Methylation of H3 K4 and K79 is not strictly dependent on H2B K123 ubiquitylation

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Covalent modifications of histone proteins have profound consequences on chromatin structure and function. Specific modification patterns constitute a code read by effector proteins. Studies from yeast found that H3 trimethylation at K4 and K79 is dependent on ubiquitylation of H2B K123, which is termed a “trans-tail pathway.” In this study, we show that a strain unable to be ubiquitylated on H2B (K123R) is still proficient for H3 trimethylation at both K4 and K79, indicating that H3 methylation status is not solely dependent on H2B ubiquitylation. However, additional mutations in H2B result in loss of H3 methylation when combined with htb1-K123R. Consistent with this, we find that the original strain used to identify the trans-tail pathway has a genomic mutation that, when combined with H2B K123R, results in defective H3 methylation. Finally, we show that strains lacking the ubiquitin ligase Brel1 are defective for H3 methylation, suggesting that there is an additional Brel1 substrate that in combination with H2B K123 facilitates H3 methylation.

Introduction

In eukaryotes, DNA is condensed into chromatin, the basic unit of which is the nucleosome. The DNA in a nucleosome is wrapped around an octamer of histone proteins: two each of H2A, H2B, H3, and H4. All four histones are subjected to numerous covalent modifications, including acetylation, phosphorylation, mono-, di-, and trimethylation, ubiquitylation, and sumoylation. These modifications can have profound consequences on the ability of chromatin fibers to form higher order structures. In addition, modified histones can provide binding sites for proteins and thus help to recruit and/or retain proteins at particular regions of chromatin. The combination of modifications present on a histone constitutes a code that is read by cellular proteins (Strahl and Allis, 2000; Jenuwein and Allis, 2001).

Between 1 and 10% of histone H2B in budding yeast is ubiquitylated on K123 (Robzyk et al., 2000). This is catalyzed by Rad6 and Brel1, which encode an E2 and E3 ubiquitin ligase, respectively (Robzyk et al., 2000; Hwang et al., 2003; Wood et al., 2003a). Previous studies found that disruption of RAD6 or BREL1 or mutation of H2B K123 results in a loss of H3 methylation at K4 and K79 (Briggs et al., 2002; Dover et al., 2002; Ng et al., 2002; Sun and Allis, 2002), and this has been termed a “trans-tail pathway” (Briggs et al., 2002; Henry et al., 2003).

More recently, it appears that monomethylation of H3 K4 and K79 is unaffected by the absence of H2B ubiquitylation (Dehe et al., 2005). Although it is not clear whether dimethylation is dependent on H2B ubiquitylation, there is a consensus that this trans-tail pathway is important for trimethylation of these residues (Dehe et al., 2005; Laribee et al., 2005; Shahbazian et al., 2005; Shukla et al., 2006).

H3 K4 methylation is catalyzed by the Set1 methyltransferase and H3 K79 methylation by Dot1. In addition to effects on regulation of gene expression, these modifications contribute to DNA damage responses. dot1 or H3 K79E mutant strains are UV sensitive (Bostelman et al., 2007). Additionally, a dot1 mutant strain was found to be ionizing radiation sensitive in one study (Game et al., 2006) but only when combined with mutations in other methyltransferases, such as set1, in another (Wysocki et al., 2005). The dot1 and H3 K79A mutant strains were found to have a defect in DNA damage checkpoints (Giannattasio et al., 2005; Wysocki et al., 2005), and genetic analysis of the strains in response to UV irradiation suggests that there is also a contribution to survival after DNA damage beyond mediating checkpoint responses (Bostelman et al., 2007). Although the involvement of Set1-mediated methylation of H3 K4 in DNA damage responses is less well characterized, Set1

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appears to contribute to the intra-S phase DNA damage checkpoint (Wysocki et al., 2005).

Consistent with a dependence on K123 ubiquitylation, rad6 and bre1 mutant strains are hypersensitive to ionizing radiation, function on the same pathway as dot1 (Game et al., 2006), and, like htb1-K123R mutant cells, show DNA damage checkpoint defects (Giannattasio et al., 2005; Wysocki et al., 2005).

One of the most well-characterized DNA damage–dependent histone modifications is the phosphorylation of histone H2A (H2AX in higher eukaryotes) by the phosphatidylinositol kinase–like kinases (PIKKs; Mec1 and Tel1 in budding yeast; Downs et al., 2007). The phosphorylated motif is a well-defined consensus site for this family of kinases (S/TQ), and its presence in histone H2A variants is well conserved throughout eukaryotic evolution (Foster and Downs, 2005). Despite the rapid and robust phosphorylation of H2A in response to DNA damage, mutation of the site results in a very mild hypersensitivity to DNA-damaging agents (Downs et al., 2000; Redon et al., 2003). We noted the existence of an additional PIKK consensus site in the C-terminal tail of H2B and considered that it may play a redundant role in mediating cellular responses to DNA damage.

In this study, we investigate the role of the C-terminal tail of H2B in DNA damage responses. We found that the PIKK consensus site does not contribute to DNA damage responses but that other mutations in the C-terminal tail result in hypersensitivity to DNA damage. In contrast to previous studies (Briggs et al., 2002; Dover et al., 2002; Ng et al., 2002; Sun and Allis, 2002; Henry et al., 2003), we also found that a strain in which H2B cannot be ubiquitylated (htb1-K123R) was not defective in H3 trimethylation at K4 and K79, indicating that H3 methylation is not strictly dependent on H2B ubiquitylation. Interestingly, we found that if the K123R mutation was combined with either mutation of two serines in the H2B tail or the addition of an N-terminal Flag tag (htb1-K123R/S125A/S126A or Flag–htb1-K123R), the resulting strains were both hypersensitive to DNA damage and lost methylation at H3 K4 and K79. Moreover, we found that the original strain used to elucidate the trans-tail pathway has a mutation in the genome that, when combined with htb1-K123R, results in defective H3 methylation. Together, these data demonstrate that H2B ubiquitylation contributes to normal H3 methylation but is not the sole determinant of this pathway.

Consistent with previous results (Briggs et al., 2002; Dover et al., 2002; Ng et al., 2002; Sun and Allis, 2002; Henry et al., 2003), however, we found that disruption of the Bre1 ubiquitin ligase resulted in both hypersensitivity to DNA damage and loss of H3 K4 and K79 trimethylation. This suggests that Bre1 may have an additional target that is critical for H3 methylation.

Results and discussion

Characterization of H2B C-terminal tail mutations in DNA damage responses

We hypothesized that the PIKK consensus site on the C-terminal tail of histone H2B might be a target of Mec1 and Tel1 and function in the cellular response to DNA damage. To investigate this possibility, we made a truncation mutation of histone H2B in which the last six amino acid residues were lost (htb1-S125stop) to remove the TQ motif as well as the adjacent serine residues (Fig. 1 A). We found that this strain was hypersensitive to phleomycin (Fig. 1 B), which is consistent with a potential role in DNA damage responses. To examine its relationship with the H2A C-terminal tail, we made a strain in which both tails were truncated (hta1-S129stop/htb1-S125stop). This strain was more sensitive to DNA damage than strains harboring either single mutant, suggesting that the two tails play separate roles (Fig. 1 B). To determine whether the TQ motif of H2B is responsible for the phenotype observed, we generated an htb1-T128stop mutant strain. However, this strain appeared to behave similarly to wild type (Fig. 1 C). We considered that the motif may be functioning redundantly with the H2A SQ motif and therefore combined this mutation with an hta1-S129stop mutant. We found that htb1-T128stop/hta1-S129stop is no more sensitive to DNA damage than the hta1-S129stop single mutant in the presence of either methylmethanesulfonate or phleomycin (Fig. 1 C), suggesting that the H2B TQ motif is not contributing to cellular survival after exposure to DNA damage.

To identify the residues in the H2B tail that are important for DNA damage responses, we made a series of point mutants in the final six amino acid residues. Curiously, no combination of point mutations resulted in detectable hypersensitivity to DNA damage (Fig. 2 A and not depicted). H2B K123 had previously been shown to be ubiquitylated (Robzyk et al., 2000), and strains lacking enzymes that mediate this event, as well as an htb1-K123R mutant strain, had defective DNA damage responses (Giannattasio et al., 2005; Game et al., 2006). Therefore, we considered the possibility that our truncation mutation may have affected ubiquitylation of K123 and subsequent functions in DNA damage responses.

Notably, however, we did not detect any hypersensitivity of an htb1-K123R mutant strain to DNA damage (Fig. 2 A). We combined this mutation with a series of point mutants in the H2B tail and found that a triple htb1-K123R/S125A/S126A mutant strain (referred to as KSS hereafter) showed similar levels of hypersensitivity to phleomycin as the htb1-S125stop mutant strain (Fig. 2 A), suggesting that all three residues contribute to survival after exposure to DNA damage.

Next, we examined the ability of these strains to be ubiquitylated using N-terminally Flag-tagged constructs. We found that the slower migrating band corresponding to ubiquitylated H2B disappears in both the K123R and KSS mutant strains as expected. Mutation of the serines alone appears to have no effect on H2B ubiquitylation (Fig. 2 B).

Because H2B ubiquitylation had been implicated in DNA damage responses (Giannattasio et al., 2005), we compared our strains with those used in previous studies (Y133 and its parent strain Y131; Robzyk et al., 2000; Kao et al., 2004; Giannattasio et al., 2005; Shahbazian et al., 2005) and found that the htb1-K123R mutant in the Y133 strain background shows hypersensitivity to DNA damage relative to the Y131 parent strain background (Fig. 2 C). We also noted that although our htb1-K123R (K) and our htb1-S125A/S126A (SS) mutant strains grew as well as wild type (Fig. 2 F and not depicted), our KSS triple mutant displayed a slight slow growth phenotype (Fig. 2 D).
We also examined the phenotypes of our Flag-tagged constructs. Although the addition of the Flag tag to wild-type H2B did not affect the ability of the strain to survive when exposed to phleomycin, the Flag–htb1-K123R (Flag-K) mutant strain displayed a striking hypersensitivity (Fig. 2 E). Because the htb1-K123R parent strain was indistinguishable from wild type in this assay (Fig. 2 E), this suggests that the combination of the two alterations to the H2B gene results in impaired DNA damage responses. Interestingly, this is very similar to the phenotype seen when the K123R mutation is combined with the S125A/S126A mutations (KSS; Fig. 2 A), and we found that the Flag-KSS mutant strain was no more sensitive than the Flag-K mutant (Fig. 2 E). Moreover, the Flag-K mutant strain displays a similar slow growth phenotype to the KSS triple mutant (Fig. 2 F). Altogether, these data suggest that the addition of the Flag epitope to H2B may impair similar functions as the htb1-S125A/S126A mutations. These data also indicate that H2B K123 plays a role in DNA damage responses, but this contribution is only detectable when other H2B functions are impaired.

H3 trimethylation of K4 and K79 is not dependent on H2B ubiquitylation

As previously discussed, H3 trimethylation at K4 and K79 is dependent on H2B K123 ubiquitylation in strains derived from Y131 (Dehe et al., 2005; Laribee et al., 2005; Shahbazian et al., 2005; Shukla et al., 2006). We investigated these modifications in our H2B mutant strains. Strikingly, we found that M3 trimethylation of both K4 and K79 was indistinguishable from wild type in our K123R mutant strain (Fig. 3 A), indicating that ubiquitylation of H2B is not a prerequisite for these H3 methylation events. Consistent with previous studies, however, both K4 and K79 trimethylation was abolished in the htb1-K123R (Flag-K) mutant strain (Fig. 3 A), whereas no change was detected in the SS mutant strain. This is consistent with a lack of hypersensitivity to DNA damage in this strain. dot1 and set1 mutant strains were tested in parallel to demonstrate the specificity of the antibodies (Fig. 3 A).

Because we found that the addition of the Flag epitope to the N terminus of H2B had a similar effect on DNA damage hypersensitivity as the S5 mutation when combined with K123R in our strain background, this raised the possibility that H3 methylation may be affected as well. We found that Flag-HTB1 (F-WT) and htb1-K123R (K) mutant strains each have normal H3 K4 and K79 trimethylation, but this is lost in the Flag-htb1-K123R (F-K)
into the Y131-based parent strain, we found that similar to our strain background, this Y131-based htb1-K123R mutant strain was not hypersensitive to DNA damage and had normal H3 methylation (Fig. 3, C and D).

Because Y133 was created from Y131, once the H2B-encoding plasmids were replaced with our htb1-K123R construct, the resulting strains should be genetically identical. Therefore, one explanation for these results is that Y133 sustained a mutation in the genome that, when combined with the htb1-K123R mutation (regardless of which plasmid construct is used), results in the phenotypes seen here.

To test this, we performed a genetic cross between the Y131- and Y133-based strains. Tetrads were dissected, and the htb1-K123R mutant construct was shuffled into the haploid parental strain (Fig. 3 B). These data indicate that although H2B ubiquitylation alone is not required for H3 trimethylation, additional alterations to the H2B coding sequence in combination with htb1-K123R result in defective H3 trimethylation.

Next, we investigated the loss of H3 trimethylation in the Y133 (htb1-K123R) mutant strain background. We considered that perhaps differences in the H2B coding sequences present on the plasmids used in Y133 compared with FY406 accounted for the different phenotypes. We shuffled our htb1-K123R-encoding plasmid into the Y133 strain background, replacing the original htb1-K123R plasmid in this strain, and found that the resulting strain was still hypersensitive to phleomycin and still had defective H3 methylation (Fig. 3, C and D). Interestingly, however, when we shuffled our htb1-K123R mutant construct into the Y131-based parent strain, we found that similar to our strain background, this Y131-based htb1-K123R mutant strain was not hypersensitive to DNA damage and had normal H3 methylation (Fig. 3, C and D).

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daughter strains. Hypersensitivity to phleomycin and defective H3 methylation segregated 2:2 (Fig. 4 and not depicted). In contrast, spores derived from Y131 crossed with itself as a control consistently showed no hypersensitivity to phleomycin and had normal H3 methylation when carrying the htb1-K123R mutation (Fig. 4 and not depicted). These data demonstrate that the defect in H3 trimethylation in Y133 is a product of the htb1-K123R mutation in combination with a mutation in the genome.

In this study, we show that H3 can be trimethylated at K4 and K79 in strains that cannot be ubiquitylated on H2B K123. However, this is not entirely contradictory with previous studies (Briggs et al., 2002; Dover et al., 2002; Ng et al., 2002; Sun and Allis, 2002; Henry et al., 2003; Dehe et al., 2005; Laribee et al., 2005;
H3 trimethylation of K4 and K79 is dependent on the Bre1 ubiquitin ligase

Notably, multiple groups using different strain backgrounds found that strains lacking genes required for H2B ubiquitylation such as RAD6 and BRE1 are hypersensitive to DNA damage and lose H3 K4 and K79 methylation (Dover et al., 2002; Sun and Allis, 2002; Wood et al., 2003b; Laribee et al., 2005; Game et al., 2006). We created a bre1 mutant strain in W303 and found that, consistent with previous studies (Dover et al., 2002; Sun and Allis, 2002; Wood et al., 2003b; Laribee et al., 2005; Game et al., 2006), H3 K4 and K79 trimethylation is absent in this strain (Fig. 5 A).

Next, we examined whether this was related to H2B serines 125 and 126 by making a bre1/KSS mutant strain. First, we found that bre1 mutant cells are hypersensitive to phleomycin, again suggesting that the loss of H3 methylation results in hypersensitivity to DNA damage. Importantly, we also found that the bre1/KSS mutant strain is no more sensitive to phleomycin than either the KSS or the bre1 mutant strains (Fig. 5 B). This raises the possibility that Bre1, H2B K123, and S125/S126 function in the same pathway to regulate H3 methylation status.

Because trimethylation is lost in the absence of Bre1, but not in the htb1-K123R mutant strain, this suggests that Bre1 has

Figure 4. The Y133 strain carries a mutation in the genome that, in combination with htb1-K123R, results in hypersensitivity to phleomycin and loss of H3 trimethylation. (A) Serial dilutions of midlog cultures of Y131- or Y133-based strains and four independent segregants from the Y131 x Y131 cross or the Y131 x Y133 cross were plated onto media in the absence or the presence of phleomycin. All strains contain pJD173 (HFA1-htb1-K123R) as the sole source of H2B. (B) Western blot analysis of the strains in A using an antibody raised against trimethylated H3 K4 (H3K4me3), trimethylated H3 K79 (H3K79me3), or H3.

Figure 5. The E3 ubiquitin ligase Bre1 is required for H3 K4 and K79 trimethylation. (A) Western blot analysis of wild-type [WT; JDY183], bre1 (bre1Δ; JDY193), dot1 (dot1Δ; SKY2849), and set1 (set1Δ; SKY2856) mutant yeast strains using an antibody raised against either trimethylated H3 K4 (H3K4me3), trimethylated H3 K79 (H3K79me3), or H3. (B) DNA damage hypersensitivity of strains lacking BRE1 combined with mutations in H2B. The strains used were wild type [WT; JDY183], bre1 (bre1Δ; JDY193), htb1-S125A/S126A (SS; JDY185), htb1-S125A/S126A/bre1 (SS bre1Δ; JDY195), htb1-K123R (K; JDY186), htb1-K123R/bre1 (K bre1Δ), and htb1-K123R/S125A/S126A/bre1 (KSS bre1Δ; JDY197).
a contribution to the regulation of H3 methylation beyond the ubiquitylation of H2B K123. Notably, the absence of ubiquitin from a reconstituted in vitro transcription system results in the loss of H3 trimethylation of K4 (Pavri et al., 2006), suggesting that the putative second target is either another residue in the nucleosome or the methyltransferase itself. This is consistent with a recent study in which Bré1 was found to ubiquitylate the Swd2 subunit of COMPASS (complex proteins associated with Set1) to facilitate H3 K4 trimethylation (Vitaliano-Prunier et al., 2008). Additionally, H2B was recently found to be ubiquitylated at other sites, which may be important for H3 methylation (Geng and Tansey, 2008). Although the trans-tail pathway exists to regulate the methylation status of histone H3, it is clearly more complex than originally thought and requires activities beyond ubiquitylation of histone H2B at K123.

**Materials and methods**

**Strains and plasmids**

Yeast strains and plasmids used in this study are listed in Table I. F. Winston (Harvard Medical School, Boston, MA) provided the strain FY406. M. Grunstein (University of California, Los Angeles, Los Angeles, CA) provided the strains YM5037 and YM5038. M.A. Osley (University of New Mexico, Albuquerque, NM) provided the strains Y131 and Y133. S. Kron (University of Chicago, Chicago, IL) provided the strains SKY2849, SKY2856, and SKY2859.

**DNA damage sensitivity assays**

Midlog cultures were diluted to a density corresponding to an absorbance of 0.3 at 600 nm, and fivefold serial dilutions were plated onto medium containing the indicated DNA damage–inducing agents. Phleomycin was

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**Table I. *Saccharomyces cerevisiae* strains used in this study**

| Strain        | Genotype                                                   | Source                        |
|---------------|------------------------------------------------------------|-------------------------------|
| FY406         | MATa his3*Δ200 leu2*Δ1 lys2*Δ128Δ trp1*Δ63 ura3*Δ52 [hta1-htb1Δ::LEU2 (hta2-htb2Δ::TRP1, <pAB6 [URA3, HTA1-HTB1]) | Hirschhorn et al., 1995       |
| JDY183        | As FY406 except pID150 [HTA1-HTB1, HIS3] replaces pAB6   | Downs et al., 2000            |
| JDY184        | As FY406 except pID157 [HTA1-htb1-S125stop, HIS3] replaces pAB6 | This study                    |
| JDY5          | As FY406 except pID149 [hta1-S129stop-HTB1] replaces pAB6 | This study                    |
| JDY6          | As FY406 except pID167 [hta1-S129stop-htb1-S125stop] replaces pAB6 | This study                    |
| JDY7          | As FY406 except pID168 [HTA1-htb1-T128stop, HIS3] replaces pAB6 | This study                    |
| JDY8          | As FY406 except pID180 [hta1-S129stop-htb1-T128stop, HIS3] replaces pAB6 | This study                    |
| JDY185        | As FY406 except pID172 [HTA1-htb1-S125A/S126A, HIS3] replaces pAB6 | This study                    |
| JDY186        | As FY406 except pID173 [HTA1-htb1-K123R, HIS3] replaces pAB6 | This study                    |
| JDY187        | As FY406 except pID174 [HTA1-htb1-K123R-S125A/S126A, HIS3] replaces pAB6 | This study                    |
| JDY630        | As FY406 except pID183 [HTA1-Flag-HTB1, HIS3] replaces pAB6 | This study                    |
| JDY206        | As FY406 except pID287 [HTA1-Flag-htb1-S125A/S126A, HIS3] replaces pAB6 | This study                    |
| JDY251        | As FY406 except pID288 [HTA1-Flag-htb1-K123R, HIS3] replaces pAB6 | This study                    |
| JDY253        | As FY406 except pID253 [HTA1-Flag-htb1-K123R/S125A/S126A, HIS3] replaces pAB6 | This study                    |
| Y131          | MATa ura3-1 leu2-3,-12 ade2-1 trp1-1 his3-11,15 can1-100 hta1-htb1::LEU2, hta2-htb2Δ <pRS426-HTA1-HTB1[URA3] | Koo et al., 2004; Robzyk et al., 2000 |
| Y132          | MATa ura3-1 leu2-3,-12 ade2-1 trp1-1 his3-11,15 can1-100 hta1-htb1::LEU2, hta2-htb2Δ <pRS426-HTA1-htb1-K123R[URA3] | Koo et al., 2004; Robzyk et al., 2000 |
| YM5037        | As Y131 except DOT1-13Myc::KANMX                           | Shahbazian et al., 2005       |
| YM5038        | As Y133 except DOT1-13Myc::KANMX                           | Shahbazian et al., 2005       |
| JDY631        | As YM5037 except pID150 [HTA1-HTB1, HIS3]                  | This study                    |
| JDY632        | As YM5037 except pID172 [HTA1-htb1-S125A/S126A, HIS3]       | This study                    |
| JDY633        | As YM5037 except pID173 [HTA1-htb1-K123R, HIS3]            | This study                    |
| JDY634        | As YM5038 except pID173 [HTA1-htb1-K123R, HIS3]            | This study                    |
| JDY635        | As YM5037 except pID174 [HTA1-htb1-S125A/S126A, HIS3]       | This study                    |
| W303a         | Matα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535 | R. Rothstein*                |
| JDY286        | bre1Δ::KANMX in W303a                                       | This study                    |
| SKY2849       | Matα ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1 dot1Δ::TRP1 | Wysocki et al., 2005           |
| SKY2856       | Matα ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1 set1Δ::HIS3MX6 | Wysocki et al., 2005           |
| SKY2859       | Matα ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1 dot1Δ::TRP1 set1Δ::HIS3MX6 | Wysocki et al., 2005           |
| JDY193        | bre1Δ::KANMX and pID150 [HTA1-HTB1, HIS3] in FY406          | This study                    |
| JDY195        | As JDY193 except pID172 [HTA1-htb1-S125A/S126A, HIS3]       | This study                    |
| JDY196        | As JDY193 except pID173 [HTA1-htb1-K123R, HIS3]            | This study                    |
| JDY197        | As JDY193 except pID174 [HTA1-htb1-K123R/S125A/S126A]       | This study                    |
| JDY771        | Matα/Matα URA3-1/SUZ3-1 leu2-3,-112 ade2-1/ade2-1 trp1-1/trp1-1 his3-11,15 his3-11,15 can1-100 DOT1-13Myc::KANMX/URA3::KANMX hta1-htb1::LEU2/hta1-htb1::LEU2, hta2-htb2Δ/hta2-htb2Δ <pRS426-HTA1-HTB1[URA3]> | This study                    |
| JDY772        | Matα/Matα URA3-1/SUZ3-1 leu2-3,-112 ade2-1/ade2-1 trp1-1/trp1-1 his3-11,15 his3-11,15 can1-100 DOT1-13Myc::KANMX/URA3::KANMX hta1-htb1::LEU2/hta1-htb1::LEU2, hta2-htb2Δ/hta2-htb2Δ <pRS426-HTA1-HTB1-K123[URA3]> | This study                    |

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used in plates at concentrations between 0.1 and 20 μg/ml. Typically, plates with 0.5 μg/ml phleomycin are shown. The plates were incubated at 30°C for 2–3 d.

**Western blot analyses**

Midlog cultures were treated with or without 20 μg/ml phleomycin for 2 h unless otherwise indicated. Cells were prepared for analysis by methods described in methods. Plates were electrophoresed on 15% SDS-PAGE, transferred to nitrocellulose, and analyzed with the following antibodies: 1:500 anti–Flag M2 (Sigma-Aldrich), 1:1,000 anti-H3 (Abcam), 1:2,000 anti-H3K4me3 (Millipore), 1:500 anti-H3K79me3 (Abcam), and 1:2,000 anti-H2B (generating against recombinant H2B). HRP-conjugated anti–mouse (Abcam) or anti–rabbit (Abcam) secondary antibodies were used, and bands were visualized by chemiluminescence (Thermo Fisher Scientific).

**Genetic crosses**

The Y131- and Y133-based strains (YM5037 and YM5038) were transformed with pJD150 (HHT1-HTB1, HIS3) and were 5-fluoroorotic acid selected. The mating type of YM5037 was switched, and this strain was crossed with YM5037 or YM5038 to create the diploid strains JDY771 and JDY772, respectively. The strains were grown in rich media, and single colonies were screened for Ura+ His+ phenotypes, reflecting the sole presence of the URA3-based plasmid encoding wild-type H2B. These were sporulated, tetrads were dissected, and sets of four segregants were transformed with pJD173 (HHT1-H1b, K123R, HIS3) and were 5-fluoroorotic acid selected.

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