAACCATGGTCTTTGTGC	*UBP8* KO genotyping middle of gene reverse
JL102	TCCAGCCTTTGCTCTTTTGT	*UBP8* KO genotyping 3' reverse
JL118	TCCTGACTATGCGGGCTATC	HA tag forward on pRG145
JL119	GGAATGCCTTCCTTGTCTTG	*UBI4* reverse on pRG145
JL120	GAACGAAGGAAGGAGCACAG	*URA3* forward on pRG145
JL121	AAGGCAGCGTTTTGTTCTTG	*URA3* reverse on pRG145
JL153	ACCCAATTCAAAGCAGAACCTTTTCTAATT TAATTCTCACTTATACAGCTGAAGCTTCGTA CGC	*CHD1* forward KanR replacement
JL154	TATGGGGGGAAGGAACAATGGAAAATGTG GTGAAGAAAAATTGTTGCATAGGCCACTAG TGGATCTG	*CHD1* reverse KanR replacement
JL155	TCGAAACTGTACCACGAGAAAC	*CHD1* KO genotyping 5' forward
JL156	ATCTTCGGCGGTAACGTATG	*CHD1* KO genotyping middle of gene reverse

JL157	GATGGCATCACGACAGACAC	*CHD1* KO genotyping 3' reverse
JL163	TGGTTAGACGCTCAATGTCG	*HTB1* genotyping 5' forward
JL164	CAGTGTCAGGGTGAGTTTGC	*HTB1* KO genotyping 3' reverse
JL165	GCAAACTCACCCAGACACTG	*HTB2* genotyping 5' forward
JL166	TTTTGTTTAGCGGACGTGTG	*HTB2* KO genotyping 3' reverse
JL167	GGACCAAATAGGCAATGGTG	*LEU2* genotyping 5' forward
JL168	AAGGAAGACGATGGTGATGG	*LEU2* KO genotyping 3' reverse
JL169	AGAAAGCATGCGAGATCGAG	*HTB2* KO genotyping 5' forward
JL170	ATTCAGTACAATAGAACAGTGCTCATAATAGTATAAAGGGTCACACAGCTGAAGCTTCGTACGC	*PAF1* forward KanR replacement
JL171	AAGAACTACAGGTTTAAAATCAATCTCCCTTCACTTCTCAATATTGCATAGGCCACTAGTGGATCTG	*PAF1* reverse KanR replacement
JL172	CGAAAAGCACCGATCCTTAG	*PAF1* KO genotyping 5' forward
JL173	CATCCGAATCTCCATCATCC	*PAF1* KO genotyping middle of gene reverse
JL174	CATATTGGCAATTGGGAAGG	*PAF1* KO genotyping 3' reverse
JL175	CTGATTGCTCTATACTCAAACCAACAACAACTTACTCTACAACTACAGCTGAAGCTTCGTACGC	*HTB2* forward KanR replacement
JL176	TAATAAAAGAAAACATGACTAAATCACAATACCTAGTGAGTGACGCATAGGCCACTAGTGGATCTG	*HTB2* reverse KanR replacement
JL177	CACCGCTTCGCCTAATAAAG	*HTB2* KO genotyping 5' forward

JL178	ACGGCATGTTTAGCCAATTC	*HTB2* KO genotyping middle of gene reverse
JL179	GCTTTCAGTCCGAAAACAGC	*HTB2* KO genotyping 3' reverse
JL187	CCTATATAGACAAGTCAAACCACAAATAAACCATACACACATACACAGCTGAAGCTTCGTACGC	*HTB1* forward KanR replacement
JL188	CCATATAAATAATAATATTAATTATAACCAAAGGAAGTGATTTCAGCATAGGCCACTAGTGGATCTG	*HTB1* reverse KanR replacement
JL189	TGGTTAGACGCTCAATGTCG	*HTB1* KO genotyping 5' forward
JL191	GCGCATTCCCTCTATGAGAC	*HTB1* KO genotyping 3' reverse
JL198	CCTCACTAAAGGGAACAAAAGC	T3
JL199	TAATACGACTCACTATAGGGCGA	T7
JL202	TATCCACGCCCTCCTACATC	*hphR* genotyping 5' reverse
JL203	ACTCGCCGATAGTGGAAACC	*hphR* genotyping 3' forward
JL204	GCAAACTCACCCTGACACTG	*HTB1* KO genotyping middle of gene reverse
JRB93	GGGGACGAGGCAAGCTAAACA	KanR genotyping middle of gene reverse
JRB94	CGACATCATCTGCCCAGATGC	KanR genotyping middle of gene forward
KE100	GACAACAGAGACAAGAGG	Dam1 forward to genotype HA-Dam1 tag
KE137	GTCCGGGACGTCATAGGG	HA tag reverse to genotype HA-Dam1 tag
dam1-500F-AS	GTAACGTGGTATGGCCTTTC	Forward primer to clone HA-Dam1 500 bp upstream of start site

dam1-500R-AS	CTCCCGTCTCTTCCATAAAA	Reverse primer to clone HA-Dam1 500 bp downstream of stop site

as described below.

yJL25 *rad6Δ* and yJL26 *rad6Δ* were created using the *RAD6* deletion plasmid p46 (a gift from Mary Ann Osley) linearized by digestion with BamHI (New England Biolabs) and transformed into yeast strains ZK1 and ZK2. Transformants were selected for by growth on ura- SC plates and the genotype was confirmed by immunoblotting for loss of H3K4me2.

yJL40 and yJL41 were created by mating strain ZK4 to strain Y133, respectively. The resulting diploid cells were selected for by growth on ade-/lys- SC plates and sporulated. Haploid cells were isolated by tetrad dissection using a Singer MSM dissecting microscope (Singer Instruments). Haploids containing the pRS426-*HTA1-htb1K123R* 2μ plasmid as well as *hta1-htb1Δ::LEU2* were selected for by growth on ura-/leu- SC plates. The lack of a wild-type *HTB1* allele was confirmed by immunoblotting for loss of H3K4me2. The presence or absence of the *ipl1-2* allele was tested by failure of the haploid to grow at 37°C and confirmed by sequencing. Later sequencing revealed that the pRS426-*HTA1-htb1K123R* 2μ plasmid was in fact pRS426-*hta1-4K/R-htb1K123R*. Therefore yJL41 contains point mutations at lysines at K119, K120, K123, and K126 within *HTA1* and K123 within *HTB1*.

yJL42 and yJL82 was created by mating ZK5 to Y133 and Y131, respectively. The resulting diploid cells were selected for by growth on ade-/leu- SC plates and sporulated. Haploid cells were isolated by tetrad dissection using a Singer MSM dissecting

microscope (Singer Instruments). Haploids containing the pRS426-*HTA1-HTB1*or

pRS426-*HTA1-htb1K123R* 2µ plasmid as well as *hta1-htb1∆::LEU2* were selected by

growth on ura-/leu- SC plates. The lack of a wild-type *HTB1* allele was confirmed by

immunoblotting for loss of H3K4me2. The presence of the *HA-DAM1* allele was

determined by PCR for the HA tag using primers listed in Table 4 and confirmed by

immunoblotting for HA. Later sequencing revealed that the pRS426-*HTA1-htb1K123R*

2µ plasmid was in fact pRS426-*hta1-4K/R-htb1K123R*. Therefore yJL42 contains point

mutations at lysines at K119, K120, K123, and K126 within *HTA1* and K123 within

HTB1.

yJL27 *htb1K123R* and yJL28 *htb1K123R* were created by gene replacement of the *HTB1*

coding region with *KanMX*. Positive transformants were transformed with the 2µ

plasmid pJL2 containing *htb1K123R*. Finally, gene replacement of *HTB1* with *HphMX3*

was performed as previously described (Goldstein and McCusker, 1999). Positive

transformants were confirmed by immunoblotting for loss of H3K4me2 and by

sequencing.

 yJL39 *htb1K123R* was created by mating ZK5 with Y131 (from Mary Ann Osley). The

resulting diploids were selected for on ura-/trp- SC plates and sporulated. Haploid cells

were isolated by tetrad dissection using Singer MSM dissecting microscope (Singer

Instruments). Haploids containing the pRS426-*HTA1-HTB1* 2µ plasmid as well as *hta1-

htb1∆::LEU2* were selected by growth on ura-/leu- SC plates. Cells were tested for the

presence of the *HA-DAM1* allele by PCR with primers specific to the *DAM1* coding

region and specific to the HA epitope. Colonies were tested for deletion of the *HTA2-HTB2* locus by failure to grow on 5-FOA and sequencing was performed for further confirmation. Next, HA-Dam1 *hta1-htb1Δ::LEU2*, *hta2-htb2Δ*, pRS426-*HTA1-HTB1* cells underwent a two-step plasmid shuffle with pJL7 containing *HTA1-htb1K123R* first by selection for growth on his- SC plates, followed by growth on 5-FOA plates. Positive transformants were confirmed by sequencing and loss of H3K4me2 by immunoblotting.

yJL45, yJL46, and yJL47 were constructed by mating CC 1077-6C to Sc657, Sc660, and Sc671, respectively. The resulting diploid cells were selected for by growth on ade-/lys- SC plates and sporulated. Haploid cells were isolated by tetrad dissection using a Singer MSM dissecting microscope (Singer Instruments). The presence of the *ipl1-2* allele was tested by failure of the haploid to grow at 37°C and confirmed by sequencing. The presence of *sug1-3*, *sug1-25*, or *sug2-1* was determined by sequencing.

yJL49, yJL50, yJL51, and yJL52 were constructed by transforming TAP-Spc24, TAP-Nuf2, TAP-Duo1, and TAP-Ipl1, respectively, with pJL1. Positive transformants were selected for by growth on ura- SC plates and confirmed by immunoblotting for HA-ubiquitin.

Strains yJL39 and yJL40 were created by transforming strains YTX031 and YTX032, respectively, with pJL8 and selecting for growth on trp- SC plates. Positive transformants were confirmed by immunoblotting for HA-Dam1.

Strains yJL41, yJL42, and yJL43 were created by transforming strains SBY3, SBY164, and SBY1117, respectively, with pJL9 and selecting for growth on ura- SC plates. Positive transformants were confirmed by immunoblotting for HA-Dam1.

Plasmid Construction

All plasmids used in this study are listed in Table 5. All restriction enzymes were from New England Biolabs. To construct plasmid pJL1, 3XHA-Ubiquitin along with the *TDH3* promoter was excised from plasmid pRG145 (courtesy of Richard Gardner) with HindIII/KpnI. The resulting fragment was ligated into pRS426 with T4 ligase (Invitrogen).

To construct plasmid pJL2, the pRS426-*hta1-4K/R – htb1K123R* plasmid was isolated from strain Y133 (courtesy Mary Ann Osley) (Robzyk et al., 2000) using Zymoprep Yeast Plasmid Miniprep II (Zymo Research) following the manufacture's instructions. The entire *htb1K123R* coding region as well as 783 base pairs 5' of the start site was amplified by PCR from the pRS426-*hta1-4K/R – htb1K123R* plasmid using an upstream primer containing a BamHI restriction site and the downstream primer containing a XhoI restriction site. The resulting 1.2kb fragment was digested with the aforementioned restriction enzymes, and then ligated with T4 ligase (Invitrogen) into XhoI-BamHI digested pRS426.

Plasmid pJL5 was constructed by digesting pRS426-*hta1-4K/R – htb1K123R* with Xho1 and Not1. The excised fragment containing the entire *htb1K123R* was ligated into

Table 5: List of plasmids used in this study

Name	Genotype	Source
pJL1	pRS426-p*TDH3*-ubiquitin-3XHA, *URA3*	This study
pRG145	YIp–p*TDH3*-ubiquitin-3XHA, *URA3*	Gardner lab
pJL2	pRS426-*htb1K123R, URA3*	This study
pJL5	pRS426-*HTA1-htb1K123R, URA3*	This study
pJL7	pRS413-*HTA1-htb1K123R, HIS3*	This study
pJL8	pRS414-*DAM1-HA3, TRP1*	This study
pJL9	pRS416-*DAM1-HA3, URA3*	This study
pSB246	yiplac211-*CSE4-myc12, URA3*	(Buvelot et al., 2003)
pRS426-*hta1-4K/R – htb1K123R*	pRS426-*hta1-4K/R – htb1K123R, URA3*	(Robzyk et al., 2000)
p46	pRS406-*rad6*::hisG-*URA3*-hisG	Osley lab
p*CUP1*	pRS426-*pCUP1-His7-ubiquitin-G76A, URA3*	(Geng and Tansey, 2008)

pRS426-*HTA1-HTB1* isolated from Y131 (courtesy Mary Ann Osley) (Robzyk et al., 2000) that was also digested with Xho1 and Not1 resulting in pRS426-*HTA1-htb1K123R*.

Plasmid pJL7 was constructed by digesting pJL5 with XhoI and NotI and ligating the excised fragment into pRS413 at the XhoI and NotI sites.

Plasmid pJL8 was constructed by amplifying the entire HA-Dam1 coding region as well as 500 base pairs upstream of the start site contain the endogenous promoter and 500 base pairs downstream of the stop site from genomic DNA isolated from strain ZK5. The resulting fragment (containing HA-Dam1) was ligated into pCRII-TOPO (Invitrogen) following the manufacture's instructions. The resulting plasmid was digested with NsiI. The excised 2.4 kb fragment containing HA-Dam1 from pCRII-TOPO was ligated into pRS414 digested with PstI resulting in plasmid pJL8.

pJL9 was constructed by digesting pJL8 with SalI and XbaI and ligating the 2.4 kb fragment containing HA-Dam1 into the same sites in pRS416.

Sequencing

All sequencing was performed by the DNA Analysis core facility at MDACC. Sequencing alignments were done by weighted ClustalV using Lazergene 8 (DNASTART).

10-fold serial dilution growth assays

10-fold serial dilution growth assays with the *ipl1-2* temperature sensitive allele were performed as previously described (Zhang et al., 2005).

Immunoprecipitation and immunoblotting

Immunoprecipitation of Dam1 and immunoblotting with the Dam1K233me2 specific antibody was performed as previously described (Zhang et al., 2005) with the following changes. 500mL or 1L cultures of yeast were grown to an OD_{600} of 1.0. After preclearing with Protein A agarose beads (Millipore), whole cell extracts were incubated with HA affinity beads (Roche) overnight. For co-immunoprecipitation of HA-Dam1 with histones, lysates were incubated with 100μg/mL ethidium bromide on ice for thirty minutes prior to preclearing. Immunoblotting with the Dam1K233me2 antibody (Zhang et al., 2005) for Figures 13 and Figure 20 was done at a concentration of 1:2500 in 5% milk/TBST overnight. Immunoblotting with the Dam1K233me2 antibody generated for this work (see below) (Figures 6, 10, 15, and 23) was performed at a concentration of 1:5000 in 5% milk/TBST overnight. Immunoprecipitated HA-Dam1 and immunoprecipitation inputs were immunoblotted with yeast specific histone H2A, yeast specific H2B, and H3 (Active Motif) 1:2000 overnight.

For immunoblotting from total protein extracts from COMPASS deletion strains, yeast cultures of indicated strains were grown to 1.5 OD_{600} , harvested, and then pellets were frozen with liquid nitrogen. The pellets were then lysed with NaOH, and proteins were

precipitated with TCA as previously described (Ooi et al., 1996). Total protein extracts

were immunoblotted with H3K4me2 and H3 antibodies (Active Motif) 1:5000 overnight.

For all other immunoblotting from total protein extracts, 5-10mL overnight yeast cultures

were harvested, washed with ddH$_2$O, and resuspended in 200µL of 2X protein extraction

buffer (200mM Tris pH 8.0, 400mM (NH$_4$)$_2$SO$_4$, 10mM MgCl$_2$, 1mM EDTA pH 8.0,

10% glycerol, 1X PMSF, 1X pepstatin, 1X, leupeptin, 1X benzamadine). The

resuspended pellet was shaken on a Vortexer with glass beads for ten minutes at 4°C,

after which another 200µL of 2X protein extraction buffer was added. The extract was

removed from the beads and centrifuged at 14,000 RPM for five minutes at 4°C. The

supernatant was transferred to a new Eppendorf tube, 4X Laemmli sample buffer was

added, and the samples were incubated at 100°C for five minutes. Quantification of

immunoblots was performed using ImageJ (**http://rsbweb.nih.gov/ij/**).

Dam1K233me2 antibody purification

Streptavidin beads (Sigma) were washed three times with 50mM sodium phosphate pH

7.5. 10mg of either biotinylated N-CFVLNPTNIGMSKSSQ-C or biotinylated N-

CFVLNPTNIGMSK((CH$_3$)$_2$)SSQ-C Dam1 peptide (Keck Biotechnology Resource

Laboratory, Yale University) was dissolved in 1mL of 50mM sodium phosphate pH 7.5.

All of the following steps were carried out at 4°C. The peptides were incubated with the

streptavidin beads overnight. After incubation, the beads were washed twice for five

minutes with 50mM sodium phosphate pH 7.5, and followed by a single wash for five

minutes with 1M NaCl$_2$, 50mM sodium phosphate pH 7.5. The beads were next

incubated with ten bead-volumes of 100mM ethanolamine pH 7.5 overnight. The next

day, the beads were washed twice with PBS pH 7.4, and then transferred to a small Poly-

prep column (Bio-rad). The column was washed with ten bead-volumes of 10mM Tris

pH 7.5, ten bead-volumes of 100mM glycine pH 2.5, ten bead-volumes of 10mM Tris pH

8.8, followed by ten bead-volumes of 10mM Tris pH 7.5. Whole serum was diluted 1:10

in 10mM Tris pH 7.5 and ten bead-volumes were added to the column containing the

dimethylated Dam1 peptide bound to streptavidin beads. The serum and peptide-bound

beads were incubated overnight. The next day, the column was washed with twenty

bead-volumes of 10mM Tris pH 7.5 followed by twenty bead-volumes of 500mM $NaCl_2$,

10mM Tris pH 7.5. The antibodies were eluted with ten bead-volumes of 100mM

glycine pH 2.5 into a tube containing one bead-volume of 1M Tris pH 8.0. The column

was washed with 10mM Tris pH 8.8, and then with 10mM Tris pH7.5. The column was

then stored in 10mM Tris pH 7.5, 500mM $NaCl_2$, 0.02% NaN_3 at $4^{o}C$.

 The eluant from the methylated Dam1 column was then added to the column containing

the unmethylated Dam1 peptide bound to streptavidin beads and incubated for two hours.

The column was washed with twenty bead-volumes of 10mM Tris pH 7.5 followed by

with twenty bead-volumes of 500mM $NaCl_2$, 10mM Tris pH 7.5. The antibodies were

eluted with ten bead-volumes of 100mM glycine pH 2.5 into a tube containing one bead-

volume of 1M Tris pH 8.0. The columns were then washed with 10mM Tris pH 8.8, then

with 10mM Tris pH7.5. Incubation with the unmethylated Dam1 peptide was repeated

twice more. The columns were then stored in 10mM Tris pH 7.5, 500mM $NaCl_2$, 0.02%

NaN_3 at $4^{o}C$.

Dam1K233me2 antibody generation

An additional Dam1K233me2 specific antibody was generated for this study.

Biotinylated peptides C-PTNIGMSK((CH$_3$)$_2$)SSQGH-N and C-PTNIGMSKSSQGH-N

were constructed by Ynzym Antibodies. Ynzym then used the dimethylated Dam1

peptide to raise polyclonal antibodies in rabbits. The antisera was purified against the

dimethylated Dam1 peptide and negatively selected against the unmethylated peptide by

Ynzym.

Tandem affinity purifications (TAP)

Cell extracts were made from twelve liters of yeast cultures grown to an OD$_{600}$ of 1.0.

Cells were collected by centrifugation and washed once with sterile ddH$_2$O. The cells

were resuspended in ≈ 0.2 volumes of lysis buffer (50mM Tris-HCl pH 7.4, 100mM

NaCl, 5mM EDTA, 1mM DTT, 10mM iodoacetate, 10mM N-ethylmaleimide, 1X PMSF,

1X leupeptin, 1X pepstatin A, 1X benzamidine). The cells were flash frozen in liquid

nitrogen and then ground with dry ice in a Waring blender. The lysed cells were

transferred to centrifuge tubes and thawed at 4°C. When partially thawed, ≈ 1 volume of

lysis buffer was added to the lysates. When completely thawed, the lysates were

centrifuged at 15,000rpm for fifteen minutes then ultracentrifuged in a Ti-70 rotor for two

hours at 34,000rpm. Glycerol was added to the supernant to a 7.5% final concentration,

Triton X-100 to a 1% final concentration, and NaCl to final concentration of 400mM.

This solution was the binding buffer. The lysate was then incubated with 150μL agarose

beads for one hour, then IgG sepharose for four hours at 4°C. The IgG sepharose was

transferred to a micro Bio-rad column and washed 3X for ten minutes with the binding

buffer, then 1X with TEV buffer (50mM Tris-HCl pH 7.4, 0.1% Triton X-100, 150mM NaCl, 1mM DTT) for five minutes. The IgG sepharose was then incubated in 100U AcTEV protease in 300μL TEV buffer overnight at 4°C. The TEV eluate was collected and the IgG sepharose was washed with 300μL calmodulin binding buffer (10mM Tris-HCl, 0.1% Triton X-100, 2mM CaCl$_2$, 10mM β-ME). The 300μL wash of calmodulin binding buffer was added to the TEV elute. 100μL of calmodulin agarose suspension was added to a new column and the suspension was eluted. The calmodulin was then equilibrated with 500μL of calmodulin binding buffer and the calmodulin agarose was incubated with 500μL of the TEV eluate and 500μL of calmodulin binding buffer for two hours at 4°C. Next, the calmodulin beads were washed 2X for five minutes with ten column volumes of calmodulin binding buffer and then for five minutes with ten column volumes of calmodulin wash buffer (10mM Tris-HCl pH 7.4, 0.1% Triton X-100, 500mM NaCl, 0.1mM CaCl$_2$, 10mM β-ME, 1X PMSF). The calmodulin beads were incubated for five minutes with two column volumes of calmodulin elution buffer (10mM Tris-HCl pH 7.4, 0.1% Triton X-100, 150mM NaCl, 3mM EGTA, 10mM β-ME, 1X PMSF). The eluate was collected and designated the first fraction. Five subsequent fractions were collected of two column volumes each of calmodulin elution buffer. The samples were then boiled for five minutes in 4X Laemmli sample buffer and then run on 4%-12% Nupage gels (Invitrogen). Gels were then either immunoblotted for α-CBP (Openbiosystems) or α-HA (Roche) at a 1/5000 dilution overnight in 5% milk with 1X TBST or silver stained.

For silver staining, 4%-12% Nupage gels (Invitrogen) gels were incubated for fifteen minutes in 50% methanol on an orbital shaker, fifteen minutes in 5% methanol, and fifteen minutes in 32μM DTT. The gel was rinsed 2X for a few seconds each with ddH$_2$O and then a small amount of silver staining solution (0.1% AgNO$_3$). The gel was incubated shaking in silver staining solution for fifteen minutes. The gel was rinsed 2X for a few seconds in ddH$_2$O and then 2X in developing solution (3% Na$_2$CO$_3$, 0.05% CH$_2$O). The gel was incubated in the developing solution until bands were of the desired intensity. Most of the developing solution was decanted and citric acid (C$_6$H$_8$O$_7$) was added to stop the reaction. ddH$_2$O was added and washed 3X in ddH$_2$O for fifteen minutes each.

Prior to mass spectroscopy analysis, 4%-12% Nupage gels (Invitrogen) were stained with colloidal blue using the colloidal blue staining kit (Invitrogen) according to the manufacture's instructions. The bands were cut out of the gels, placed in siliconized tubes, and trypsin digested prior to mass spectroscopy. For the trypsin digestion, the bands were incubated in 400μL destain solution (50% methanol, 5% acetic acid). After destaining, the gel slice was dehydrated with 200μL acetonitrile, and then air-dried at room temperature. The gel slices were incubated with 10mM DTT for thirty minutes and then in 50mM iodoacetamide for thirty minutes. The gel was washed in 200μL 100mM ammonium bicarbonate for ten minutes, twice for five minutes in 200μL acetonitrile, and then air-dried again. 20μL trypsin (Promega) (10μg/mL in 50mM ammonium bicarbonate) was added to the slices at 4°C to rehydrate. After rehydration, excess trypsin was removed, 20μL 50mM ammonium bicarbonate was added, and incubated

overnight at 37°C. The next day, 10μL of 5% formic acid was added, the samples were

vortexed briefly, centrifuged at 150,000 RPM for two minutes, and the supernatant was

transferred to a new siliconized tube. Another 10μL of 5% formic acid was added to the

gel, the samples were vortexed briefly, centrifuged at 150,000 RPM for two minutes, and

the supernatant was transferred to the same tube containing the supernant from the

previous step bringing the total volume to 20μL. The trypsinized samples were sent to

Dr. Maria Persons at the University of Texas at Austin who performed the mass

spectroscopy.

In vitro ubiquitination assays

In vitro ubiquititination assays were performed using the ubiquitin conjugation reaction

buffer kit (Boston Biochem) according to the instructions provided by the manufacture.

For the ubiquitination ligation, the reactions were incubated at 30°C for one hour.

Mammalian ubiquitin and the yeast E1 enzyme Ube1 were obtained from Boston

Biochem. TAP-Rad6 and TAP-Bre1 purified using the TAP tag were the E2 and E3

enzymes in this assay. Ndc80 and Nuf2 complexes purified using the TAP tag from

strains yJL50 and yJL51 were used as substrates along with recombinant yeast histone

generated by Dr. Diane Edmondson. To analyze the results of the *in vitro* ubiquitination

assays, the samples were run on a 4-12% NuPages gels (Invitrogen) and immunoblotted

overnight with α-HA (Roche) at 1:5000, α-CBP (Sigma) 1:2500, or α-H2B antibody

1:1000 raised previously by the Dent lab.

Chapter 3

Results

Suppression of *ipl1-2* by deletion of *SET1* is independent of H3K4 methylation

In investigating possible functional connections between different histone H3 modifying

enzymes, our lab discovered a genetic interaction between the Set1 methyltransferase and

the Ipl1 Aurora kinase (Zhang et al., 2005). Set1 is the sole methyltransferase of lysine

(K) 4 on the histone H3 tail, and Ipl1 phosphorylates serine (S) 10 (Briggs et al., 2001;

Wei et al., 1999). Our lab determined that deletion of *SET1* suppresses the temperature

sensitive (ts) growth defects of *ipl1-2*, a well-characterized hypomorphic allele of *IPL1*

that contains a point mutation within its kinase domain (Chan and Botstein, 1993; Zhang

et al., 2005). A possible mechanism for this suppression is that deletion of *SET1*

abolishes H3K4 methylation, somehow affecting *ipl1-2* cell viability at higher

temperatures. To investigate this possibility, I compared the ability of a K4 mutation in

H3 to suppress the *ipl1-2* mutation with the suppression of *ipl1-2* by *SET1* deletion. In

collaboration with a former student, Ke Zhang, I performed plate spot assays comparing

the growth of wild-type, *h3K4R*, *ipl1-2*, and *ipl1-2 h3K4R* strains (Figure 3). In these

ipl1-2 suppression assays, cells were 10-fold serially diluted, spotted on rich media

(YPD) plates, and grown at permissive and restrictive temperatures for 2-3 days.

At 25°C, all the strains grew normally indicating that equal numbers of cells for each

strain were loaded onto the plates (Figure 3). At higher temperatures, deletion of *SET1*

strongly suppressed the temperature sensitive phenotype of *ipl1-2*, as we had previously

Figure 3. *h3K4R* does not suppress *ipl1-2* to the degree of *set1Δ*

Wild-type, *h3K4R*, *ipl1-2*, *ipl1-2 h3K4R*, and *ipl1-2 set1Δ* yeast strains were serially diluted 10-fold, spotted onto rich media (YPD) plates, and grown at 25°C or 32.5°C for 3 days.
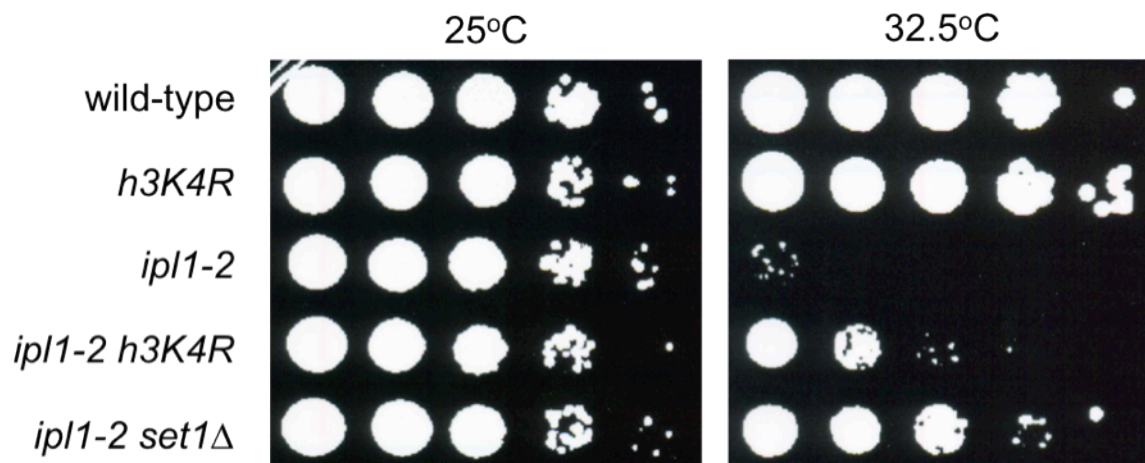
Figure 3

determined (Zhang et al., 2005). However, mutation of H3K4 to arginine only partially

suppressed the *ipl1-2* temperature sensitive phenotype and was approximately 10-100

fold weaker than deletion of *SET1* (Figure 3). These results, together with results

described elsewhere (Zhang et al., 2005), strongly indicate suppression of *ipl1-2* by *set1Δ*

is not mediated through loss of H3K4 methylation, implying that effects on another

shared substrate, such as Dam1, are important in regulating Ipl1 functions.

SET1 is the only known or putative lysine methyltransferase that suppresses the

ipl1-2 ts phenotype

The modulation of Ipl1 function by Set1 raises the question of whether Set1 is the only

methyltransferase in yeast that regulates Ipl1 function. There are seven SET domain-

containing proteins and one non-SET domain containing lysine methyltransferase in *S.*

cerevisiae (Briggs et al., 2001). Other than Set1, only two other lysine

methyltransferases have been identified. Set2 methylates histone H3K36 and Dot1

methylates the H3 C-terminal core on lysine 79 (Feng et al., 2002; Ng et al., 2002a;

Strahl et al., 2002; van Leeuwen et al., 2002). Substrates for Set3 through Set7 are yet to

be identified. To determine whether any of these other known or putative

methyltransferases regulate Ipl1 function, I individually deleted each of these SET

domain-containing genes and the *DOT1* gene from wild-type and *ipl1-2* cells and

performed *ipl1-2* suppression assays (Figure 4). Deletion of these known and putative

methyltransferases had little to no effect on *ipl1-2* temperature sensitivity compared to

the suppression observed upon deletion of *SET1* (Figure 4). This suggests that Set1 is

the primary lysine methyltransferase in yeast that regulates Ipl1 function.

Figure 4. Deletion of known and putative methyltransferases do not suppress *ipl1-2*

The indicated yeast strains were serially diluted 10-fold, spotted onto rich media (YPD) plates, and grown at 25°C or 32°C for 3 days.
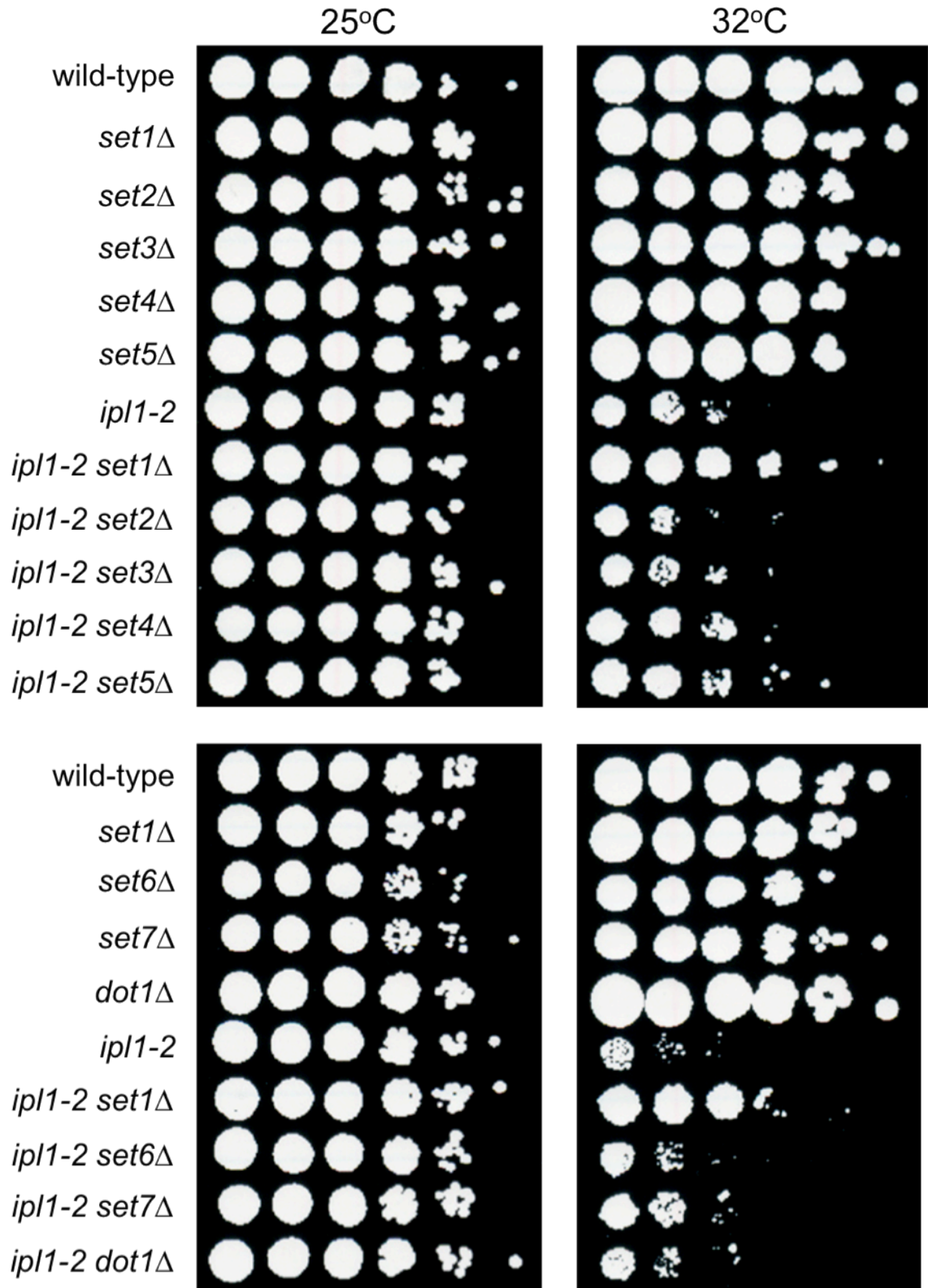
Figure 4

The COMPASS complex is essential for Dam1 methylation

Set1 functions within an eight-member complex termed COMPASS (Miller et al., 2001).

In addition to Set1, several additional non-catalytic COMPASS subunits are required for

H3K4 methylation (Dehe et al., 2006; Krogan et al., 2003b; Schneider et al., 2005). To

determine whether Set1 methyltransferase activity at the kinetochore occurs within the

context of the COMPASS complex, I performed *ipl1-2* suppression assays in cells

deleted for individual COMPASS subunits to determine whether loss of any of these

genes suppresses the *ipl1-2* temperature sensitive phenotype as does deletion of *SET1*

(Figure 5). I created strains bearing deletions in *SWD1*, which is required for all H3K4

methylation, *SDC1*, which is required for H3K4 di- and trimethylation, *BRE2*, which is

required for full dimethylation and whose deletion completely abolishes trimethylation,

and *SPP1*, which is required only for H3K4 trimethylation (Dehe et al., 2006; Krogan et

al., 2003b; Schneider et al., 2005). My results demonstrate that deletion of the *SWD1*,

SDC1, and *BRE2* COMPASS subunits, but not *SPP1*, suppresses the *ipl1-2* temperature

sensitive phenotype to a degree similar to that observed upon deletion of *SET1* itself

(Figure 5). These results suggest that Set1 requires the COMPASS complex to function

at the kinetochore, and imply that mono- or dimethylation of a COMPASS substrate is

important in regulating Ipl1 functions.

To determine directly whether suppression of the temperature-sensitive phenotype of the

mutant *ipl1-2* allele by deletion of members of the COMPASS complex is due to changes

in Dam1K233 dimethylation, I examined Dam1 methylation levels by

immunoprecipitating HA-Dam1 expressed from the native *DAM1* locus from cells

Figure 5. Deletion of COMPASS subunits suppresses *ipl1-2*

The indicated COMPASS deletion yeast strains or deletion of *PAF1* were serially diluted

10-fold, spotted onto rich media (YPD) plates, and grown at 25°C or 32.5°C for 3 days.

	25°C	32.5°C
WT *IPL1*		
swd1Δ IPL1		
sdc1Δ IPL1		
spp1Δ IPL1		
bre2Δ IPL1		
paf1Δ IPL1		
WT *ipl1-2*		
swd1Δ ipl1-2		
sdc1Δ ipl1-2		
spp1Δ ipl1-2		
bre2Δ ipl1-2		
paf1Δ ipl1-2		
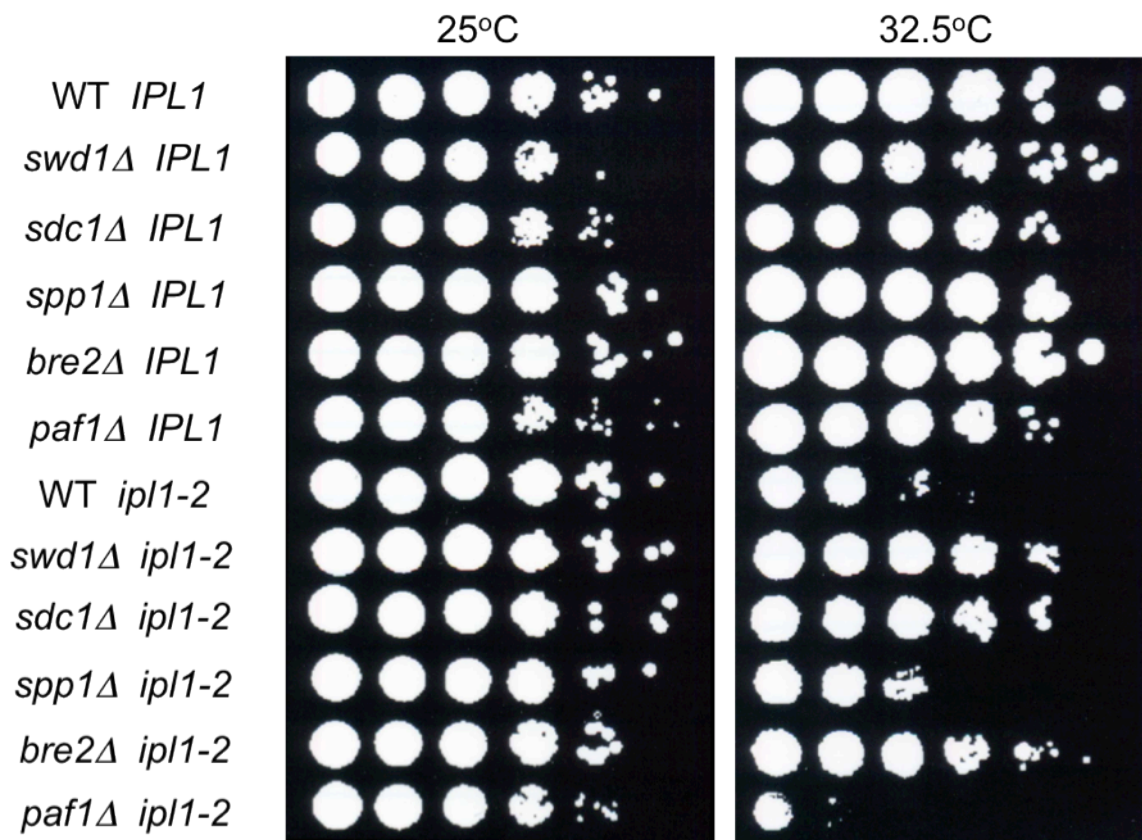
Figure 5

containing the same COMPASS subunit deletions used in the *ipl1-2* suppression assays

described above. I then immunoblotted with a Dam1K233me2 specific antibody (Figure

6). If methylation is reduced upon deletion of COMPASS subunits, then Set1 normally

methylates Dam1 as part of the COMPASS complex. My data revealed that similar to

the *set1Δ* control, *SWD1*, *SDC1*, and *BRE2* are essential for Dam1K233 dimethylation

(Figure 6). However, loss of *SPP1* has no effect on Dam1K233 methylation, consistent

with my genetic findings above (Figure 5 and 6). These results demonstrate that

suppression of the *ipl1-2* temperature phenotype is tightly linked to Dam1 methylation

status and suggest that suppression of *ipl1-2* can serve as an indicator of a requirement

for Dam1 methylation.

To compare the role of the COMPASS subunits in Dam1K233 dimethylation to H3K4

methylation, I made total protein extracts from HA-Dam1 COMPASS deletion cells and

immunoblotted with an antibody specific for H3K4 dimethylation. As expected, deletion

of *SWD1* completely abolished H3K4me2 while deletion of *SPP1* had little effect on

H3K4me2 levels (Figure 7). Contrary to previously published results, deletion of either

SDC1 or *BRE2* completely abolished H3K4 dimethylation (Figure 7). Other labs had

previously determined that deletion of either of these two COMPASS subunits only

partially decreased the levels of H3K4me2 (Dehe et al., 2006; Krogan et al., 2002;

Mueller et al., 2006). The difference in results may reflect a difference in strain

background or the antibody used to immunoblot for H3K4me2. My results show a

striking correlation between the roles of these factors in H3K4 methylation and their roles

in Dam1K233 dimethylation (Figure 8). Deletion of *SWD1* abolishes the integrity of the

Figure 6. Set1 requires the COMPASS complex to methylate Dam1

Endogenously tagged HA-Dam1 was immunoprecipitated from wild-type or COMPASS

deletion strains. Immunoblots were then probed with either a HA or Dam1K233me2

specific antibody. The HA-Dam1 *set1Δ* strain serves as a negative control. The HA

blots serve as loading controls.

α-HA IP

HA-Dam1 strains

HA-Dam1 strains

untagged wild-type bre2Δ set1Δ   untagged wild-type set1Δ swd1Δ sdc1Δ spp1Δ

α-Dam1K233me2

α-HA

1.0 0.1 0.1   1.0   0.1   0.1   0.3   0.7
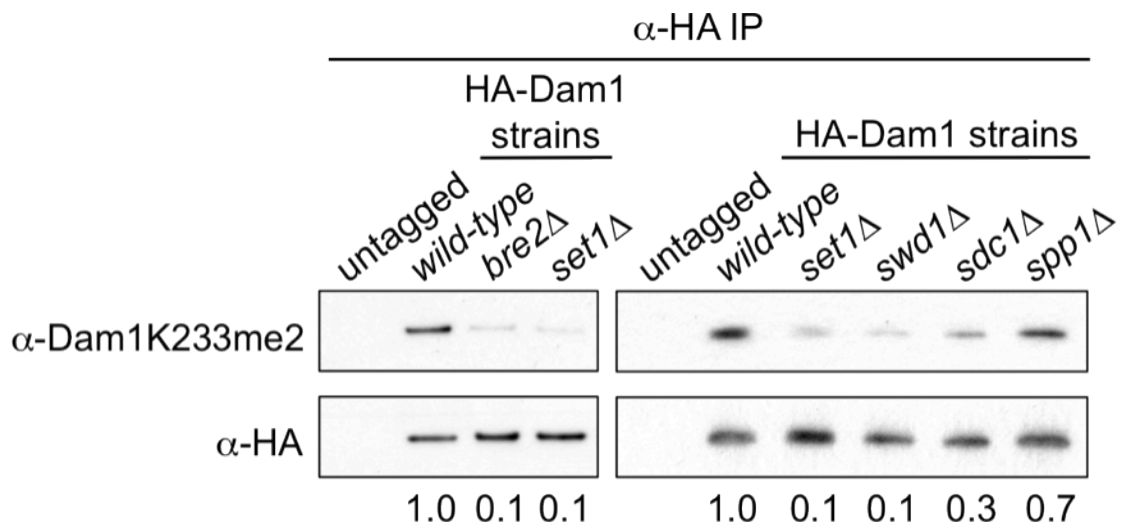
Figure 6

Figure 7. Role of the COMPASS complex in H3K4 dimethylation

Total protein extracts of COMPASS deletions in HA-Dam1 strains were immunoblotted

for either H3K4me2 or H3. H3 serves as the loading control.

HA-Dam1 strains

untagged / wild-type / swd1Δ / bre2Δ / sdc1Δ / spp1Δ / set1Δ / dam1k233R

α-H3K4me2

α-H3

Figure 7

Figure 8. Comparison of the roles of the COMPASS complex in *ipl1-2* suppression, Dam1K233me2, and H3K4me2

Summary of COMPASS complex requirements for Dam1K233me2, *ipl1-2* suppression, and H3K4me2.

	ipl1-2 suppression	Dam1K233me2	H3K4me2
set1Δ	+	-	-
swd1Δ	+	-	-
bre2Δ	+	-	-
sdc1Δ	+	-	-
spp1Δ	-	+	+

Figure 8

COMPASS complex (Dehe et al., 2006), therefore loss of Dam1K233me2 in the *swd1Δ* strain suggests that a functional COMPASS complex is required for the catalytic activity of Set1 regardless of substrate. Similarly, loss of Dam1 dimethylation upon deletion of either *BRE2* or *SDC1* indicates that Set1-mediated dimethylation requires this heterodimer for both of its substrates identified to date (Figure 7).

The Paf1 complex is required for Dam1 methylation

The Paf1 transcription elongation complex is required for Set1 and COMPASS recruitment to histones for H3K4 methylation (Krogan et al., 2003b). The Paf1 complex is also required for H2BK123 ubiquitination through an unknown mechanism (Shilatifard, 2006). If deletion of members of the Paf1 complex suppresses *ipl1-2* temperature sensitivity, this would imply that the Paf1 complex is more generally required for Set1 functions. I therefore deleted *RTF1* and *PAF1* and a rotation student I supervised, Shanzhi Wang, deleted *CTR9* from *ipl1-2* cells. Deletion of any of these three subunits abolishes H3K4 methylation (Krogan et al., 2003b). However, deletion of *PAF1* or *CTR9* did not suppress the *ipl1-2* temperature sensitive phenotype (Figure 9). In contrast, deletion of *RTF1* did suppress the *ipl1-2* temperature sensitive phenotype (Figure 9). These findings indicate either that Rtf1 has a unique function in regulating Ipl1, or that other phenotypes associated with loss of Paf1 and Ctr9 mask the ability to score suppression of the *ipl1-2* mutant phenotype. In fact, loss of either *PAF1* or *CTR9* leads to cell wall defects that result in temperature sensitivity (Betz et al., 2002). These defects are suppressed by the addition of an osmotic stabilizer such as sorbitol to the growth medium (Betz et al., 2002). When I repeated the *ipl1-2* suppression assays on

Figure 9. Deletion of Paf1 complex members suppress *ipl1-2*

The indicated yeast strains were serially diluted 10-fold, spotted onto rich media (YPD) or YPD containing 1M sorbitol, and grown at 25°C or 31°C for 3 days.
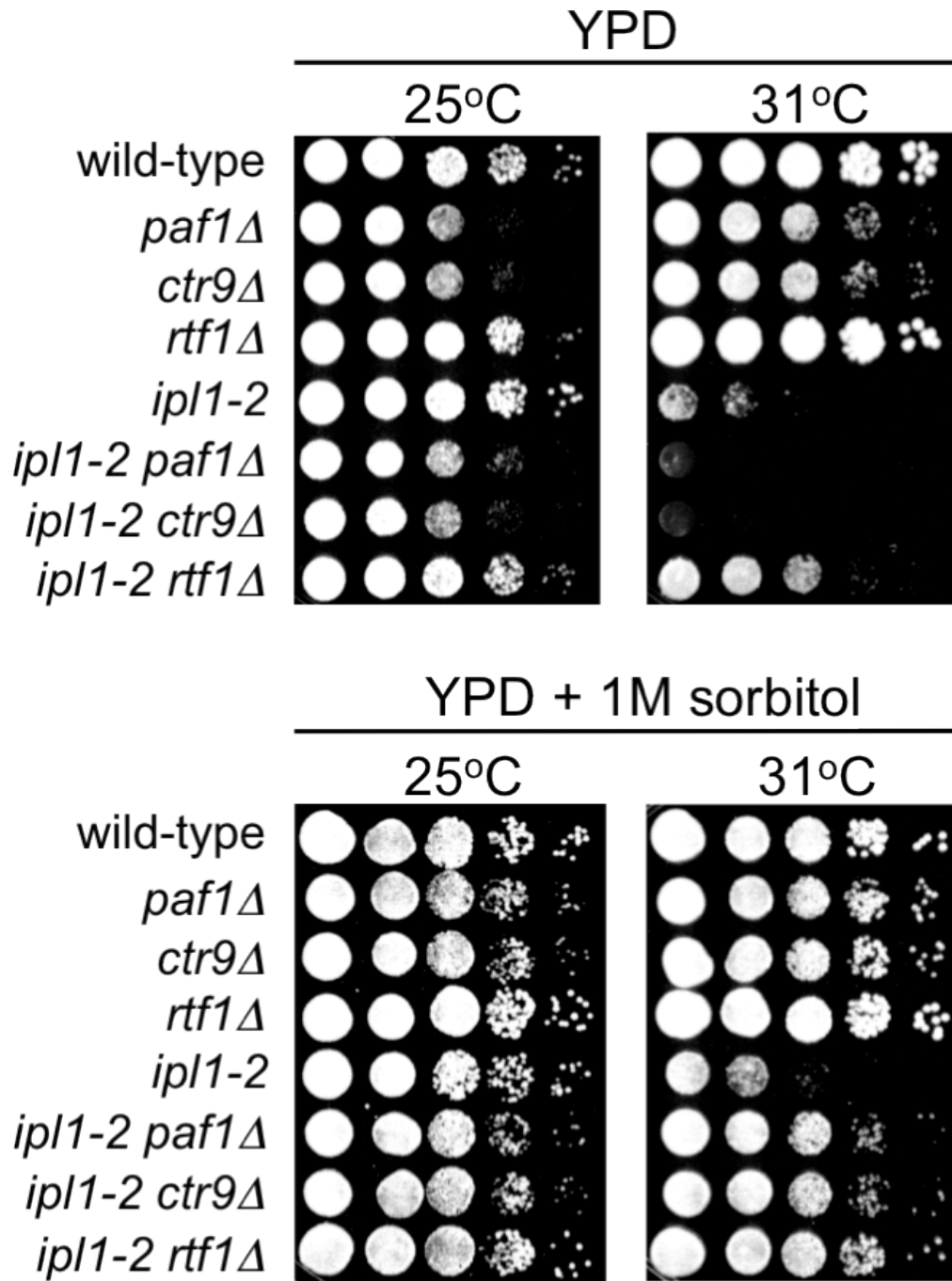
Figure 9

media containing sorbitol, deletion of either *PAF1* or *CTR9* suppressed the *ipl1-2* temperature sensitivity to a degree similar to that observed upon deletion of *RTF1* (Figure 9). Importantly, the addition of sorbitol did not affect the temperature sensitivity of the cells containing only the *ipl1-2* allele. Together, my data indicate that the Paf1 complex negatively regulates Ipl1 functions at the kinetochore, as does Set1.

To directly determine whether the Paf1 complex is required for Dam1K233 methylation, I deleted *CTR9* and *RTF1* from HA-Dam1 cells. I then immunoprecipitated HA-Dam1 followed by immunoblotting for Dam1K233 methylation. Deletion of either of these Paf1 complex subunits resulted in loss of Dam1K233 dimethylation (Figure 10). Together, my results indicate that the Paf1 complex plays an integral role in Set1-mediated methylation regardless of the substrate and shows for the first time that the Paf1 complex has functions outside of its roles in transcription.

Dam1 methylation is independent of transcriptional elongation

Our finding that the Paf1 transcriptional elongation complex is essential for Dam1 methylation raises the question of whether transcription is required for Dam1 methylation. The H3K4 methylation pathway at active gene promoters is dependent on transcription as H2BK123 ubiquitination and Set1 recruitment to specific gene promoters is dependent on Kin28 mediated phosphorylation of serine 5 in the C-terminal domain (CTD) of RNA polymerase (Krogan et al., 2003a; Ng et al., 2003b; Xiao et al., 2005). Phosphorylation of RNA polymerase by Kin28 is required for progression of transcriptional initiation to elongation (Cismowski et al., 1995; Valay et al., 1995). To

Figure 10. Paf1 complex is required for Dam1 methylation

HA-Dam1 was immunoprecipitated from wild-type or Paf1 complex deletion strains then

probed with either a HA or Dam1K233me2 specific antibody. The HA-*dam1K233R*

strain serves as a negative control along with *set1Δ*. The HA blot serves as a loading

control.
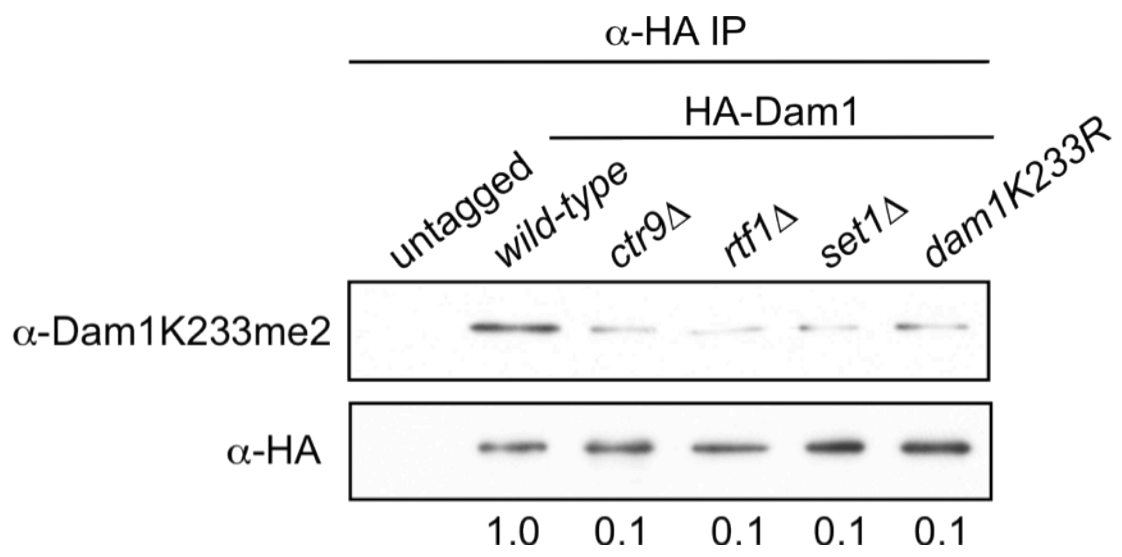
Figure 10

determine whether Dam1 methylation is dependent on transcription, Dam1K233 dimethylation levels were measured in the *kin28-ts16* mutant strain. Cells containing the *kin28-ts16* temperature sensitive allele are deficient in RNA polymerase II CTD serine 5 phosphorylation (data not shown) (Xiao et al., 2003). No difference in Dam1 methylation levels was observed upon inactivation of Kin28 at the restrictive temperature of 37°C (Figure 11). We were unable to determine whether the *kin28-ts16* allele suppresses the *ipl1-2* temperature sensitive phenotype at the restrictive temperature since the *ipl1-2* mutant is inviable at 37°C. These results indicate that Dam1 methylation, in contrast to H3K4 methylation, is not dependent on active transcription.

Methylation of Dam1 requires kinetochore association

The above results indicate that several transcription factors are required for Dam1 methylation, raising the question of whether this modification occurs at kinetochores, where Dam1 is localized (Cheeseman et al., 2001b). To address this question, we asked whether mutations that disconnect the kinetochore from the centromere (in *NDC10*) or the Dam1 complex from the rest of the kinetochore (in *NDC80*) affect Dam1K233 methylation (Janke et al., 2002). At the restrictive temperature, *ncd10-1* cells exhibited characteristic clusters of cells characteristic of their inability to complete cytokinesis, and the *ndc80-1* cells were arrested in metaphase as expected (data not shown) (Bouck and Bloom, 2005; McCleland et al., 2003). Dam1 methylation was severely decreased upon inactivation of either Ndc10 or Ndc80 at 37°C (Figure 12). As with the *kin28-ts16* mutant, we were unable to determine whether *ndc10-1* or *ndc80-1* suppress *ipl1-2* due to the high temperature required to induce the *ndc10-1* and *ndc80-1* phenotypes. Together

Figure 11. Mutation of *KIN28* has no effect on Dam1 methylation

HA-Dam1 was immunoprecipitated from wild-type or *kin28-ts16* cells incubated at either

25°C or cells heat shocked at 37°C for 3 hours. The immunoprecipitated HA-Dam1 was

then probed with either a HA or Dam1K233me2 specific antibody.
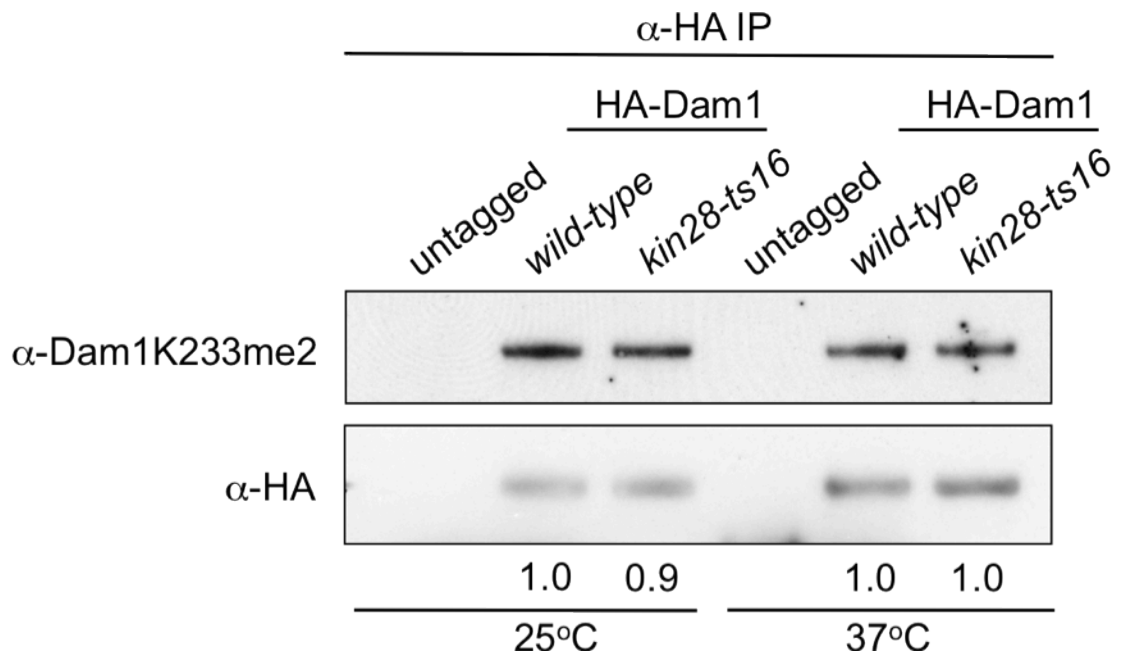
Figure 11

Figure 12. *NDC10* **and** *NDC80* **are required for Dam1 methylation**

HA-Dam1 was immunoprecipitated from wild-type, *ndc10-1*, or *ndc80-1* cells incubated at either 25°C or heat shocked at 37°C for 3 hours. The immunoprecipitated HA-Dam1 was then probed with either a HA or Dam1K233me2 specific antibody.
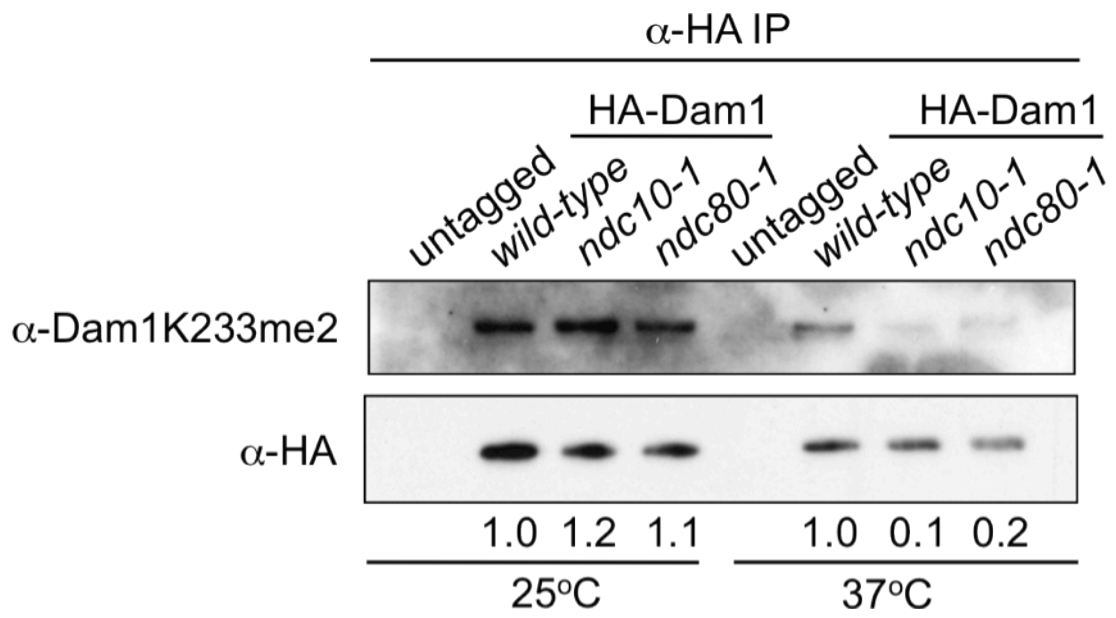
Figure 12

with my results above, these findings strongly indicate that the Paf1 complex functions at the kinetochore, in the absence of active transcription, to promote methylation of Dam1.

Dam1 methylation is regulated by Rad6 and Bre1

Ubiquitination of histone H2BK123 is required for H3K4 methylation, constituting the only known example of regulatory cross-talk *in trans* between histone modifications (Shilatifard, 2006). H2B ubiquitination is catalyzed by the E2 ubiquitin conjugating enzyme Rad6 and the E3 ubiquitin ligase Bre1. Deletion of *RAD6*, *BRE1*, or mutation of H2BK123 to arginine, which cannot be ubiquitinated, abolishes H3K4 methylation (Shilatifard, 2006). Since Rad6 and Bre1 play such a pivotal role in regulating H3K4 methylation, I determined whether these enzymes were also required for Set1 function at the kinetochore. I deleted either *RAD6* or *BRE1* from wild-type and *ipl1-2* cells and performed *ipl1-2* suppression assays. Each of these suppression assays contained multiple individual isolates of *ipl1-2 rad6Δ* or *ipl1-2 bre1Δ* (Figure 13 and 14). My results clearly indicate that loss of either of these enzymes suppresses the *ipl1-2* temperature sensitive phenotype, suggesting that both Rad6 and Bre1 are required for Set1 function in opposing Ipl1 at the kinetochore (Figure 13 and 14).

To directly determine whether Rad6 and Bre1 are required for Dam1K233 dimethylation, I deleted *RAD6* and *BRE1* from cells containing HA-Dam1. I immunoprecipitated HA-Dam1 from these cells and immunoblotted with the Dam1K233me2 specific antibody.

Figure 13. Deletion of *RAD6* suppress *ipl1-2*

Wild-type, *rad6Δ*, *ipl1-2*, or *ipl1-2 rad6Δ* yeast strains were serially diluted 10-fold,

spotted on rich media, and grown at 25°C or 32°C for 3 days.   Shown are three individual

isolates of *rad6Δ* and *ipl1-2 rad6Δ* strains.
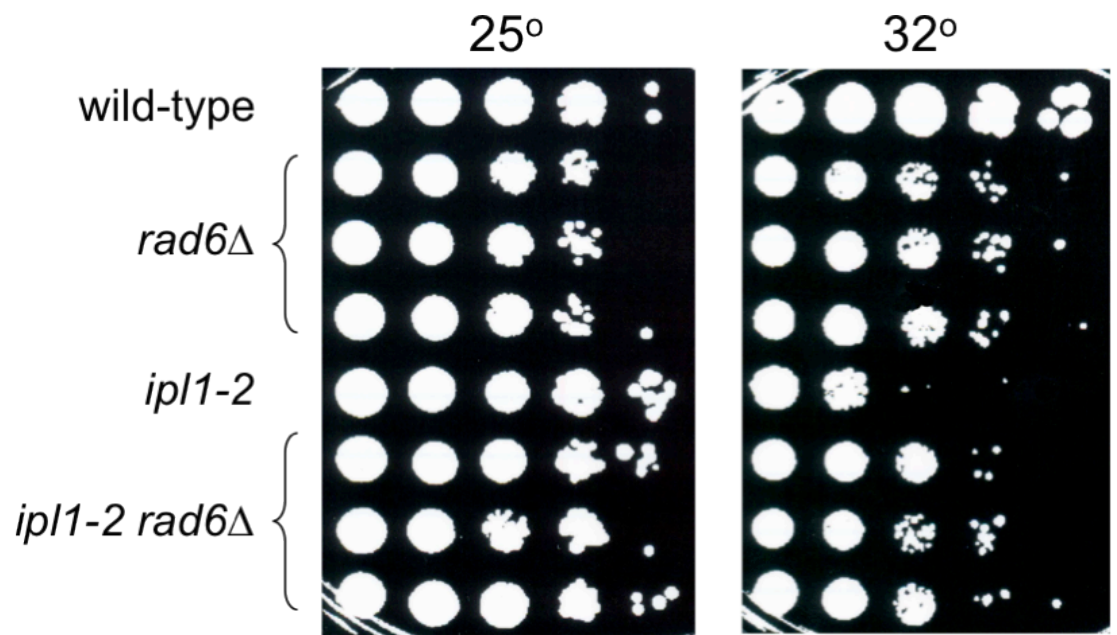
Figure 13

Figure 14. *bre1Δ* suppresses *ipl1-2*

Wild-type, *bre1Δ*, *ipl1-2*, or *ipl1-2 bre1Δ* yeast strains were serially diluted 10-fold,

spotted on rich media, and grown at 25°C or 32°C for 3 days.   Shown are three individual

isolates of *bre1Δ* and *ipl1-2 bre1Δ* strains.
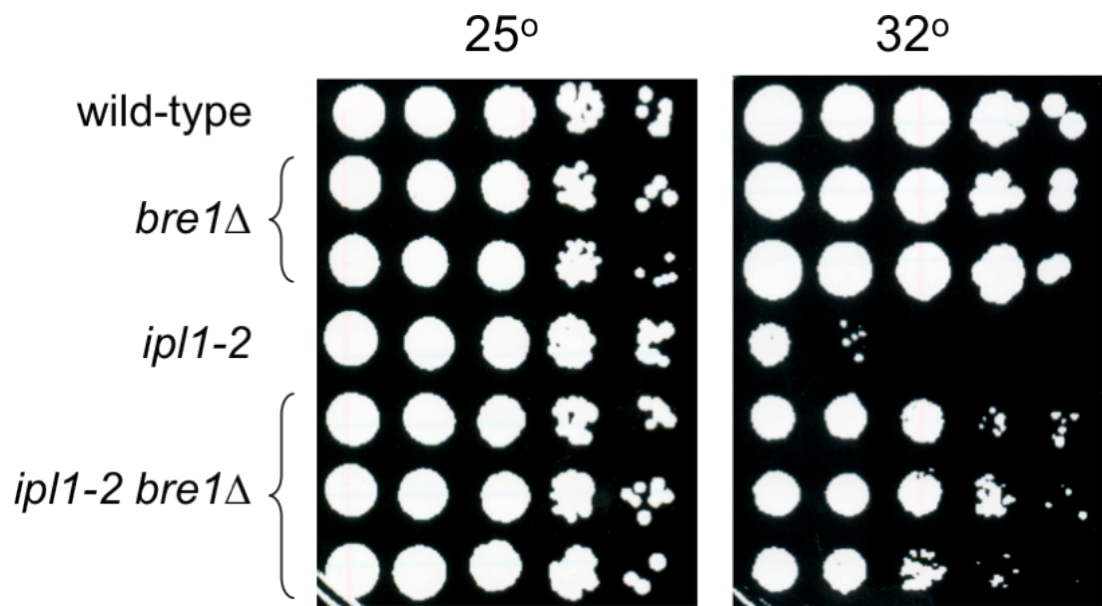
Figure 14

My results showed that both Rad6 and Bre1 are required for Dam1K233 methylation (Figure 15). These data suggest that Rad6-Bre1 mediated ubiquitination of H2B or another Rad6-Bre1 substrate regulate Dam1K233 methylation.

Other E3 ligases of Rad6 do not suppress *ipl1-2* temperature sensitivity

Rad6 functions with two other E3 ligases; Ubr1 and Rad18, which participate in N-rule mediated protein degradation and the DNA damage response, respectively. In order to determine whether Bre1 was the only E3 ligase of Rad6 that functions in regulating Dam1 methylation, I deleted either *UBR1* or *RAD18* from *ipl1-2* cells and performed an *ipl1-2* suppression assay. Deletion of either of these genes did not suppress *ipl1-2* suggesting that Bre1 is the sole E3 ligase of Rad6 that regulates Dam1 methylation (Figure 16).

Ubp8 regulates proper levels of Dam1K233 methylation

Removal of the ubiquitin moiety from H2BK123 is performed by the deubiquitinating enzymes Ubp8 and Ubp10 (Henry et al., 2003; Zhang et al., 2008). Deletion of either *UBP8* or *UBP10* results in increased levels of H2B ubiquitination and subsequently, increased levels of H3K4 methylation at gene promoters (Shukla et al., 2006). If deletion of these enzymes also resulted in an increase in Dam1 methylation, this would be further evidence that persistent ubiquitination of H2B (or another protein) continuously promotes Dam1 methylation. Therefore, I immunoprecipitated Dam1 from *ubp8Δ* and *ubp10Δ* cells and immunoblotted for Dam1K233me2. The level of Dam1K233 methylation in

Figure 15. Rad6 and Bre1 are required for Dam1 methylation

HA-Dam1 was immunoprecipitated from wild-type, *rad6Δ*, or *bre1Δ* strains then immunoblotted with either a HA or Dam1K233me2 specific antibody. The HA-Dam1 *set1Δ* strain serves as a negative control. The HA blot serves as a loading control. ND indicates that the Dam1K233me2 was not detectable by quantitation.

α-HA IP

HA-Dam1

untagged   wild-type   set1Δ   rad6Δ   bre1Δ

α-Dam1K233me2

α-HA

1.0   0.4   0.3   ND
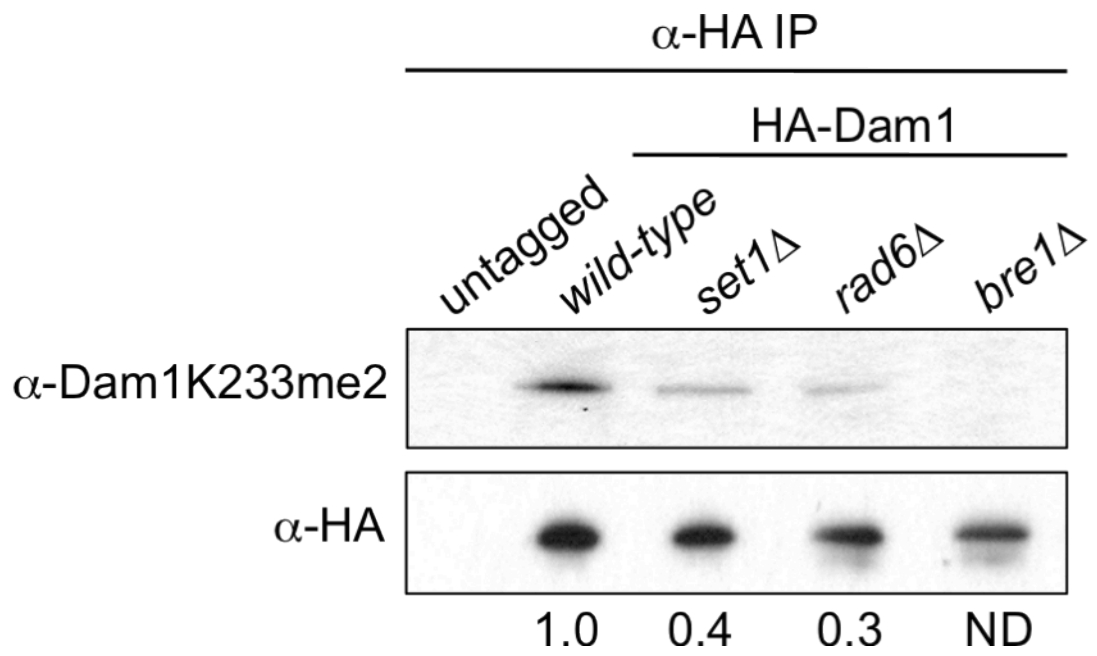
Figure 15

Figure 16. Deletion of *RAD18* or *UBR1* do not suppress *ipl1-2*

The indicated yeast strains were serially diluted 10-fold, spotted on rich media, and grown at 25°C or 32°C for 3 days. Shown are two individual isolates of *ubr1Δ* and *ipl1-2 ubr1Δ* strains and three individual isolates of *rad18Δ* and *ipl1-2 rad18Δ* strains.
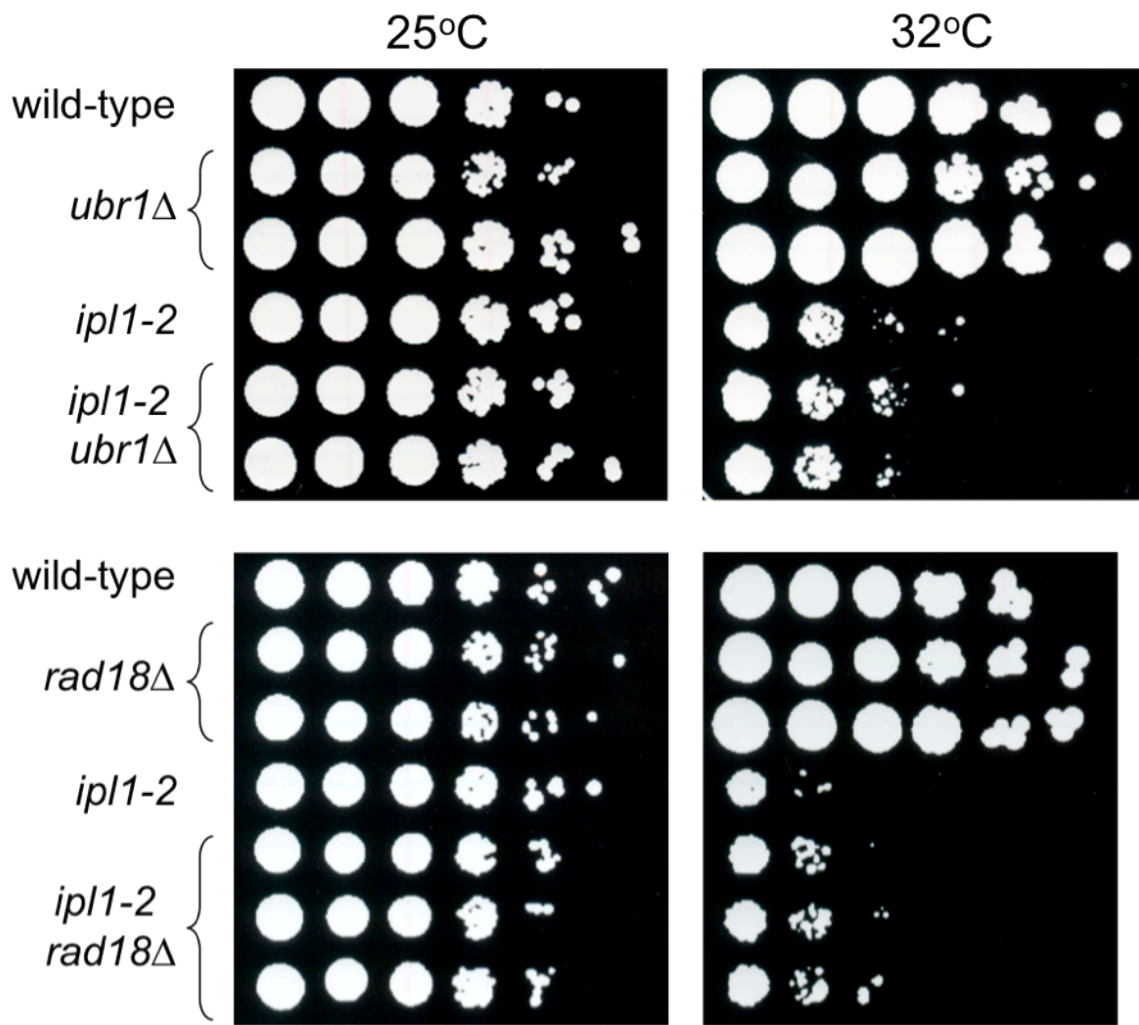
Figure 16

ubp10Δ cells was slightly diminished compared to wild-type levels of methylation (Figure 17). However, deletion of *UBP8* resulted in a substantial increase in Dam1K233 methylation (Figure 17). My findings suggest that Ubp8 is important for modulating proper levels of Dam1 methylation, likely through removal of a ubiquitin moiety ligated to its substrate by Rad6 and Bre1. Furthermore, my data illustrate the importance of H2BK123 ubiquitination in regulating Dam1K233me2.

Deletion of *CHD1* does not suppress the *ipl1-2* temperature sensitive phenotype

Chd1, like Ubp8, is a member of the SAGA complex (Pray-Grant et al., 2005). It has been suggested that Chd1 binds to methylated H3K4 (Pray-Grant et al., 2005) although some controversy over this result remains (Flanagan et al., 2007; Okuda et al., 2007; Sims et al., 2005). The histone code hypothesis proposes that the mechanism through which lysine methylation controls downstream functions is through binding of an effector molecule. It is currently unknown whether any protein binds to Dam1K233me2. Since Ubp8 regulates methylation of Dam1K233 and Ubp8 catalytic activity is dependent on the presence of at least a sub-module of the SAGA complex, Chd1 seemed a likely candidate for binding to Dam1K233me2. I therefore deleted *CHD1* from wild-type and *ipl1-2* cells and performed an *ipl1-2* suppression assay. If Dam1K233 dimethylation inhibited Ipl1 phosphorylation of flanking serines though facilitation of Chd1binding to Dam1K233me2, then I would expect loss of *CHD1* to suppress the *ipl1-2* temperature sensitive phenotype. However deletion of *CHD1* had no effect on *ipl1-2* temperature sensitivity (Figure 18). While this does not necessarily rule out the possibility that Chd1 binds to Dam1K233me2, it suggests that Chd1 does not modulate Ipl1 function *in vivo*.

Figure 17. Ubp8 regulates Dam1 methylation levels

HA-Dam1 was immunoprecipitated from wild-type, *ubp8Δ*, or *ubp10Δ* strains then immunoblotted with either a HA or Dam1K233me2 specific antibody. The HA blot serves as a loading control.
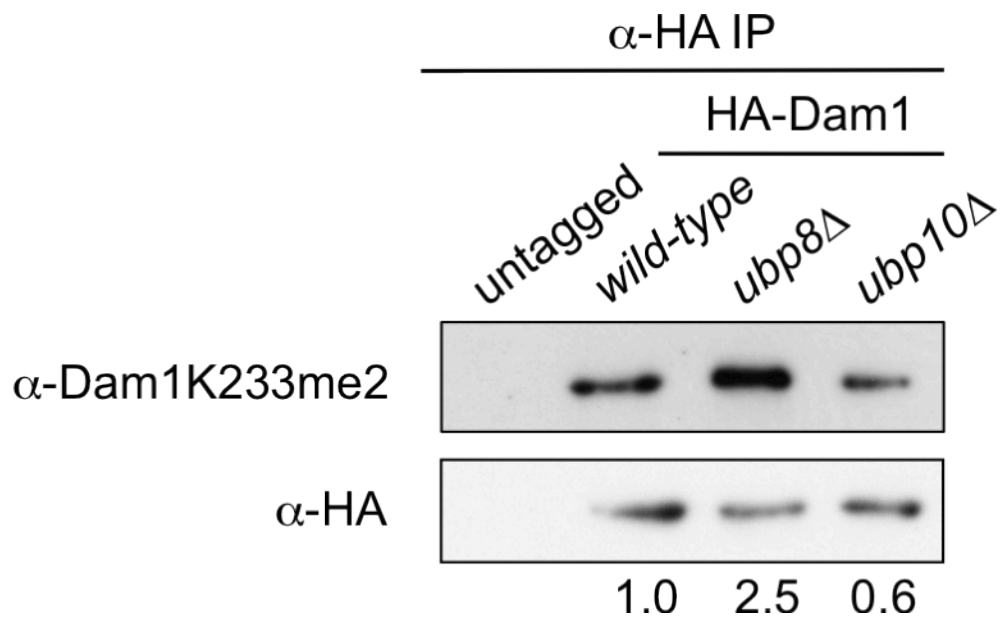
Figure 17

Figure 18. *chd1Δ* **does not suppress** *ipl1-2*

The indicated yeast strains were serially diluted 10-fold, spotted on rich media, and

grown at 25°C or 32°C for 3 days.   Shown are four individual isolates of *ipl1-2 chd1Δ*.
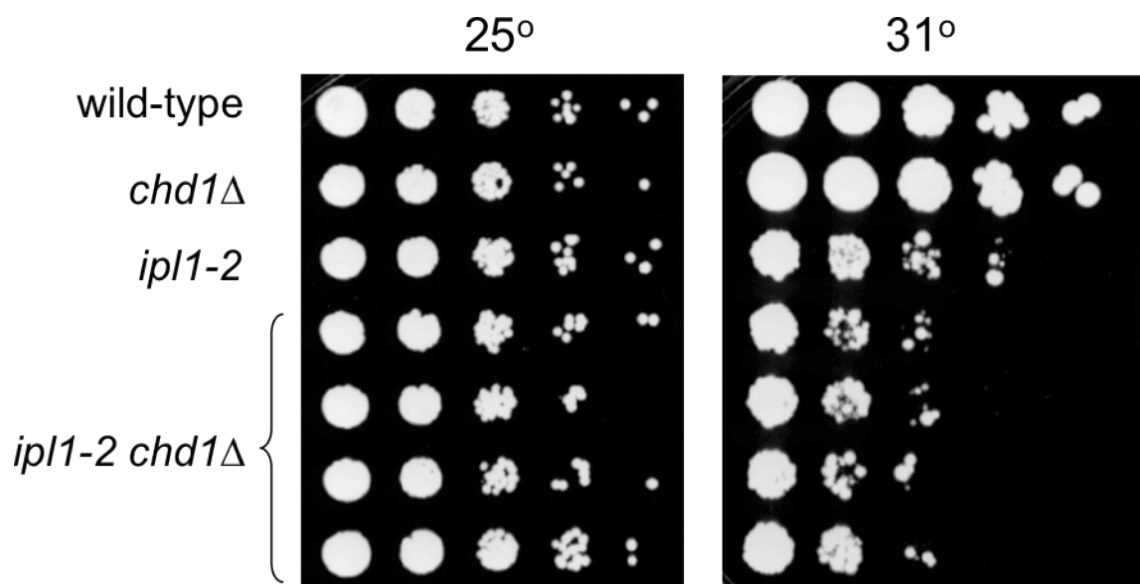
Figure 18

Dam1 and Ndc80 complexes are not ubiquitinated by Rad6-Bre1

My data thus far suggest that ubiquitination of some unknown substrate is required for Dam1K233 dimethylation. Our lab had previously shown that ubiquitination of H2BK123 was likely not required for Dam1K233 dimethylation as mutation of H2BK123 to arginine (R), which is unable to be ubiquitinated, did not suppress the *ipl1-2* temperature sensitive phenotype (Zhang et al., 2005). By using the role of H2BK123 ubiquitination as a prerequisite for H3K4 methylation as a model, I reasoned that a protein closely associated with Dam1, or even Dam1 itself, was likely a novel substrate for Rad6-Bre1 mediated ubiquitination. The two most likely candidates were the ten-member Dam1 complex or the closely associated Ndc80 complex. In support of this idea, Bre1 interacts with the Ndc80 complex member Nuf2 as determined by yeast-two hybrid analysis (Newman et al., 2000).

To determine whether a member of the Dam1 complex was ubiquitinated, I transformed strains containing a TAP-tagged member of the Dam1 complex with an episomal plasmid bearing HA tagged ubiquitin driven by the *TDH3* promoter. I then performed immunoprecipitations for the TAP tag from strains containing HA-ubiquitin, the Dam1 complex member TAP-Duo1, or TAP-Duo1 HA-ubiquitin. I confirmed that I had successfully immunoprecipitated TAP-Duo1 by immunoblotting for protein A (Figure 19). To determine whether ubiquitinated proteins were associated with TAP-Duo1, I immunoblotted for HA-ubiquitin using an HA antibody. Several bands were present in the lane containing TAP-Duo1 HA-ubiquitin that were not present in the TAP-Duo1 lane (Figure 19). These bands were likely ubiquitinated proteins that associate with Duo1.

Figure 19. The Dam1 complex is not ubiquitinated

TAP-Duo1 was purified from cells expressing only TAP-Duo1 or TAP-Duo1 and HA-ubiquitin under control of the *TDH3* promoter. Cells expressing only HA-ubiquitin serves as a negative control. Bands in the HA blot that were present in the TAP-Duo1 HA-ubiquitin lane but not in the TAP-Duo1 were lane were identified by mass spectroscopy. These bands are marked with asterisks.
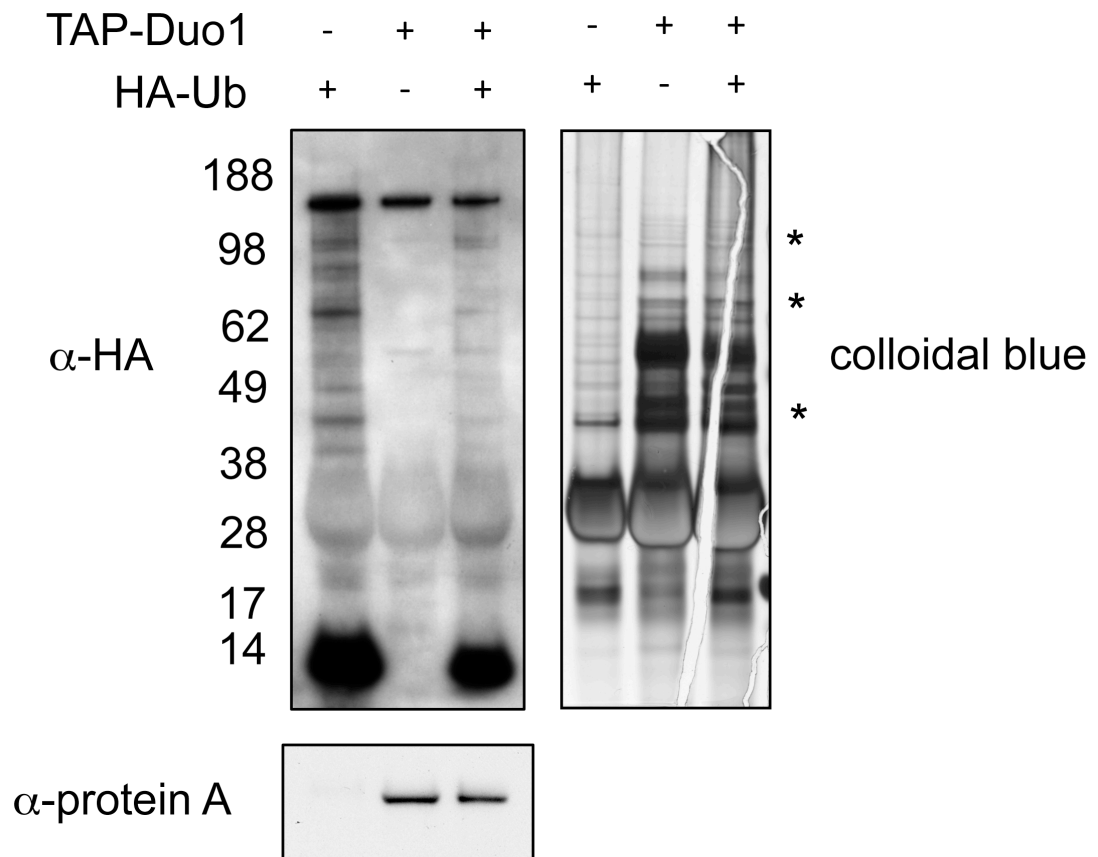
Figure 19

The bands were cut out of a sister gel stained with colloidal blue and analyzed by mass spectroscopy by Dr. Maria Persons at the University of Texas at Austin. None of the proteins identified by mass spectroscopy were members of the Dam1 complex or other kinetochore members. In addition, I performed a similar experiment with the Ndc80 complex in which I did a TAP purification from cells containing HA-ubiquitin, TAP-Nuf2, or TAP-Nuf2 HA-ubiquitin (data not shown). Bands that were only present in the TAP-Nuf2 HA-ubiquitin lane were sent to Dr. Persons for identification by mass spectroscopy, but again failed to reveal a member of the Ndc80 complex or other member of the kinetochore suggesting the Dam1 and Ndc80 complexes may not be ubiquitinated (data not shown).

One reason I may not have detected ubiquitination of either the Dam1 or Ndc80 complexes was that the ubiquitination was either too transient or too labile a modification to be detected by the methods described above. However, Rad6 ubiquitinates H2B *in vitro* suggesting that Rad6 and Bre1 may also be able to ubiquitinate a member of the Dam1 or Ndc80 complexes *in vitro* (Sung et al., 1988). Therefore, I performed *in vitro* ubiquitination assays using purified Dam1 or Ndc80 complexes as substrates and TAP-Rad6 and TAP-Bre1 as the E2 and E3 ligases. However, Rad6 and Bre1 failed to ubiquitinate either of these complexes *in vitro* (data not shown).

Since I had previously shown that Ubp8 regulates Dam1K233 dimethylation, likely through deubiquitination, I reasoned that deletion of *UBP8* would increase the likelihood of detecting ubiquitinated proteins. I therefore deleted *UBP8* and *BRE1* from HA-Dam1

strains containing His-ubiquitin-G76A. This mutation within ubiquitin inhibits its removal from substrates by deubiquitinating enzymes. I immunoprecipitated HA-Dam1 and immunoblotted for His-ubiquitin. If Dam1 or any other protein co-immunoprecipitated by Dam1 was ubiquitinated by Rad6-Bre1, I expected this ubiquitination to be increased in the *ubp8Δ* lane and abolished in the *bre1Δ* lane. However, I was unable to detect any ubiquitination of HA-Dam1 or any other protein co-immunoprecipitated by Dam1 (data not shown). All together, despite using three different strategies, I was unable to identify a novel substrate(s) for Rad6 and Bre1 at the kinetochore.

Lysines within histones H2A and H2B are required for Dam1 methylation

As I was not able to identify a novel Rad6-Bre1 substrate at the kinetochore, I decided to re-examine whether H2BK123 ubiquitination is required for Dam1 methylation. To do this, I repeated the *ipl1-2* suppression assays containing *h2bK123R* as the only source of histone H2B. As we had previously observed, mutation of H2BK123 to arginine did not suppress the *ipl1-2* temperature sensitive phenotype (data not shown) (Zhang et al., 2005). I therefore decided to test whether the *h2bK123R* strains lacked H3K4 dimethylation, which would confirm the *h2bK123R* mutation. Surprisingly, in all the isolates tested, H3K4 dimethylation was still present, suggesting either these isolates were not in fact *h2bK123R* mutants or that some other issue existed with these isolates (Figure 20).

Figure 20. H3K4me2 is still present in *ipl1-2 h2bK123R* strains

Total protein extracts of *ipl1-2 h2bK123R* mutants were immunoblotted for either

H3K4me2 or H3. H3 serves as the loading control. The *ipl1-2* and *h2bK123R* strains

serve as negative and positive controls.
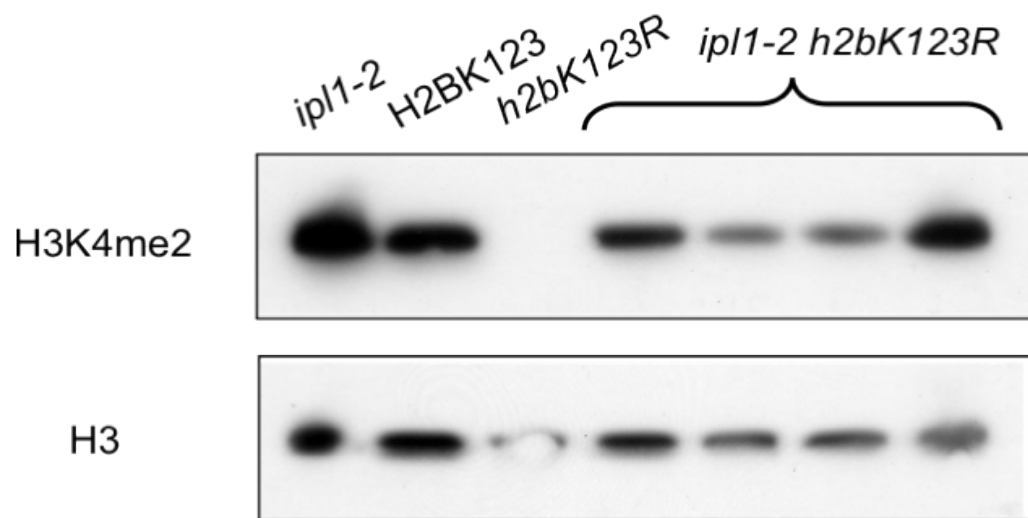
Figure 20

I therefore decided to construct new *h2bK123R ipl1-2* strains by mating *h2bK123R* cells

with *ipl1-2* cells. In order to make sure that the only source of H2B contained the K123R

point mutation, I made total protein lysates and immunoblotted for H3K4me2 to confirm

that methylation was abolished in these isolates, which it was (data not shown). To

determine whether H2BK123 modulates Ipl1 function, I performed *ipl1-2* suppression

assays. Surprisingly, seven of the nine isolates suppressed the *ipl1-2* temperature

sensitive phenotype (Figure 21). This was surprising because in the past when doing

ipl1-2 suppression assays with other gene deletions or point mutants, all isolates gave the

identical phenotype. This suggested to me that perhaps other functions of H2BK123

confounded our genetic analysis or that something was still amiss with the *h2bK123R*

strains.

To determine whether there was something amiss with the genetic analysis, I decided to

directly assess Dam1K233 dimethylation in the *h2bK123R* strain. I therefore constructed

a HA-Dam1 *h2bK123R* strain. I immunoprecipitated HA-Dam1 and then immunoblotted

with the Dam1K233me2 specific antibody. In all isolates tested, Dam1K233me2 was

abolished in the *h2bK123R* background similar to deletion of *SET1* suggesting that

H2BK123 ubiquitination is indeed required for Dam1K233 dimethylation (Figure 22).

I then went back to my *h2bK123R ipl1-2* strains and sequenced the plasmid containing

H2A and H2B to confirm the presence of the *h2bK123R* point mutation. The H2B gene

contained the K123R mutation as expected (Figure 23). To my surprise the H2A also

Figure 21. Some *h2bK123R* isolates suppress *ipl1-2*

The indicated yeast strains were serially diluted 10-fold, spotted on rich media, and grown at 25°C or 31°C for 3 days. Shown are nine individual isolates of *ipl1-2 h2bK123R*.
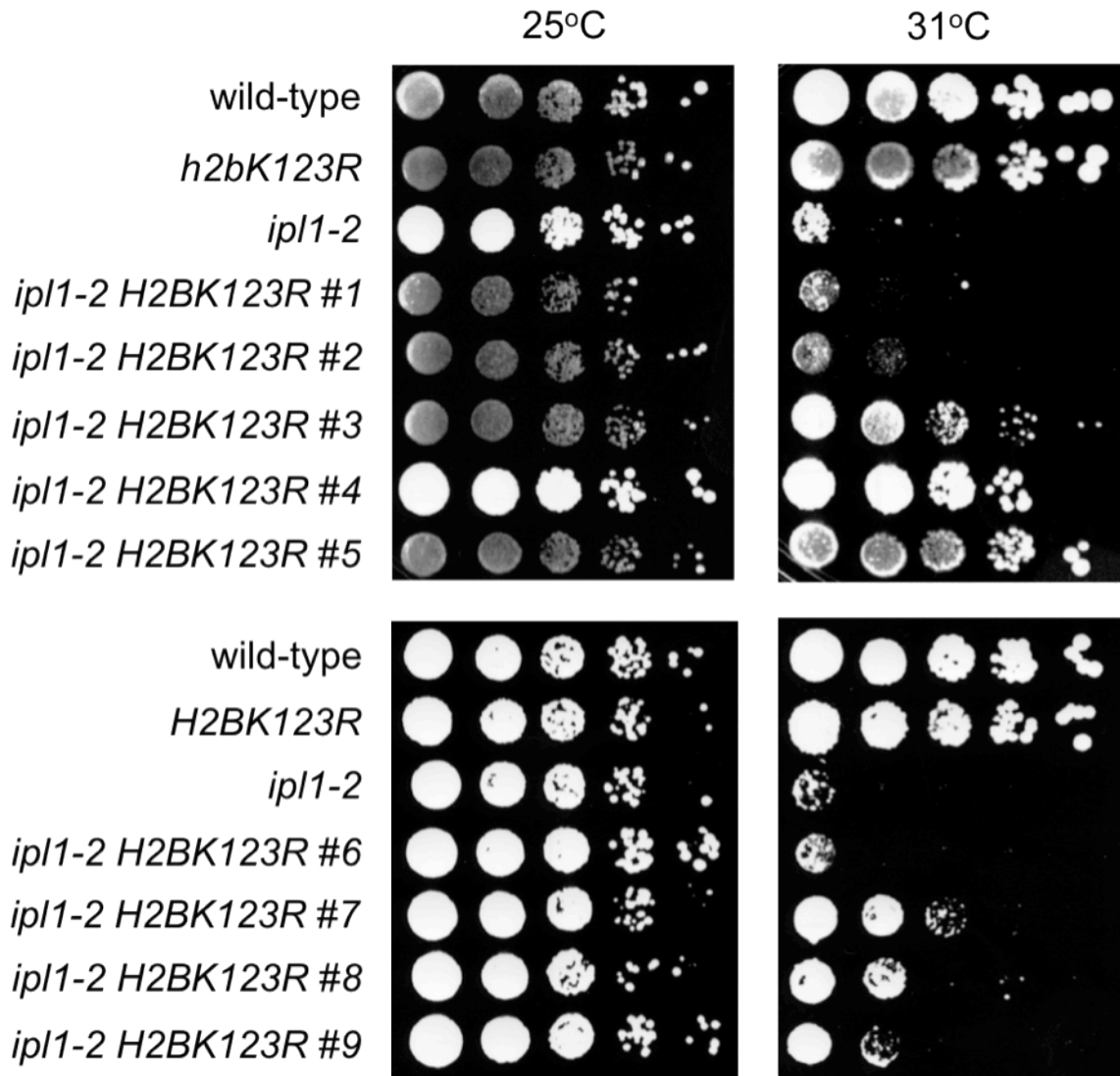
Figure 21

Figure 22.   Lysines within H2A and H2B are important for Dam1 methylation

HA-Dam1 was immunoprecipitated from wild-type or *h2bK123R* strains then
immunoblotted with either a HA or Dam1K233me2 specific antibody. Shown are three
individual isolates of HA-Dam1 *h2bK123R*. The HA blot serves as a loading control and
set1Δ as a negative control.
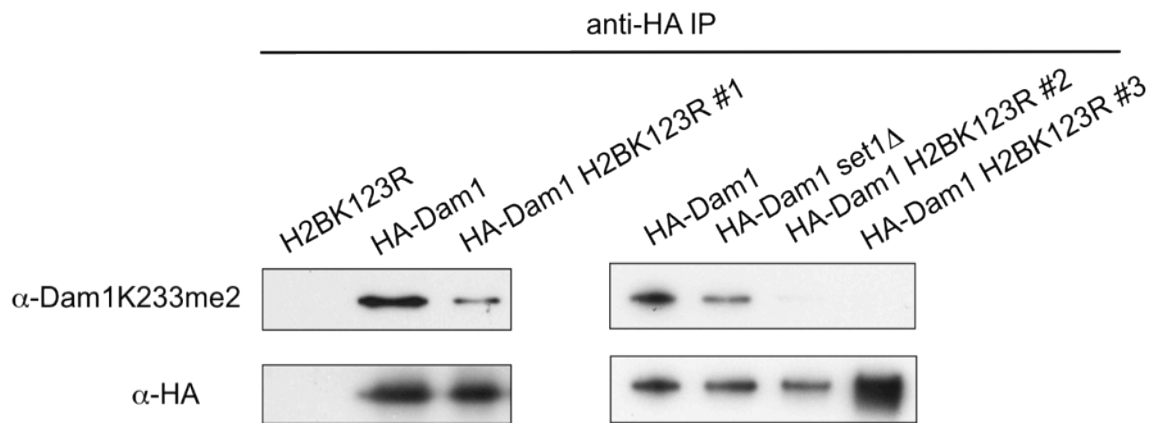
Figure 22

Figure 23. Sequencing of *ipl1-2 h2bK123R* isolates

The entire *HTA1-HTB1* locus was sequenced from the *ipl1-2 h2bK123R* strains in Figure

21 as was wild-type (Y131) and *h2bK123R* (Y133). Shown is the sequence of the 3'

region of both genes. Highlighted in red are point mutations from lysine to arginine

within Y133 and the *ipl1-2 h2bK123R* strains at K123 within *HTB1* and K119, K120,

K123, and K126 within *HTA1*.
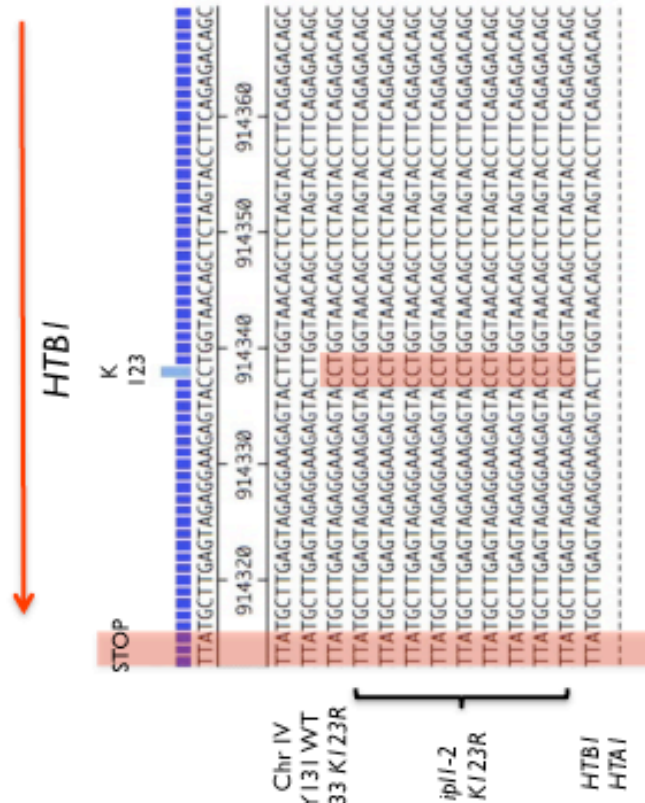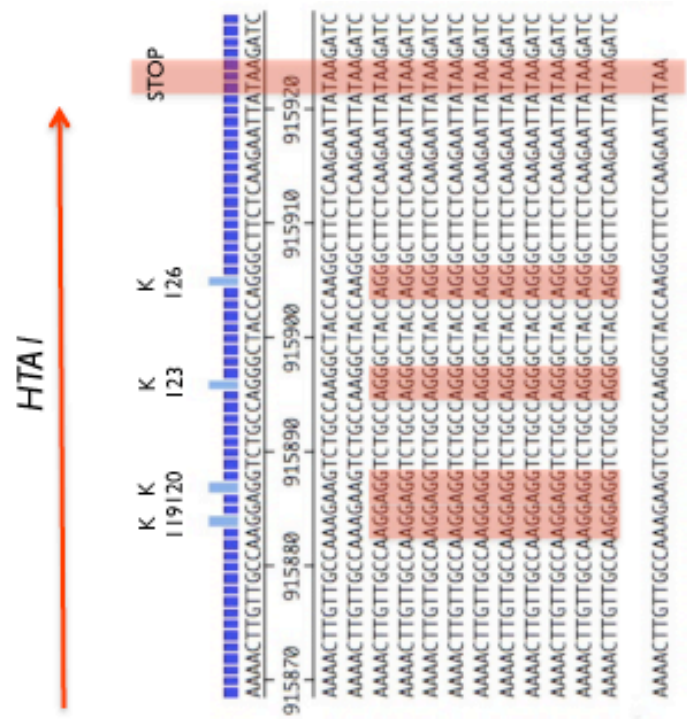
Figure 23

contained four lysine to arginine point mutations at K119, K120, K123, and K126 (Figure 23). It was previously published that mutation of these five lysines within H2A and H2B leads to pronounced mitotic and meiotic defects (Robzyk et al., 2000), which would compromise my ability to measure the effects of the *h2bK123R* mutation on *ipl1-2* temperature sensitivity and likely explains why only some, but not all of the *h2bK123R* isolates suppressed the *ipl1-2* temperature sensitive phenotype. I then sequenced the HA-Dam1 *h2bK123R* strains used in the experiments described in the preceding paragraph and found that all five of these lysines within H2A and H2B were mutated to arginine (data not shown). Therefore, I was unable to conclude that the mutation of H2BK123 to arginine was responsible for the loss of Dam1K233 dimethylation rather than the other four K to R point mutations within H2A.

Ubiquitination of histone H2BK123 regulates Dam1 methylation

To determine whether the *h2bK123R* mutation alone could suppress the *ipl1-2* phenotype, I constructed new *ipl1-2* strains containing only the *h2bK123R* point mutation. Sequencing confirmed the presence of the *h2bK123R* mutation and no other mutation in H2B or H2A and immunoblotting for H3K4 dimethylation revealed that methylation was abolished in the *ipl1-2 h2bK123R* mutants (data not shown). In all isolates tested, the *h2bK123R* mutation suppressed the *ipl1-2* temperature sensitive phenotype suggesting a role for this lysine in regulating Set1 functions at the kinetochore (Figure 24). I next constructed new HA-Dam1 *h2bK123R* strains and again sequenced to confirm no other mutations were present and confirmed loss of H3K4 dimethylation (data not shown). When I immunoprecipitated HA-Dam1 and immunoblotted with the Dam1K233me2

Figure 24.   Mutation of H2BK123 suppresses *ipl1-2*

The indicated yeast strains were serially diluted 10-fold, spotted on rich media, and

grown at 25$^{\text{o}}$C or 31.5$^{\text{o}}$C for 3 days.   Shown are two individual isolates of *ipl1-2*

h2bK123R.
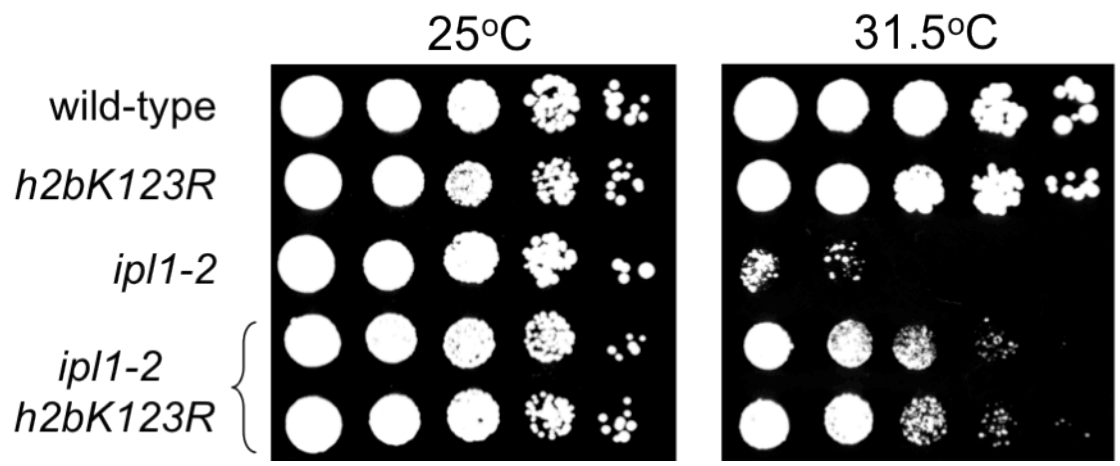
Figure 24

specific antibody, Dam1K233 dimethylation was abolished similar to that in the *set1Δ* and *dam1K233R* control strains (Figure 25). These data, together with the role of Rad6, Bre1 and Ubp8 in Dam1 methylation, strongly argue that ubiquitination of H2BK123 is a prerequisite for Dam1K233 methylation (Figures 15, 17, and 25). My results demonstrate for the first time that cross-talk between posttranslational modifications can occur *in trans* between a histone protein and a non-histone protein.

Histone and Set1 association with Dam1 is dependent on H2BK123

Intriguingly, histones H2A, H2B, and H4 were identified by others upon mass spectroscopy of immunoprecipitates of Dam1 complexes (Janke et al., 2002). I confirmed Dam1-histone interactions using our *HA-DAM1* allele. Histones H2A and H2B co-immunoprecipitated with HA-Dam1 (Figure 26). Importantly this association is dependent on Dam1 as immunoprecipitation with HA conjugated beads in a strain containing untagged Dam1 or in a control strain containing the exosome Ski7 protein endogenously tagged with HA coimmunoprecipitated little to no H2A or H2B (Figure 26). However, histone H3 did not appear to associate with Dam1 (Figure 26). Importantly, all histones were present at equal levels in the immunoprecipitation inputs (Figure 26).

 In *S. cerevisiae*, centromeres contain nucleosomes consisting of histones H2A, H2B, H4, and the centromere-specific H3 variant histone Cse4 (Meluh et al., 1998). The lack of association of Dam1 with H3 is consistent with the replacement of this histone by the Cse4 H3 variant at the centromere. In fact, interaction between Dam1 and Cse4 was

Figure 25.   H2BK123 is required for Dam1 methylation

HA-Dam1 was immunoprecipitated from wild-type or *h2bK123R* strains then immunoblotted with either a HA or Dam1K233me2 specific antibody. The HA blot serves as a loading control. The *set1Δ* and *dam1K233R* strains serve as negative controls.
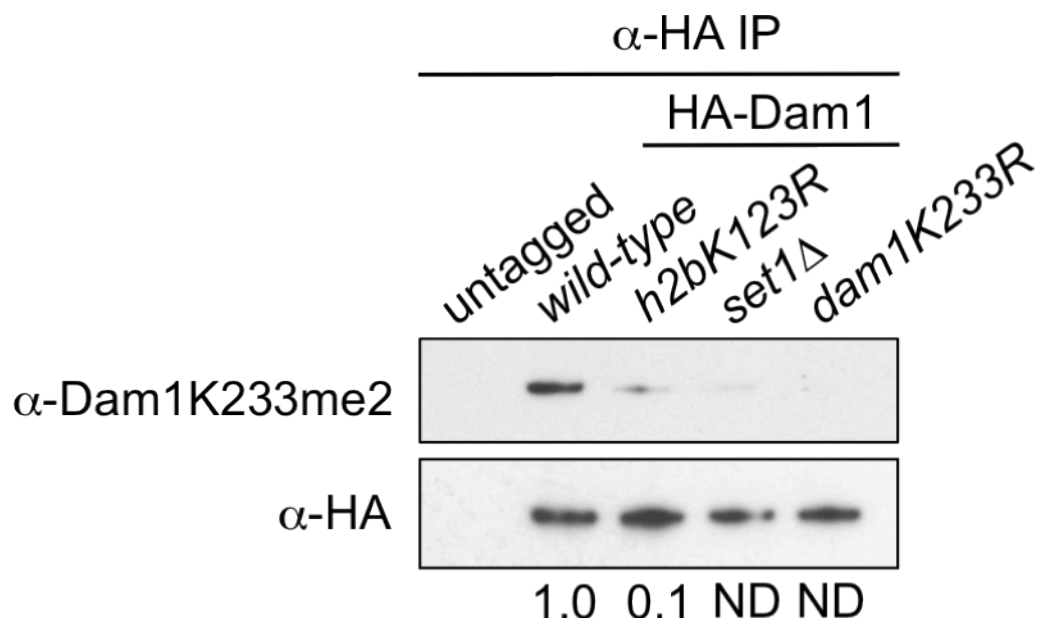
Figure 25

Figure 26.   Dam1 associates with H2A and H2B

HA-Dam1 and HA-Ski7 were immunoprecipitated and then immunoblotted for HA, with antibodies specific to yeast histones H2A or H2B, and with general H3 or H4 antibodies. The immunoprecipitant inputs were also immunoblotted for histones to confirm their presence at equal levels in all strains. HA-Ski7 serves as a control for the HA tag.
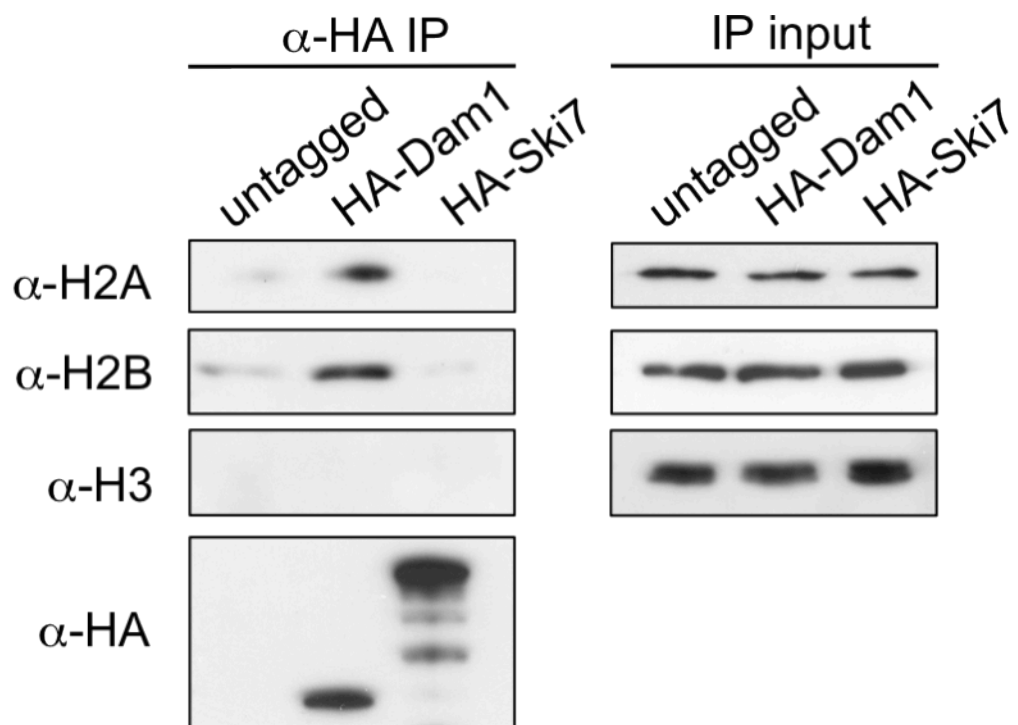
Figure 26

demonstrated by others by both a two-hybrid analysis and *in vitro* binding assays (Shang et al., 2003). I also confirmed that Cse4 and Dam1 interact as Cse4 tagged with the myc epitope at its endogenous locus coimmunoprecipitated HA-Dam1 (Figure 27). The close physical interaction observed between Dam1 and H2B suggests that H2B ubiquitination may directly trigger Dam1 methylation.

To further explore the relationship of H2B ubiquitination with Dam1 methylation, I sought to determine whether Dam1 co-immunoprecipitates with ubiquitinated H2B. Therefore, I immunoprecipitated HA-Dam1 from cells containing either wild-type H2B or in which H2BK123 was mutated to arginine. As expected, HA-Dam1 co-immunoprecipitated H2B (Figure 28). However in the *h2bK123R* point mutant, this association is abolished (Figure 28). This suggests that Dam1 associates only with the ubiquitinated isoform of H2B. We had previously shown that Dam1 and Set1 co-immunoprecipitate (Zhang et al., 2005). I therefore wished to determine whether association of Set1 with Dam1 was dependent on ubiquitinated H2B. I immunoprecipitated HA-Dam1 from either wild-type, *set1Δ*, or *h2bK123R* cells. As we had seen previously, HA-Dam1 co-immunoprecipitated Set1 and this interaction was abolished in the *set1Δ* lane (Figure 28). Intriguingly, the association between Set1 and Dam1 is abolished in the *h2bK123R* mutant suggesting that association of Set1 with Dam1 is dependent on ubiquitinated H2B (Figure 28). This is in contrast to the model in which Set1 and COMPASS recruitment to promoters is not dependent on H2BK123 ubiquitination with the exception of the Swd2 subunit (Lee et al., 2007). Together, my

Figure 27.   Dam1 associates with Cse4

Endogenously tagged myc-Cse4 under its own promoter was immunoprecipitated from either wild-type or HA-Dam1 cells and then probed with an antibody specific to either the myc or HA tags. The immunoprecipitant inputs were immunoblotted for HA to confirm their presents at equal levels in all strains.
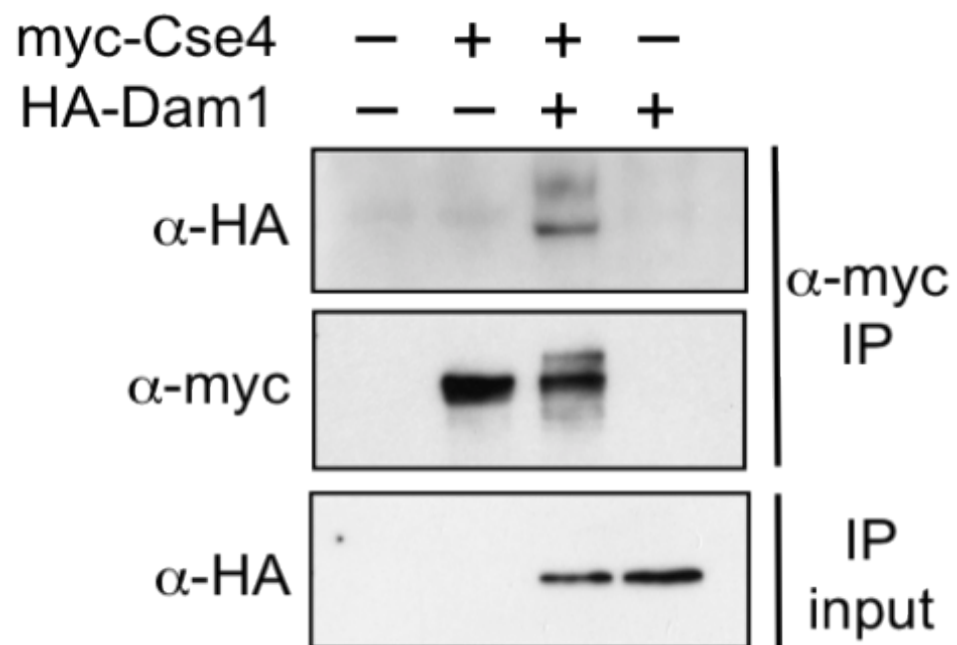
Figure 27

Figure 28. Set1 and H2B association with Dam1 is dependent on H2BK123

HA-Dam1 was immunoprecipitated from wild-type, *set1Δ*, or *h2bK123R* cells and HA-Ski7 was immunoprecipitated from wild-type cells and then immunoblotted for HA, H2B, or Set1. The immunoprecipitant inputs were immunoblotted for H2B and Set1 to confirm their presents at equal levels in all strains. HA-Ski7 serves as a control for the HA tag.
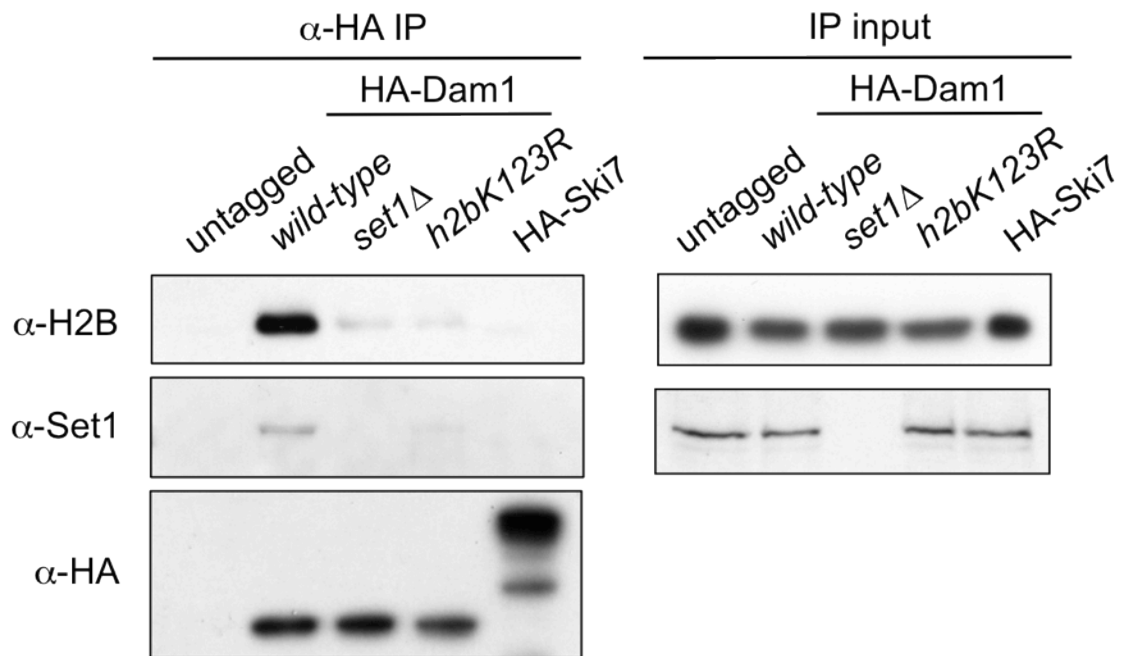
Figure 28

results in both Figure 26 and Figure 28 strongly suggest that H2BK123 ubiquitination is directly required for Dam1 methylation by Set1.

A role for Rpt4 and Rpt6 in Dam1 methylation is inconclusive

The proteosomal ATPases Rpt4 and Rpt6, which are also known as *SUG2* and *SUG1*, respectively, affect the levels of H3K4 di- and trimethylation (Ezhkova and Tansey, 2004). However, these ATPases have no effect on H2BK123 ubiquitination indicating that their requirement in H3 methylation is downstream of H2B ubiquitination (Ezhkova and Tansey, 2004). Both *RPT4* and *RPT6* are essential genes in yeast. To determine whether Rpt4 and Rpt6 also regulate Dam1 K233 dimethylation, I mated the hypomorphic alleles *sug2-1*, *sug1-3*, and *sug1-25* with the *ipl1-2* strain. After sporulation, I performed an *ipl1-2* suppression assay using the four spores from a single tetrad that resulted in a wild-type spore, a *sug2* or *sug1* mutant spore, an *ipl1-2* spore, and a spore containing both mutant alleles. Neither *sug1-3* nor *sug1-25* suppressed the temperature sensitivity of *ipl1-2* (Figure 29). However, while *sug2-1* failed to suppress *ipl1-2* from one tetrad, *sug2-1* did suppress *ipl1-2* using a second tetrad (Figure 29). The role for Rpt4 and Rpt6 in H3K4 methylation was demonstrated by immunoblotting for H3K4 methylation from cells grown at the *sug1-3*, *sug1-25*, and *sug2-1* restrictive temperature of 37°C (Ezhkova and Tansey, 2004). However, the *ipl1-2* suppression assays were performed at 32.5°C, which may not be a high enough temperature to affect the enzymatic activity of Rpt4 and Rpt6. In *ipl1-2* suppression assays performed at 37°C, both *ipl1-2* and the double mutant strains were unable to survive (data not shown).

Figure 29.   Suppression of *ipl1-2 rpt4/rpt6* mutants is inconclusive

The indicated yeast strains were serially diluted 10-fold, spotted on rich media, and grown at 25°C or 31.5^{o}C for 3 days.
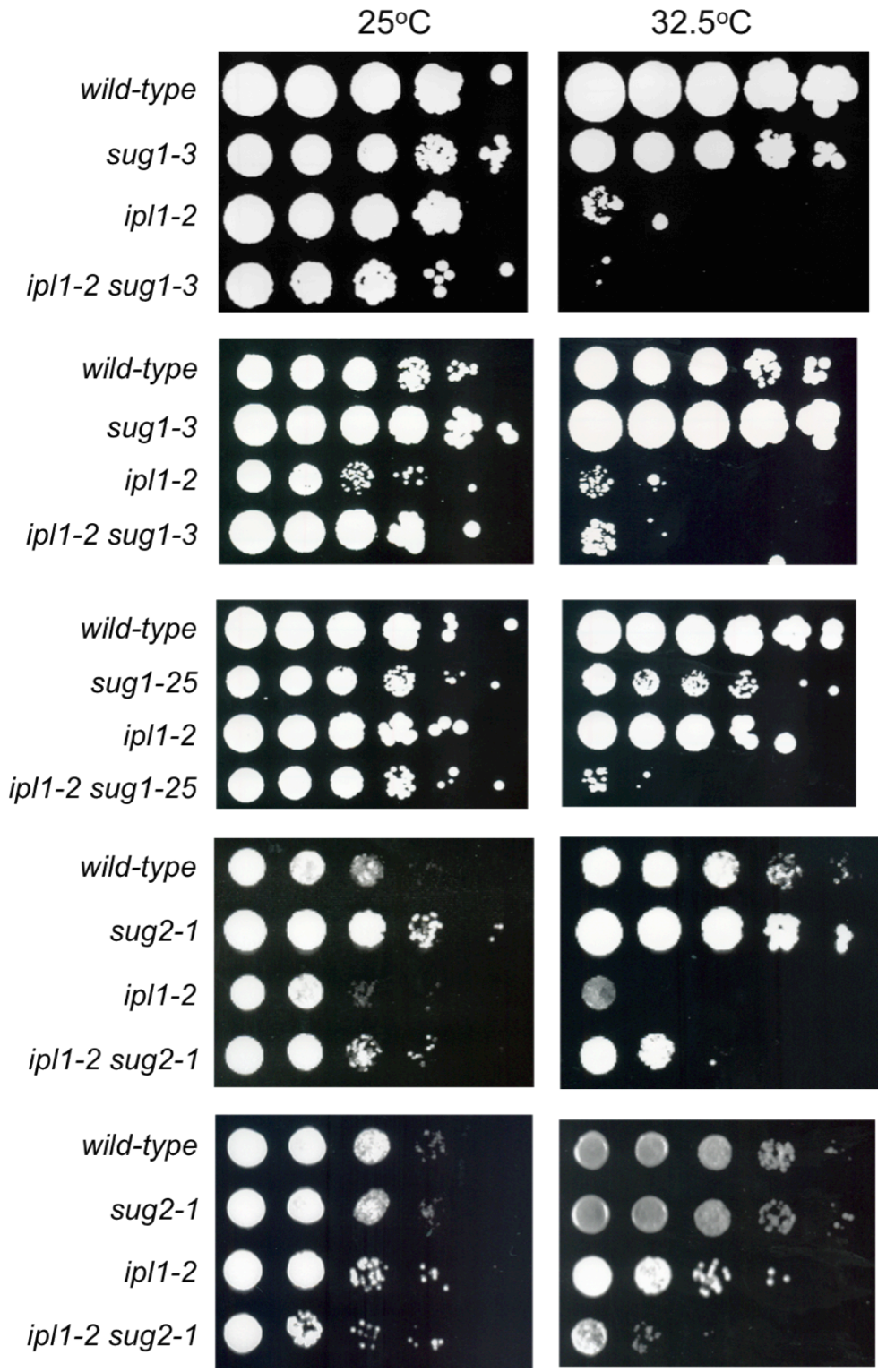
Figure 29

Due to the *ipl1-2* suppression assays being inconclusive, I mated the *sug2-1* strain with

the HA-Dam1 strain to directly determine whether the proteosomal ATPases are required

for Dam1K233 dimethylation by immunoprecipitation and immunoblotting for

Dam1K233me2. I only used the *sug2-1* strain, as the *SUG1* gene is located on the same

chromosome as Dam1 making recovery of a HA-Dam1 *sug1* mutant strain very difficult.

However in characterizing these mutants, I was never able to replicate the previously

published results that the *sug2-1* mutation decreases the levels of H3K4me2 at the

restrictive temperature of $37^{o}C$ (Figure 30). Since I was not able to replicate the previous

published results and due to the extremely slow growth of the HA-Dam1 strain at $37^{o}C$

(data not shown), I decided not to pursue the question of whether Rpt4 or Rpt6 regulate

Dam1K233 methylation further.

Figure 30. The *sug2-1* mutant has no effect on H3K4me2

Total protein extracts of either HA-Dam1 or *sug2-1* mutants were immunoblotted for either H3K4me2 or H3 after growth at the indicated temperatures. H3 serves as the loading control. Rpt4 is the same as *SUG2*. It was previously shown that H3K4me2 is markedly reduced at 37°C in the *sug2-1* mutant (Ezhkova and Tansey, 2004).
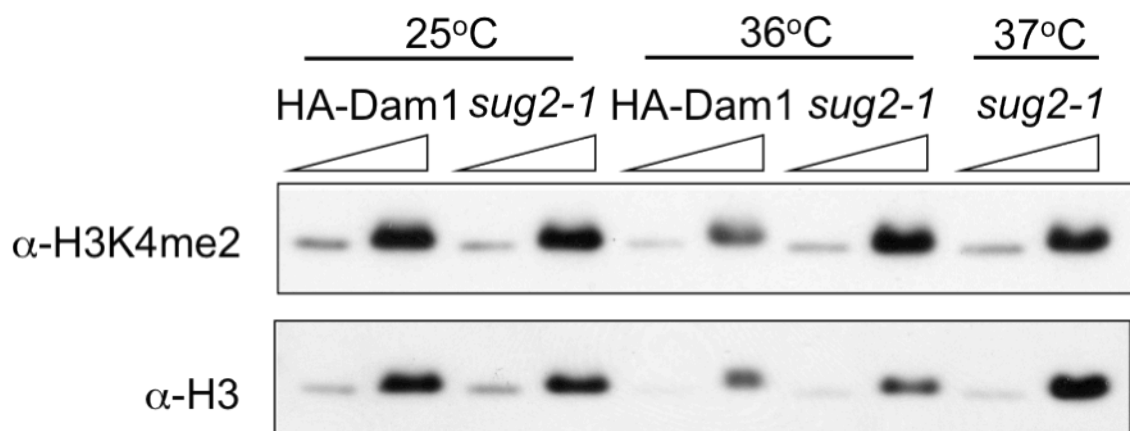
Figure 30

Chapter 4

Discussion

My studies define a conserved pathway that regulates Set1-mediated methylation regardless of substrate. All previous studies on the regulation of Set1 catalytic activity focused on methylation of histone H3 at active promoters. By studying the regulation of Dam1 methylation by Set1, I revealed unexpected roles for transcriptional regulatory factors outside of transcription in mitosis. I determined that Set1 requires a functional COMPASS complex for its catalytic activity as deletion of COMPASS subunits suppress the *ipl1-2* temperature sensitive phenotype and are required for Dam1 methylation. In addition, I demonstrated a link between suppression of *ipl1-2* and a requirement for Dam1K233 dimethylation indicating that suppression of *ipl1-2* serves as an indicator of Dam1K233 dimethylation. I determined that methylation of Dam1 is dependent on Rad6-Bre1 mediated ubiquitination of H2BK123 and the Paf1 transcriptional elongation complex. I showed that Dam1 methylation is not dependent on transcription and occurs at the kinetochore. In addition, I demonstrated a tight association between Dam1 and histone H2B that is dependent on H2BK123 ubiquitination. Together, my results indicate that cross-talk *in trans* occurs not only from H2BK123 ubiquitination to H3K4 methylation, but may also occur *in trans* outside of the nucleosome to Dam1. Importantly, this is the first example of a histone modification regulating the modification of a non-histone protein *in trans*. Moreover, my studies suggest a substrate independent pathway for Set1-mediated methylation and reveal previously unknown functions for

transcriptional regulatory proteins in mitosis. These data provide new insights to the regulation of Dam1 functions and provide a new fundamental insight to the regulation of protein functions by post-translational modifications.

COMPASS is essential for Set1-mediated methylation

The catalytic activity of Set1 toward H3K4 is dependent on an intact COMPASS complex (Dehe et al., 2006; Krogan et al., 2002; Morillon et al., 2005; Mueller et al., 2006). Deletion of either *SWD1* or *SWD3*, which together with Swd2 form the core of the COMPASS complex, completely abolishes all H3K4 methylation. *SWD2* is essential for viability and therefore cannot be deleted. However, studies with various *swd2* mutants support the argument that it is essential for at least H3K4 di- and trimethylation (Cheng et al., 2004). Deletion of either member of the *SDC1-BRE2* heterodimer affects both H3K4 di- and trimethylation but does not affect monomethylation. My results that *SWD1*, *SDC1*, and *BRE2* are required for Dam1K233 dimethylation mirror the role of these subunits in H3K4 methylation. In addition, *SPP1* has little effect on H3K4 dimethylation and deletion only results in loss of H3K4 trimethylation. I demonstrated that *SPP1* has little effect on Dam1K233 dimethylation levels indicating that the function of this subunit of COMPASS is conserved as well. I do not have an antibody specific to trimethylated Dam1K233 and I was therefore unable to test whether deletion of *SPP1* affects Dam1 trimethylation. Together, my results demonstrate that COMPASS is required for Set1 function regardless of substrate. This idea is reinforced by the lack of catalytic activity of Set1 on substrates *in vitro,* as methyltransferase assays require purification of an intact COMPASS complex (Krogan et al., 2002). In addition, my data

indicate that the COMPASS subunits role in regulating the processivity of Set1-mediated methylation is conserved for both substrates. My results further predict that Set1 will require an intact COMPASS complex for any Set1 substrates identified in the future.

Previous published reports indicate that deletion of the *BRE2* subunit has little effect on H3K4 dimethylation, but is required for H3K4 trimethylation (Dehe et al., 2006; Krogan et al., 2003b; Schneider et al., 2005). In contrast, my data demonstrate that deletion of *BRE2* results in complete loss of Dam1K233 dimethylation. This result initially suggested to me that Bre2 might confer substrate specificity for Set1 to Dam1 versus H3. However, when we tested the requirement of *BRE2* for H3K4 dimethylation with our H3K4me2 antibody and in our HA-Dam1 strain background, deletion of *BRE2* abolished H3K4 dimethylation. Therefore, Bre2 does not play a role in the substrate specificity for Set1 and it is unlikely that the COMPASS complex itself plays a role in directing Set1 to specific substrates.

A regulatory pathway required for Set1-mediated methylation regardless of substrate

Over the past decade, a large body of research has revealed a complex pathway regulating Set1-mediated methylation of H3K4 at promoters (Weake and Workman, 2008). These studies showed that ubiquitination of H2BK123 by Rad6 and Bre1, as well as the Paf1 transcription elongation complex, are essential for H3K4 di- and trimethylation (Hwang et al., 2003; Krogan et al., 2003b; Ng et al., 2003a; Robzyk et al., 2000; Wood et al., 2003a; Wood et al., 2003b). In addition, the Ubp8 ubiquitin-specific

protease regulates H2BK123 ubiquitination leading to gene-specific regulation of H3K4 methylation (Henry et al., 2003). In this dissertation, I showed that this pathway is conserved in regulating methylation of the other known substrate of Set1, the kinetochore protein Dam1, on lysine 233. Prior to my work, the consensus within the field was that the role of all these transcriptional regulatory factors in regulating H3K4 was to recruit COMPASS to promoters in order to trimethylate H3K4 at promoters and dimethylate H3K4 within the body of actively transcribed genes. My results counter this model and instead suggest a model by which this pathway is a general pathway for regulating Set1-mediated methylation regardless of substrate (Figure 31).

I discovered a slight difference between regulation of Set1-mediated methylation of H3K4 and Dam1K233 in that Ubp8, but not Ubp10, regulated Dam1 methylation. Deletion of *UBP8* results in an increase in Dam1K233 methylation. This finding suggests that in the absence of *UBP8* continuous H2B ubiquitination signals for more Dam1 methylation. This is supported by the fact that H2B at promoters is known to undergo rapid cycles of ubiquitination and deubiquitination (Henry et al., 2003). Ubp8 is part of the SAGA complex (Henry et al., 2003). Also within this complex is Gcn5 whose acetyltransferase activity is important for gene activation (Grant et al., 1997). The catalytic activity of Ubp8 requires Sgf11 for association to the rest of the SAGA complex (Ingvarsdottir et al., 2005; Lee et al., 2005). This suggests that the SAGA complex might also be closely associated with Dam1 and raises the possibility that SAGA may have other substrates at the kinetochore if not Dam1 itself. In contrast, deletion of *UBP10* did

Figure 31. Model of regulation of Set1-mediated methylation

Multiple signaling pathways control Set1-mediated methylation. Methylation of H3K4 is

dependent on H2BK123 ubiquitination and the Paf1 complex. H2BK123 is ubiquitinated

by Rad6-Bre1, which also ubiquitinates the COMPASS subunit Swd2. I showed that

H2BK123 ubiquitination also signals changes in methylation of at least one non-histone

protein, Dam1K233, providing a mechanisms for connecting changes in chromatin

structures to cellular processes independent of gene transcription.
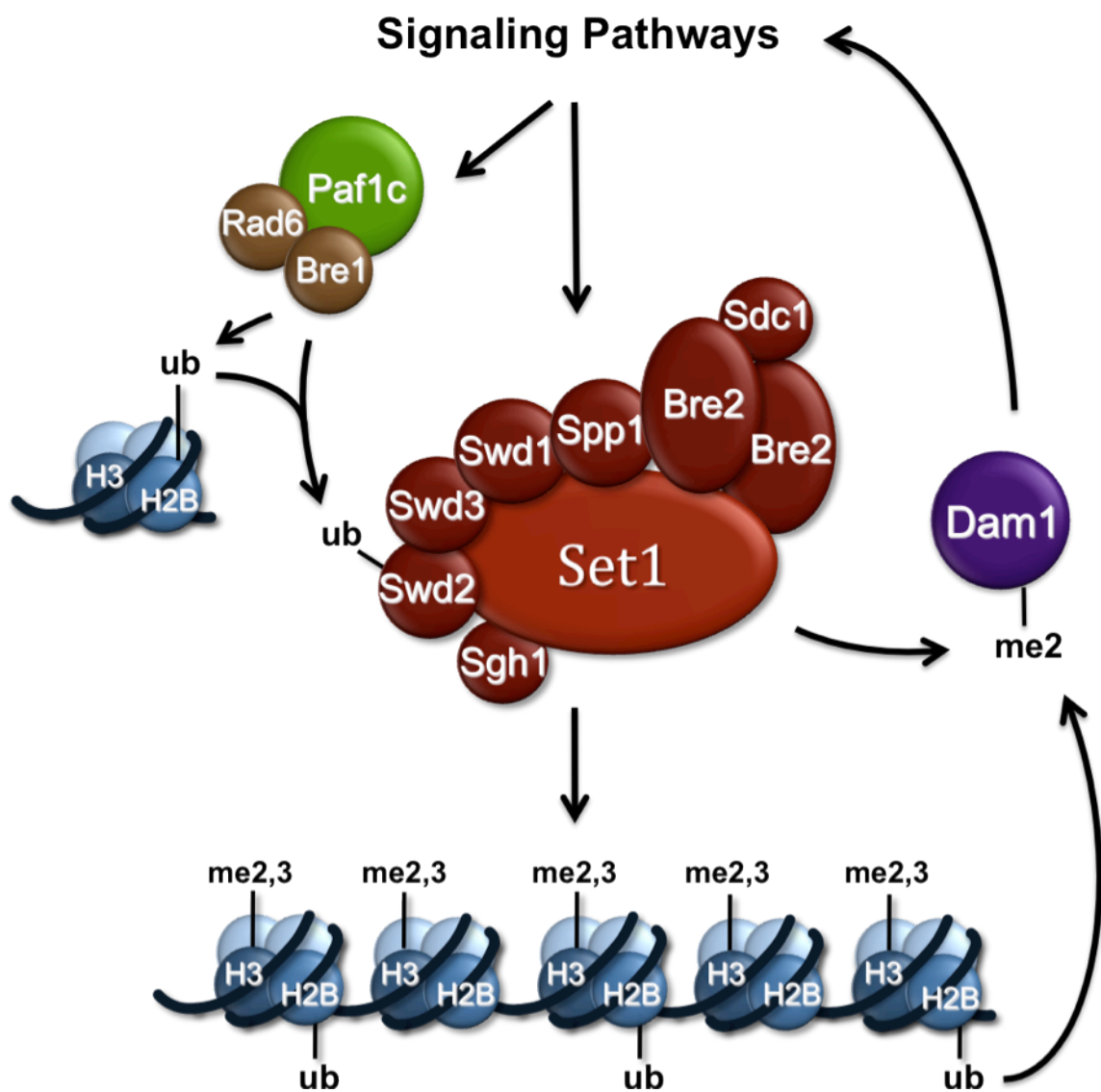
Figure 31

not lead to an increase in Dam1 methylation. While Ubp10 also regulates H2BK123 ubiquitination, this does not occur at active gene promoters. Instead, Ubp10 associates with Sir2 to maintain silenced chromatin at telomeres (Emre et al., 2005; Gardner et al., 2005). This suggests there is a specific pathway through H2BK123 deubiquitination by Ubp8 regulates Dam1 methylation.

I investigated the roles of Rpt4 and Rpt6 in H3K4 and Dam1K233 dimethylation. Also called *SUG2* and *SUG1* respectively, Rpt4 and Rpt6 are ATPases that associate with the 19S proteosome (Ferdous et al., 2001). These two proteosomal subunits are required for efficient transcriptional elongation by RNA polymerase II (Ferdous et al., 2001). In addition, mutation of these essential proteins results in decreased H3K4 di- and trimethylation, but has no effect on H2BK123 ubiquitination levels (Ezhkova and Tansey, 2004). When I tested the role of Rpt4 in H3K4 dimethylation, I determined there was no difference between wild-type cells and the *rpt4* mutants. Thinking that there was something amiss with these strains, I received new *rpt4* mutants from the Tansey lab and tested for H3K4 dimethylation. Again, I saw no difference in H3K4 methylation in the *rpt4* mutants compared to wild-type. This suggests that Rpt4 and Rpt6 do not in fact regulate H3K4 dimethylation and are unlikely to regulate Dam1K233 dimethylation.

Certain members of the H3K4 methylation regulatory pathway specifically regulate H3K4 trimethylation. Since they do not regulate H3K4 dimethylation, I have not investigated their roles in Dam1K233 dimethylation. The Bur1 and Bur2 cyclin dependent kinases phosphorylate Rad6 on S120. While phosphorylation impairs the

catalytic activity of Rad6 to ubiquitinate H2B, Bur1 and Bur2 only affect H3K4

trimethylation. The COMPASS subunit Swd2 is ubiquitinated by Rad6 and Bre1.

Similarly, Swd2 ubiquitination only affects H3K4 trimethylation and has no effect on

H3K4 dimethylation. In addition, members of the CCR4/NOT mRNA processing

complex are also required specifically for H3K4 trimethylation. The role of all these

factors in specifically regulating H3K4 trimethylation may reflect the need to fine tune

the processivity of Set1-methylation between the promoters where H3K4 trimethylation

peaks and the gene body where H3K4 dimethylation is predominant. Since these factors

are specific in regulating H3K4 trimethylation, it is unlikely that they regulate

Dam1K233 dimethylation. To date, we have been unsuccessful in raising an antibody

specific to Dam1K233me3 to test whether these factors regulate Dam1K233

trimethylation or if Dam1 is in fact trimethylated at K233. This remains an open question

that we would like to address in future studies.

Are there factors that specifically regulate Dam1K233 methylation?

Since I have shown there is a substrate-independent pathway for Set1-mediated

methylation, this raises the question of how substrate specificity of Set1 is achieved. This

might be as simple as which substrate is available for methylation by Set1. It may be that

Set1 is recruited to both active promoters and centromeres. H3 is present only at

promoters and not centromeric DNA where H3 is replaced by the centromere specific

histone variant Cse4 (Meluh et al., 1998). Dam1 localizes to centromeres by chromatin

immunoprecipitation (Jones et al., 2001). Therefore, at centromeres Dam1 is the only

substrate available for methylation by Set1. My results showing that Dam1 does not

physically interact with H3 but does interact with Cse4 support the idea that Dam1 is the primary substrate available for Set1 at centromeres.

Alternatively, substrate selection between H3 and Dam1 may be achieved by protein regulators of Dam1K233 methylation that do not regulate H3K4 methylation. To identify factors that regulate H3K4 methylation, a proteomic screen was employed using the yeast non-essential deletion library (Dover et al., 2002). Each deletion in the library was tested by immunoblotting to determine whether deletion of that particular gene affected the levels of H3K4 methylation (Dover et al., 2002). Unfortunately, a proteomic approach such as this will not be possible to identify novel regulators of Dam1 methylation. Dam1 methylation is not detectable from whole cell extracts due to the low abundance of Dam1 within the cell. Rather, Dam1 must first be immunoprecipitated for Dam1 methylation to be detected making screening of the ≈4800 gene deletion library a daunting prospect.

Another possibility to address this question is to employ a genetic screen for additional Dam1K233me2 regulatory factors. My data show a tight link between suppression of *ipl1-2* and a requirement for Dam1K233 dimethylation. Therefore, a potential approach to identify novel Dam1K233me2 regulators would be to cross the yeast deletion library into strains harboring the *ipl1-2* mutation. However, this approach is also infeasible. Most genetic screens of this nature screen for lethality (Boone et al., 2007). Even the more sensitive high throughput screens such as a synthetic genetic array analysis (SGA) still rely on synthetic sick lethality (SSL) (Tong et al., 2001). In our genetic assay, deletion of genes essential for Dam1K233 methylation suppressed the *ipl1-2* temperature

sensitivity and did not rescue the temperature sensitive phenotype. Therefore, all screening would have to be done at the non-restrictive and restrictive temperature using serial dilutions of the yeast. Unless a robotic system could be adapted for this type of screen, it would be an extremely laborious project to undertake.

However, a different type of genetic screen may be suitable for identifying novel factors required for Dam1K233 methylation should proof of principle experiments determine it to be a viable method. The balance of phosphorylation and methylation in Dam1 is highly regulated by Set1, Ipl1, and the Glc7 phosphatase (Zhang et al., 2005). The *ipl1-2* mutant has a point mutation in its catalytic domain resulting in decreased kinase function (Francisco et al., 1994). One mechanism for the temperature sensitivity of *ipl1-2* is through diminished phosphorylation of Dam1. However, deletion of *SET1* allows *ipl1-2* to better phosphorylate Dam1 resulting in suppression of *ipl1-2* temperature sensitivity (Zhang et al., 2005). In addition, combination of the *glc7-127* mutation with *set1Δ*, which would likely result in unimpeded phosphorylation of Dam1 by Ipl1, is inviable (Zhang et al., 2005). However, the *glc7-127 ipl1-2 set1Δ* triple mutant is viable and actually less temperature sensitive than the *ipl1-2* mutant alone suggesting that restoring Dam1 phosphorylation to appropriate levels is important (Zhang et al., 2005). Therefore, one might predict that overexpression of *SET1* would compromise the ability of *ipl1-2* to phosphorylate Dam1 in the double mutant cells even more severely than already is the case in the *ipl1-2* mutant alone. Given that disrupting the balance of Dam1 methylation and phosphorylation in all other mutants tested previously resulted in observable phenotypic changes (Zhang et al., 2005), I expect overexpression of *SET1* would enhance

the temperature sensitivity of *ipl1-2*. This would lead to lethality of the double mutant at

a lower temperature than the *ipl1-2* mutant alone or perhaps even inviability. In addition,

overexpression of other Dam1 methylation regulators such as COMPASS complex

members, *BRE1*, or *RTF1* would be tested for enhancement of *ipl1-2* temperature

sensitivity. If the phenotype is severe enough, an overexpression screen could be

performed in which a library of overexpressed genes is crossed into the *ipl1-2*

background to identify genes that enhance the *ipl1-2* temperature sensitive phenotype.

Currently, an overexpression library is available that covers approximately 97% of the

yeast genome making this a feasible genetic strategy to identify addition Dam1K233

methylation regulators (Jones et al., 2008). In addition, such an approach would be

unbiased, and would recover both essential and non-essential genes in the yeast genome.

Factors important for transcription have other functions in mitosis

Rad6 functions with other E3 ligases outside of transcription. Rad6 pairs with Ubr1 and

functions in the N-end rule pathway for protein mediated degradation (Dohmen et al.,

1991). The N-end rule pathway degrades proteins containing destabilized N-terminal

amino acids. A second E3 partner of Rad6 is Rad18 (Bailly et al., 1997). Rad6-Rad18

are involved in DNA repair through ubiquitination of PCNA (Hoege et al., 2002).

However, when I deleted either of these other E3 ligases of Rad6, neither suppressed

ipl1-2 comparable to deletion of *BRE1* suggesting that the role of Rad6 in regulating

Dam1 methylation is only through partnering with Bre1. In addition, others have shown

that deletion of *UBR1* or *RAD18* has no effect on H2BK123 ubiquitination or H3K4

methylation (Hwang et al., 2003). Bre1 is not reported to have any other function in

yeast other than partnering with Rad6 to ubiquitinate H2B. Together, my results show

for the first time functions for the Rad6-Bre1 E2-E3 complex outside of transcription.

The Paf1 complex plays a role in transcriptional elongation. Deletion of either *PAF1* or

CTR9 leads to slow growth, large cell size, and temperature sensitivity (Mueller and

Jaehning, 2002; Shi et al., 1996). However, deletion of the Paf1 complex only affects the

expression of a subset of genes (Porter et al., 2002; Shi et al., 1996). In addition, deletion

of Paf1 complex members does not affect the distribution of RNA polymerase II along

coding regions (Mueller et al., 2004). The Paf1 complex plays a posttranscriptional role

in processing RNAs in 3'-end formation (Penheiter et al., 2005; Sheldon et al., 2005).

The severity of phenotypes in Paf1 complex deletions suggests alternative roles in the

cell, perhaps Dam1 methylation. Deletion of *RTF1* results in loss of association of the

other four members of the Paf1 complex from chromatin and actively transcribing RNA

polymerase II (Mueller et al., 2004). My results that Rtf1 is required for Dam1

methylation suggest that either Paf1 association with chromatin and RNA polymerase II

is necessary for Dam1 methylation or that Rtf1 has a function independent of Paf1

complex association with chromatin in regulating Set1-mediated methylation.

Interestingly, *ctr9Δ* cells have chromosome segregation defects and undergo chromosome

loss at a 110-fold higher rate than wild-type cells (Foreman and Davis, 1996). My data

indicate that deletion of *CTR9* suppresses the temperature sensitive phenotype of *ipl1-2*

cells associated with the chromosome segregation defects (Chan and Botstein, 1993;

Francisco and Chan, 1994). The findings that Set1-regulated Ipl1 functions trump the

mitotic phenotype in *ctr9Δ* cells highlight the importance of the methylation-phosphorylation switch within Dam1 (Zhang et al., 2005).

Transcription does not likely play a role in Dam1 methylation

My finding that suppression of *ipl1-2* phenotypes correlates strongly with Dam1 methylation changes indicates that the role of Rad6, Bre1, and the Paf1 complex in regulating Ipl1 functions is not likely related to changes in the gene expression profiles of yeast bearing deletions of these genes. Additionally, others have shown that deletion of *RAD6* or mutation of H2BK123 to arginine has no effect on *SET1* expression (Sun and Allis, 2002), further indicating that these proteins influence methylation of H3K4 and Dam1K233 through regulation of Set1 localization and catalytic activity (Sun and Allis, 2002).

H2BK123 ubiquitination at gene promoter requires active transcription by RNA polymerase II at the locus, as both events are dependent on phosphorylation of serine 5 in the C-terminal domain (CTD) of RNA polymerase by the Kin28 kinase (Ng et al., 2003b; Xiao et al., 2005). Phosphorylation of RNA polymerase by Kin28 is required for progression of transcriptional initiation to elongation (Cismowski et al., 1995; Valay et al., 1995). My results demonstrate that H2BK123, but not Kin28, is required for Dam1 methylation. Together, these data suggest that Kin28 is essential for H2BK123 ubiquitination at promoters, but is not required for H2BK123 ubiquitination at centromeres. The proportion of H2B ubiquitinated is quite small and therefore it would be very difficult to detect the small amount of ubiquitinated H2B remaining at the

centromere in *kin28-ts16* cells. In addition, my data indicate that in contrast to H3K4 methylation, Dam1 methylation is not dependent on active transcription.

In addition to being required for H2BK123 ubiquitination, Kin28 and active transcription are also required for Set1 recruitment to gene promoters. However, the *kin28-ts16* allele has no effect on Dam1 methylation, indicating that Kin28 is not required for recruitment of Set1 to Dam1. Biochemical and genetic studies from a number of labs also indicate that Dam1 functions primarily at the kinetochore, and our data clearly indicate that Dam1 methylation is dependent on association of Dam1 with the kinetochore through Ndc10 and Ndc80. Therefore, it seems highly likely that Rad6, Bre1, and the Paf1 complex act independently of gene promoters to activate COMPASS for Dam1 methylation and that this event occurs at kinetochores.

Interestingly, parallels between *S. cerevisiae* CEN sequences, which nucleate kinetochores, and gene promoters were noted several years ago due to the shared functions of Cbf1 at both the Met16 promoter and centromeres (Hemmerich et al., 2000). In addition, several factors that influence CEN function also regulate transcription, including Spt4 and H2A.Z (HTZ) (Basrai et al., 1996; Crotti and Basrai, 2004; Mizuguchi et al., 2007). Given these results, it is not surprising that additional transcriptional regulatory factors such as Rad6, Bre1, and the Paf1 complex would associate with centromeres.

A recent genome-wide ChIP analysis for RNA polymerase II occupancy revealed

localization of polymerase II subunits to centromeric DNA (Steinmetz et al., 2006),

although no transcripts have been reported for CEN sequences in *S. cerevisiae* and CEN

sequences provide a strong barrier to transcription initiated from external promoters

(Doheny et al., 1993). In fact, the yeast centromeric nucleosome induces positive DNA

supercoils making transcription through this region by RNA polymerase II topologically

impossible (Furuyama and Henikoff, 2009). However, my data cannot rule out the

possibility that RNA polymerase II localization to centromeres is required for Dam1

methylation. My *kin28-ts16* results, together with the lack of transcription at the

centromeres, strongly argue that transcription is not required for Dam1 methylation.

Histone H2BK123 ubiquitination undergoes cross-talk with Dam1 methylation

The Dam1 complex bridges interactions between inner kinetochore complexes and

microtubules. While it is not typically thought to be in immediate proximity to

centromeric DNA or CEN-associated histones, Dam1 localizes to centromeric sequences

by chromatin immunoprecipitation (Jones et al., 2001). In addition, my data and previous

observations indicate that Dam1 co-purifies with a subset of histone proteins, including

H2B and the centromeric H3 variant Cse4 (Janke et al., 2002). The physical interaction

between H2B and Dam1 that is dependent on H2BK123, the lack of association of Set1

with Dam1 in the *h2bK123R* strain, and the effects of the *h2bK123R* mutation on Dam1

methylation all strongly support trans-regulation of Dam1K233me2 by H2BK123

ubiquitination. Moreover, despite extensive efforts, I have not found any evidence of

Rad6-Bre1 mediated ubiquitination of kinetochore proteins known to be in proximity to

Dam1, *in vivo* or *in vitro*, further indicating the effects of Rad6 and Bre1 on Dam1 methylation are mediated through H2B.

One question that cross-talk between H2B and Dam1 raises is a requirement for co-localization of these two proteins for Dam1 to be methylated. We currently know little about the function of Dam1 methylation or even when in the cell cycle methylation occurs which will be discussed further below. Dam1 predominately associates with kinetochore microtubules, but was observed to localize to centromeric chromatin and kinetochores even upon treatment with the microtubule disruptor nocodazole (Cheeseman et al., 2001a). I showed that Dam1 colocalizes with H2B and H2A suggesting that Dam1 associates with centromeric chromatin. Interestingly, not only do Dam1 and Cse4 interact in a yeast two-hybrid analysis, but the Dam1 complex also directly interacts with Cse4 in *in vitro* binding assays (Shang et al., 2003). My data confirm that Dam1 and Cse4 interact *in vivo*. In addition, I demonstrated that Dam1 methylation is dependent on an intact kinetochore. Together my results strongly argue that Dam1 methylation occurs at the kinetochore and centromere.

Possible roles for Dam1 methylation

Methylation of Dam1K233 inhibits phosphorylation of serines S232, S234, and S235 flanking either side of Dam1K233 (Zhang et al., 2005). These serines are phosphorylated by Ipl1 and provide a mechanism by which deletion of *SET1* suppresses the temperature sensitivity of *ipl1-2* (Zhang et al., 2005). Ipl1 phosphorylates Dam1 as part of the spindle assembly checkpoint to ensure correct bipolar attachment of the kinetochore microtubules

to the kinetochores and accurate chromosome segregation (Cheeseman et al., 2002).

Phosphorylation of Dam1 disrupts protein-protein interactions within both the Dam1

complex and between the Dam1 complex and the Ndc80 complex (Shang et al., 2003).

Deletion of *SET1* not only suppresses the *ipl1-2* temperature sensitive phenotype but also

the chromosome segregation defects observed in *ipl1-2* mutants (Zhang et al., 2005).

This suggests that Dam1K233 methylation negatively regulates Ipl1 function in the

spindle assembly checkpoint. However, it is currently unknown when during the cell

cycle Dam1 is methylated. If Dam1 is methylated prior to metaphase, then methylation

may inhibit errant Ipl1-mediated phosphorylation too early in the cell cycle. In contrast,

if methylation occurs upon onset of anaphase, it may signal an end to the spindle

assembly checkpoint and prohibit any more phosphorylation of Dam1 by Ipl1.

Determining when in the cell cycle methylation of Dam1 occurs will lend valuable

insight into the role of Dam1 methylation.

Methylated lysines within histones are binding sites for various methyl-binding effector

proteins (Taverna et al., 2007). Unlike acetylation, which changes the charge of the

lysine, methylation of lysines does not affect the positive charge of lysines. The addition

of methyl groups to a residue does little to change the size of the residue and likely does

not affect the function of the protein. Insight into the exact function of Dam1K233

methylation may come through the discovery of an effector molecule that binds to this

methylated lysine. Since methylation of H3K4 and Dam1K233 is regulated by a

conserved pathway, a protein that binds to methylated H3K4 might serve as a candidate.

Chd1 has been reported to bind to methylated H3K4 (Pray-Grant et al., 2005). In

addition, Chd1 is a member of the SAGA complex like Ubp8. Since Ubp8 already

regulates Dam1 methylation though modulation of H2BK123 ubiquitin levels, Chd1

seems a strong candidate to bind to methylated Dam1. If Chd1 were an effector molecule

for Dam1K233me2, I would expect it to suppress *ipl1-2*. However, deletion of *CHD1*

did not suppress *ipl1-2* indicating that it likely does not bind to dimethylated Dam1K233.

In addition to Chd1, several other proteins bind to methylated H3K4 in both yeast and

higher eukaryotes (Taverna et al., 2007). For example, in yeast Yng1 contains a PHD

(plant homeodomain) domain that binds to trimethylated H3K4 (Martin et al., 2006). A

member of the NuA3 histone acetyltransferase complex, binding of Yng1 promotes

acetylation of H3K14 (Taverna et al., 2006). Additionally, Set3 recruitment to

promoters, together with the histone deacetylases Hos2 and Hst1, is dependent on

dimethylation of H3K4 (Kim and Buratowski, 2009). To identify proteins that bind

methylated H3K4, an *in vitro* screen was performed to determine which PHD domain-

containing proteins bind to histone H3 peptides containing methylated K4 (Shi et al.,

2007). This screen revealed several possible targets including Yng1, Pho23, Cti6, Jhd1,

Spp1, and Set3 (Shi et al., 2007). A similar *in vitro* screen with methylated Dam1K233

peptides instead of methylated H3K4 peptides would be a strong strategy to identify

Dam1K233me2 effector proteins.

In 2004, the first lysine demethylase LSD1 was discovered (Shi et al., 2004). Since then

a number of demethylase have been discovered that remove methylation from a number

of different lysines including H3K4 (Klose and Zhang, 2007). It seems likely that

Dam1K233 is also demethylated; otherwise phosphorylation would be perpetually antagonized unless Dam1 was degraded. With the exception of LSD1, all of the demethylases characterized to date contain the JmJC demethylase domain (Anand and Marmorstein, 2007). In yeast, there are five proteins that contain the JmJC demethylase domain (Klose et al., 2006). Both Jhd1 and Rph1 demethylate H3K36me while Jhd2 demethylates H3K4 (Klose et al., 2007; Liang et al., 2007; Tsukada et al., 2006; Tu et al., 2007). All five of the JmJC containing proteins are available in the yeast deletion library. In whole cell extracts, I am unable to detect Dam1K233me2 due to the low abundance of Dam1. I reasoned that if one of these five JmJC containing proteins demethylated Dam1K233, then I may be able to detect Dam1K233me2 in whole cell extracts due to the increased levels of methylation. However, I was unable to detect Dam1K233me2 in any of the five deletion strains (data not shown). It would be interesting to delete each of these demethylases in the HA-Dam1 strain background, and then immunoprecipitate HA-Dam1 and immunoblot for Dam1K233 dimethylation to determine if methylation levels are elevated in any of deletion strains compared to wild-type.

Factors required both for H3K4me and Dam1K233me are implicated in cancer
A majority of the regulatory pathway for H3K4 and Dam1K233 methylation is disregulated in cancers. Set1 itself is an ortholog of the MLL proteins whose translocations are hallmarks of certain leukemias (Tenney and Shilatifard, 2005). In addition, another human ortholog of Set1, hSET1, is overexpressed in a variety of different cancer cell lines (Yadav et al., 2009). Rad6 overexpression leads to chemoresistance and induces tumorigenesis (Lyakhovich and Shekhar, 2004; Shekhar et

al., 2002). Bre1 has been implicated as both a tumor suppressor and an oncogene. The

Bre1 ortholog RNF20 is suggested to be a tumor suppressor mediated in part by its role in

H2BK120 ubiquitination and transcription as depletion of human Bre1 increases cells

oncogenic potential (Shema et al., 2008). However, human Bre1 also polyubiquitinates

the Ebp1 tumor suppressor leading to its degradation suggesting that an overall function

for Bre1 in cancer is not yet clear (Liu et al., 2009). Multiple members of the human

Paf1 complex are overexpressed in many cancers and implicated in tumorigenesis

(Chaudhary et al., 2007). In addition, the human Paf1 complex promotes certain

leukemias by interacting with MLL fusion proteins at HOX genes (Muntean et al., 2010).

Finally, the human ortholog of Ubp8, USP22, is part of an eleven gene signature in

malignant cancers that marks poor prognosis (Glinsky, 2006). That many orthologs of

the genes involved in the regulation of Set1 functions are also involved in tumorigenesis

emphasizes the importance of understanding their functions both in transcription and

other essential cellular processes such as chromosome segregation through regulation of

Dam1 methylation.

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Vita

John Andrew Latham was born in Madison, Wisconsin on September 16, 1978. John is the son of Kathleen Boylson Latham and Dudley Eugene Latham III and the sixth of seven children. In 1986, he moved to St. Marys, Kansas and attended St. Mary's Academy. After completing high school in 1997, he attended Kansas State University in Manhattan, Kansas majoring in Biochemistry. During college, he performed research in Dr. John Tomich's laboratory in the Department of Biochemistry and in Dr. Bruce Schultz's laboratory in the Department of Anatomy and Physiology. During his undergraduate, he received an undergraduate student cancer research award, the Rodney A. Nemechek cancer research award, and was a member of the Golden Key International Honor Society. John graduated from Kansas State University in May 2003 with a degree of Bachelor of Arts in Biochemistry. In August of 2003, he entered the University of Texas Graduate School of Biomedical Sciences in Houston, Texas where he joined the laboratory of Dr. Sharon Dent at the University of Texas M.D. Anderson Cancer Center for his dissertation research. In graduate school, he was awarded the American Legion Auxiliary Fellowship in Cancer Research. John is engaged to Kristina Ann Fox.

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