The H2BK123Rgument

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The discovery of trans-regulation of histone H3K4 methylation by ubiquitination of histone H2BK123 generated much excitement in the field of chromatin biology. Recently, the veracity of this example of cross talk between histone modifications in yeast was challenged (Foster and Downs, 2009. *J. Cell Biol.* doi:10.1083/jcb.200812088) but ultimately reconfirmed in a study in this issue (Nakanishi et al., 2009. *J. Cell Biol.* doi:10.1083/jcb.200906005).

DNA is compacted in the eukaryotic nucleus through association with histone proteins to form nucleosomes, the basic repeat units of chromatin. Histones are subject to multiple posttranslational modifications, which regulate a wide range of DNA-templated processes (Kouzarides, 2007). Posttranslational modification of histones is highly regulated by factors responsible for the addition or the removal of the modifying group. In addition, many modifications undergo cross talk in which modification of one residue affects the occurrence of modification on another residue (Latham and Dent, 2007). In most cases, such cross talk occurs between modifications on the same histone. However, it can also occur in trans, as provided by the striking example of the regulation of H3K4 (histone H3 lysine 4) di- and trimethylation by ubiquitination of histone H2B (for review see Weake and Workman, 2008).

In *Saccharomyces cerevisiae*, H2BK123 ubiquitination is catalyzed by the E2 ubiquitin–conjugating enzyme Rad6 and the E3 ubiquitin ligase Bre1 (Robzyk et al., 2000). A few years ago, multiple groups reported that deletion of either *RAD6* or *BRE1* or mutation of H2BK123 results in loss of H3K4 di- and trimethylation by the Set1 methyltransferase (Dover et al., 2002; Sun and Allis, 2002; Wood et al., 2003). Additional work revealed that H2BK123 ubiquitination is also required for di- and trimethylation of another residue in H3, K79, by another methyltransferase, Dot1 (Briggs et al., 2002; Ng et al., 2002). Methylation of H3K4 is mediated by the Set1 methyltransferase functioning within a larger complex called COMPASS, which is required for di- and trimethylation of H3K4. Subsequently, studies from several groups have revealed a complicated series of events that coordinate these modifications at gene promoters and coding regions to regulate transcription initiation and elongation (for review see Weake and Workman, 2008). This series of events appears to be conserved, as H2B ubiquitination also stimulates H3 methylation in human cells (McGinty et al., 2008; Kim et al., 2009).

Given the number of independent findings from multiple research groups that indicate that H2B ubiquitination directs H3K4 methylation, a recent study by Foster and Downs (2009) challenging this dogma was quite surprising. While investigating the role of the H2B C-terminal tail in the DNA damage response, Foster and Downs (2009) discovered that H3K4 or H3K79 trimethylation persisted in a strain engineered to contain a mutation of H2BK123 to arginine (H2BK123R). However, when the authors combined the H2BK123R mutation with mutations that converted H2BS125 and S126 (serines 125 and 126) to alanine, H3K4 and H3K79 trimethylation was lost. Mutation of the serines alone did not cause a change in H3 methylation at these sites. Similarly, the addition of an N-terminal Flag tag to H2B in combination with the K123R point mutation also caused a loss of H3K4 and K79 trimethylation, whereas the addition of a Flag tag to wild-type H2B had no effect. This finding led the authors to propose that on its own, H2BK123R is not sufficient to abolish H3 trimethylation. Rather, a second change in the H2B coding sequence is required together with the H2BK123R mutation for loss of H3K4 or H3K79 trimethylation.

Why were these results so different from those of other groups? Foster and Downs (2009) thought the answer might lie in the fact that they created their H2BK123R mutation in a different yeast strain background than that used by most other groups (Table I). Numerous papers studying the details of the H2B–H3 trans-tail pathway have used strains derived from a strain called Y131, which was used in the initial report that H2BK123 is ubiquitinated by Rad6 (Robzyk et al., 2000). In Y131, the two endogenous H2B gene copies are deleted, and wild-type H2B expressed from an episomal plasmid provides the sole source of this histone. In a derivative of this strain (called Y133), H2BK123R replaces wild-type H2B on the episome. Foster and Downs (2009) used a similar plasmid-based method to construct an H2BK123R mutant in a different starting strain called FY406 and directly compared H3 methylation in their strain to the Y133 strain (Table I). As expected from

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Table I. **Yeast strain background affects histone H3 methylation**

Strain background	Plasmid	H3K4me3 or H3K79me3
Foster and Downs, 2009		
FY406	H2BK123R	Present
FY406	H2BK123R, S125A, S126A	Absent
FY406	Flag-H2B	Present
FY406	Flag-H2BK123R	Absent
Y131	H2B[a]	Present
Y133	H2BK123R[a]	Absent
Y131	H2BK123R from FY406 strain	Present
Y133	H2BK123R from FY406 strain	Present
Nakanishi et al., 2009		
Y131	H2BK123A	Absent
FY406	H2BK123A	Absent
Y131 or FY406 (in glucose)	Flag-H2B	Present
Y131 or FY406 (in glucose)	Flag-H2BK123R	Absent
Y131 (in galactose)	Flag-H2B	Present
Y133 (in galactose)	Flag-H2BK123R	Present

H3K4me3, H3K4 trimethylation; H4K79me3, H4K79 trimethylation. Sources of the plasmids are the indicated authors unless stated otherwise.
[a]Source of the indicated plasmids is Robzyk et al. (2000).

previous studies, H3K4 or H3K79 trimethylation was abrogated in Y133, but curiously, these modifications persisted in the FY406-derived strain (Briggs et al., 2002; Dover et al., 2002; Sun and Allis, 2002).

Foster and Downs (2009) reasoned that there might be a second alteration of the H2B coding sequence together with the H2BK123R mutation in the Y131 strains that caused the defective H3 trimethylation (Table I). To test this, they performed a series of plasmid swap experiments, placing their H2BK123R plasmid into the Y131 and Y133 backgrounds. Because trimethylation was only abolished in the Y133 background and not in Y131, regardless of the H2BK123R plasmid source, the authors concluded that rather than a second change to the H2B coding region, some other, unidentified change in the Y133 genome was required together with the H2BK123R mutation to cause a loss of H3 methylation, bringing the entire idea of trans-regulation of these histone modifications into question. However, the role of ubiquitination (of something) in regulating H3K4 and H3K79 trimethylation is beyond dispute, as Foster and Downs (2009) still saw loss of trimethylation when the ubiquitin E3 ligase *BRE1* was deleted.

Given the surprising nature of these results and the importance of this question to the field, Nakanishi et al. (see p. 371 of this issue) decided to independently test the effects of H2B mutations on H3 methylation. They constructed new H2BK123 point mutations, converting the lysine to alanine, and introduced these into both the original parent Y131 strain as well as in the FY406 strain used by Foster and Downs (2009). In both cases, the H2BK123A mutation caused a complete loss of H3K4 di- and trimethylation and H3K79 trimethylation, which is contrary to the results of Foster and Downs (2009). In two additional strain backgrounds, H2BK123 lysine to arginine mutations, which mimic the mutations made by Foster and Downs (2009), also completely abolished H3K4 di- and trimethylation and H3K79 trimethylation. Nakanishi et al. (2009) confirmed that H2B was not ubiquitinated in these strains using a new antibody specific to H2BK123ub1 raised for this study. This antibody will provide a valuable reagent to the field because all previous studies of H2B ubiquitination in yeast relied on epitope-tagged versions of both H2B and ubiquitin. Interestingly, Nakanishi et al. (2009) found that the addition of an N-terminal Flag tag to H2B had no affect on H2BK123 ubiquitination or H3 methylation, which is again in contradiction to the results of Foster and Downs (2009) and argues against a role for another portion of the H2B coding sequence in addition to H2BK123 in regulating H3 methylation.

Puzzled by the difference between their results and those of Foster and Downs (2009), Nakanishi et al. (2009) went on to investigate whether there was anything unusual about the Y131 strain background. In the course of these experiments, they discovered that the Flag-H2BK123R–containing Y131 strain contained near wild-type levels of methylation of H3K4 and H3K79 when grown continuously in galactose media. Further detective work and genomic sequencing revealed an intact *HTA2-HTB2* locus under control of the bidirectional *GAL1/10* promoter, which had replaced the endogenous *HTA2-HTB2* promoter. Therefore, growth of these cells in galactose allows expression of wild-type H2B together with the plasmid-borne K123R mutant. Thus, expression and ubiquitination of wild-type H2B facilitates H3 methylation, explaining the aforementioned finding.

Nakanishi et al. (2009) show quite conclusively that mutation of H2BK123 to an unmodifiable residue results in a loss of H3K4 di- and trimethylation and H3K79 trimethylation regardless of strain background. Their discovery of the galactose-inducible copy of wild-type *HTA2-HTB2* in Y131 may explain the persistence of H3K4 trimethylation seen by Foster and Downs (2009) when the H2BK123R plasmid was swapped into that strain, although Foster and Downs (2009) make no mention of growing the strains in the course of their experiments in media containing any other sugar source than glucose. The question

remains why Foster and Downs (2009) observed trimethylation of these two H3 residues in the H2BK123R strain in the FY406 background. Others have shown that H2BK123 is required for H3K79 dimethylation using an independently derived version of H2BK123R in the same FY406 background (Ng et al., 2002). Perhaps another mutation in the genome of the FY406 isolate used by Foster and Downs (2009) suppressed the effects of the H2BK123R mutation? If so, identification of the suppressing mutation might provide an interesting addition to our understanding of the molecular steps between H2B ubiquitination and H3 methylation. Similarly, it is unclear how the addition of an N-terminal Flag tag to H2B in the Foster and Downs (2009) strain or mutation of H2B S125 and S126 to alanine combined with the H2BK123R mutation caused loss of H3K4 and H3K79 trimethylation. Additional studies are required to sort out these remaining issues.

Although the mechanism by which H2BK123 ubiquitination promotes H3 methylation is just beginning to be understood (Lee et al., 2007; Vitaliano-Prunier et al., 2008), the study performed by Nakanishi et al. (2009) again demonstrates that H2BK123 ubiquitination is indeed required for H3K4 and H3K79 di- and trimethylation. These two papers highlight the importance of using multiple independent isolates of histone mutations (or any mutations) in multiple yeast strain backgrounds to confirm linkage of engineered mutations to the resulting phenotypes. This degree of care is especially important for mutations in essential factors like histones that may promote inadvertent acquisition of second-site suppressor mutations and for strains that have passed through many laboratories over the course of many years. Finally, the unexpected finding of the galactose-inducible promoter at the *HTA2-HTB2* locus in the Y131 strain stresses the importance of clearly describing and archiving the derivation of strains.

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