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REVIEW

Conservation and divergence of the histone H2B monoubiquitination pathway from yeast to humans and plants

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Abstract **Histone ubiquitination plays a critical role in the regulation of transcription, and histone H2B monoubiquitination (H2Bub1) is mainly associated with transcriptional activation. Recent studies in yeast, humans, and *Arabidopsis* have revealed the conservation of chromatin modification via H2Bub1 during evolution. Rad6-Bre1 and their homologs are responsible for H2B monoubiquitination in diverse eukaryotic organisms, and the PAF complex is required for H2Bub1 to proceed. H2Bub1 is involved in many developmental processes in yeast, humans, and *Arabidopsis*, and it activates gene transcription by regulating the H3K4 methylation state. Notably, the level of H3K4 methylation is entirely dependent on H2Bub1 in yeast and humans, whereas the H3K4 methylation level of only a small number of genes in *Arabidopsis* is dependent on H2Bub1. In this review, we summarize the enzymes involved in H2B monoubiquitination and deubiquitination, and discuss the biologic functions of H2Bub1 in different organisms. In addition, we focus on recent advances in our understanding of the molecular mechanisms that enable H2Bub1 to perform its function.**

Keywords H2B monoubiquitination, H2B deubiquitination, H3K4 methylation, PAF complex, transcriptional requlation

Introduction

DNA is packaged in the nuclei of eukaryotic cells as chromatin, the basic unit of which is the nucleosome that is composed of 146 base pairs of DNA wrapped around a core histone octamer (containing two copies each of the histone proteins H2A, H2B, H3, and H4) (Luger et al., 1997; Kornberg and Lorch, 1999). Recent studies have shown that modifications to DNA or histones, or alterations in chromatin structure fundamentally affect gene expression. For example, covalent histone modifications, including the site-specific acetylation, methylation, phosphorylation, ubiquitination, and ADP-ribosylation of histone tails, can directly alter nucleosome or chromatin structure, or alter the accessibility of certain transcriptional regulators to DNA, thereby activating or repressing transcription (Jenuwein and Allis, 2001; Turner, 2002; Kouzarides, 2007; Li et al., 2007; Pfluger and Wagner, 2007).

Histone ubiquitination involves the attachment of a single ubiquitin moiety to the C-terminal tail of a histone protein. Similar to polyubiquitination, which targets proteins for degradation, histone monoubiquitination is catalyzed sequentially by a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), and a ubiquitin ligase (E3) (Weake and Workman, 2008). Ubiquitin is a highly conserved eukaryotic protein consisting of 76 amino acids. In the ubiquitination pathway, ubiquitin is first activated in an ATP-dependent manner by an E1, after which the ubiquitin is passed to an E2. The E2 carries the activated ubiquitin to an E3, which specifically recognizes the target protein and mediates the transfer of ubiquitin from the E2 to a lysine residue in the target. Histone ubiquitination is a reversible process; the attached ubiquitin can be removed from substrates by ubiquitin-specific proteases (UBPs in yeast).

Histone ubiquitination occurs mainly on histones H2A and H2B. H2A is the first ubiquitinated protein to be identified (Goldknopf et al., 1975); about 5%–15% of the total H2A in mammalian cells is monoubiquitinated at Lys-119 (Zhang, 2003). However, the monoubiquitinated H2A (H2Aub1) has not been detected in yeast or higher plants (Sridhar et al., 2007; Weake and Workman, 2008).

Histone H2B monoubiquitination (H2Bub1) was discovered in mouse cells about 30 years ago (West and Bonner, 1980). Although H2Bub1 is less abundant than H2Aub1 (1%–2% compared with 5%–15% for H2Aub1), it is widely

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distributed throughout eukaryotic organisms from yeast to humans and plants (Zhang, 2003; Osley, 2004, 2006; Osley et al., 2006; Weake and Workman, 2008). The ubiquitination site in *S. cerevisiae* H2B is Lys-123, which corresponds to Lys-119 in *S. pombe*, Lys-120 in humans, and Lys-146 in *Arabidopsis* (Robzyk et al., 2000; Tanny et al., 2007; Kim et al., 2005; Zhu et al., 2005b; Cao et al., 2008; Sridhar et al., 2007). In this paper, we review recent advances in our understanding of the biochemical pathways underlying H2B monoubiquitination and deubiquitination, the biologic functions of H2Bub1, and the molecular mechanisms that enable H2Bub1 to perform its functions in such eukaryotic organisms as yeast, humans, and *Arabidopsis*. Moreover, conservation and divergence of the H2Bub1 pathway among different organisms is discussed.

Biochemical pathways underlying H2B monoubiquitination and deubiquitination

As mentioned above, an enzyme cascade involving an E1, E2, and E3 catalyzes the attachment of ubiquitin to target proteins, while UBPs remove ubiquitin from substrates. The eukaryotic E1 is highly conserved; all ubiquitination processes share the same E1, while a variable E3 recruits the substrate and interacts with a specific E2 to ubiquitylate the target. Therefore, the identification of the specific E2s, E3s, and UBPs that are responsible for H2B monoubiquitination and deubiquitination will assist us in determining the biochemical pathway underlying H2Bub1.

The E2 for H2Bub1

The factors responsible for H2Bub1 were first identified in *S. cerevisiae*; Rad6 works as an E2 to target H2B for monoubiquitination *in vitro* and *in vivo* (Table 1; Robzyk et al., 2000). Global H2Bub1 is undetectable in *rad6*Δ mutants, suggesting that *RAD6* encodes the sole E2 responsible for H2B ubiquitination in budding yeast (Robzyk et al., 2000; Hwang et al., 2003; Wood et al., 2003a).

Rad6 is a highly conserved protein, with homologs in a variety of eukaryotic organisms. Three homologs of Rad6 exist in *Arabidopsis*: UBC1, UBC2, and UBC3. We found that UBC1 and UBC2 are redundantly responsible for H2Bub1 *in vivo*, while UBC3 is not involved in this process (Table 1; Cao et al., 2008). Two other studies have since demonstrated that UBC1 and UBC2 are the E2s for H2Bub1 in *Arabidopsis* (Gu et al., 2009; Xu et al., 2009).

In human cells, Zhu et al. (2005b) demonstrated that a Rad6 homolog, UbcH6, ubiquitinated H2B *in vitro*; the *in vivo* E2 was identified later by Kim et al. (2009), who found that homologs of Rad6–hRad6A and hRad6B were the functional E2s responsible for H2B monoubiquitination *in vivo*. hRad6A and hRad6B, but not UbcH6, can fully complement the function of yeast Rad6 in H2B monoubiquitination. In addition, hRad6A and hRad6B redundantly ubiquitylate H2B in human cells (Table 1; Kim et al., 2009). Interestingly, Rad6 has two functional homologs in both humans and *Arabidopsis*, and both E2s redundantly catalyze H2B ubiquitination in the two species (Cao et al., 2008; Gu et al., 2009; Kim et al., 2009; Xu et al., 2009). Evidences from different organisms suggest that Rad6 performs its function in a single-moiety manner.

Notably, the E2 activity of Rad6 is not specific for H2Bub1, but also ubiquitylates other cellular targets by interacting with different E3s, including Rad18, Ubr1, and Rad5 (Osley, 2006). Thus, apart from its function in H2B monoubiquitination, Rad6 is also involved in DNA damage repair and protein degradation (Osley, 2006).

The E3 for H2Bub1

Bre1 is the E3 that interacts with Rad6 to specifically monoubiquitylate H2B in yeast (Table 1; Hwang et al., 2003; Wood et al., 2003a). *BRE1* encodes a protein with a C3HC4 (RING) finger domain at its C terminus, which has been identified in many E3s. The deletion of *BRE1* eliminates genome-wide H2B ubiquitination in budding yeast (Hwang et al., 2003; Wood et al., 2003a).

The structure and biochemical activity of Bre1 are

Table 1 Enzymes involved in H2B monoubiquitination and deubiquitination in yeast, humans and *Arabidopsis*

	H2B ubiquitination		H2B deubiquitination
	E2	E3	
S. cerevisiae	Rad6 (Robzyk et al., 2000)	Bre1 (Hwang et al., 2003; Wood et al., 2003a)	Ubp8 (Henry et al., 2003; Daniel et al., 2004) Ubp10 (Emre et al., 2005; Gardner et al., 2005)
S. pombe	Rhp6 (Tanny et al., 2007)	Brl1, Brl2 (Tanny et al., 2007)	
human	hRad6A, hRad6B (Kim et al., 2009)	RNF20/hBre1A, RNF40/hBre1B (Zhu et al., 2005b; Kim et al., 2009)	Usp22 (Zhang et al., 2008; Zhao et al., 2008)
Arabidopsis	UBC1, UBC2 (Cao et al., 2008; Gu et al., 2009; Xu et al., 2009)	HUB1, HUB2 (Liu et al., 2007; Fleury et al., 2007; Cao et al., 2008)	SUP32/UBP26 (Sridhar et al., 2007)

conserved among eukaryotes. Two Bre1 homologs have been identified in several species: Brl1 and Brl2 in *S. pombe*, RNF20/hBre1A and RNF40/hBre1B in humans, and HUB1 and HUB2 in *Arabidopsis* (Table 1). H2Bub1 is prevented by a mutation in either homolog, suggesting that both are required for H2Bub1 to occur (Zhu et al., 2005b; Cao et al., 2008; Fleury et al., 2007; Liu et al., 2007; Tanny et al., 2007; Kim et al., 2009;). It has been reported that these homologous E3s form a complex in various organisms. For example, Zhu et al. (2005b) purified an RNF20/hBre1A and RNF40/hBre1B E3 complex from human cells with a molecular weight that was about four times that of each individual E3, whereas we found in *Arabidopsis* that HUB1 and HUB2 interacted both with each other and with themselves *in vivo*, indicating that E3 activity in this species is mediated by a hetero-tetramer composed of two copies of each E3 (Cao et al., 2008). Kim et al. (2009) confirmed that human RNF20/hBre1A forms a complex with a stoichiometric amount of RNF40/hBre1B through its N-terminal region, which is consistent with our observation in *Arabidopsis*. Interestingly, although *BRE1* encodes the only E3 capable of ubiquitylating H2B in *S. cerevisiae*, we found that Bre1 was able to self-associate *in vivo* (our unpublished data). Therefore, instead of acting in a single-moiety manner, Bre1 performs its function as part of an E3 complex composed of two or more Bre1 molecules (in *S. cerevisiae*) (our unpublished data), or two Bre1 homologs (in *S. pombe*, humans, and *Arabidopsis*) (Zhu et al., 2005b; Tanny et al., 2007; Cao et al., 2008; Kim et al., 2009).

The E3 activity of Bre1 is specific for histone H2B. Bre1 binds to the chromatin of target genes and then recruits Rad6 through a direct interaction, which facilitates the transfer of ubiquitin to H2B (Wood et al., 2003a; Kim et al., 2009).

Other factors promoting H2Bub1

The recruitment of Rad6 and Bre1 to target chromatin is not sufficient to ubiquitylate H2B; the initiation of transcription by Pol II and a number of other factors involved in transcription are also required for efficient H2B monoubiquitination (Osley, 2006; Weake and Workman, 2008; Kim et al., 2009). The most important regulatory factor among various species, including yeast, humans, and *Arabidopsis*, in H2Bub1 formation is the PAF complex, an evolutionarily conserved complex that associates with the initiating and elongating RNA Pol II during transcription. A direct interaction between the PAF complex and Rad6-Bre1 has been detected (Xiao et al., 2005; Kim et al., 2009). In yeast, humans, and *Arabidopsis*, the deletion or knockdown of PAF components markedly reduces global H2Bub1 (Ng et al., 2003; Wood et al., 2003b; Zhu et al., 2005a; Pavri et al., 2006; Kim et al., 2009; Schmitz et al., 2009; our unpublished data). The reason that the PAF complex is required for the ubiquitination of H2B lies in three aspects: first, the recruitment of Rad6-Bre1 to the Pol II transcription machinery depends on the PAF complex (Xiao et al., 2005; Kim et al., 2009); second, the PAF complex is essential for the catalytic activity of Rad6-Bre1 in H2B monoubiquitination (Wood et al., 2003b; Xiao et al., 2005); and third, the PAF complex mediates the association of Rad6 with Pol II, and it is required for the movement of Rad6 with the elongating Pol II into the coding region of genes (Xiao et al., 2005).

Ubiquitin-specific proteases responsible for the deubiquitination of H2Bub1

Two ubiquitin-specific proteases that target monoubiquitinated H2B *in vitro* and *in vivo* have been identified in *S. cerevisiae*: Ubp8 and Ubp10 (Table 1; Henry et al., 2003; Daniel et al., 2004; Emre et al., 2005; Gardner et al., 2005; Osley, 2006; Weake and Workman, 2008). Ubp8 is a component of the SAGA complex (Spt-Ada-Gcn5 acetyltransferase), and Ubp8 activity is dependent on the integrity of SAGA (Henry et al., 2003; Daniel et al., 2004; Ingvarsdottir et al., 2005; Lee et al., 2005). Ubp8 specifically deubiquitylates monoubiquitinated H2B in transcriptionally active genes, the deletion of Ubp8 causes increased H2Bub1 in yeast (Henry et al., 2003; Daniel et al., 2004). The deletion of *UBP10* also increases H2Bub1; however, it functions independently of SAGA (Emre et al., 2005). Moreover, Ubp10 primarily targets monoubiquitinated H2B in transcriptionally silent regions, but deubiquitylates monoubiquitinated H2B in euchromatin as well (Gardner et al., 2005). USP22, a human homolog of Ubp8, is a subunit of the human SAGA complex, which deubiquitylates H2B (Table 1; Zhang et al., 2008; Zhao et al., 2008). In *Arabidopsis*, recent data indicate that the ubiquitin protease SUP32/UBP26 deubiquitylates H2B, and that H2Bub1 is increased in *sup32* mutant plants (Table 1; Sridhar et al., 2007).

The biologic functions of H2Bub1

H2B monoubiquitination generally promotes transcription in various organisms, while the abolishment of H2Bub1 represses target gene expression (Kao et al., 2004; Cao et al., 2008; Minsky et al., 2008). Thus, H2Bub1 participates in many diverse developmental processes in different species by regulating divergent target genes.

H2B monoubiquitination is associated primarily with transcriptionally active chromatin, and H2Bub1 stimulates transcription elongation in yeast (Osley, 2006; Osley et al., 2006; Weake and Workman, 2008). In *S. cerevisiae*, H2Bub1 functions in cell size control (Hwang et al., 2003). Cells in which global H2Bub1 is abolished (e.g., *htb-K123*R, *rad6*Δ, and *bre1*Δ) display a large-cell phenotype, grow poorly on media containing raffinose or galactose, and exhibit heightened sensitivity to 6-azauracil (Henry et al., 2003; Hwang et

al., 2003; Kao et al., 2004; Xiao et al., 2005). A mutant strain of *S. pombe* lacking H2Bub1 grows slowly at 30°C, suggesting that H2Bub1 is required for normal growth in that species (Tanny et al., 2007).

Similar to yeast, monoubiquitinated H2B associates preferentially with the transcribed regions of highly expressed genes, and it is linked with global transcriptional elongation in human cells, while only a subset of genes are transcriptionally affected by the abrogation of H2Bub1 (Minsky et al., 2008; Shema et al., 2008). In humans, H2Bub1 specifically activates the developmentally important *HOX* genes (Zhu et al., 2005b). A recent study showed that H2Bub1 may contribute to cancer development, as the depletion of RNF20/hBre1A increases cell migration and promotes transformation and tumorigenesis (Espinosa, 2008; Shema et al., 2008).

H2Bub1 is involved in multiple developmental processes in *Arabidopsis*. We found that plants with impaired or no H2Bub1 (e.g., HUB1 or HUB2 deletion, UBC1 and UBC2 double mutation, and H2BK146A overexpression in which ubiquitin is unable to attach to the mutated H2B) expressed lower levels of *FLC*/*MAF4*,*5* (central repressors of the floral transition), resulting in an early-flowering phenotype (Cao et al., 2008). Gu et al. (2009) and Xu et al. (2009) also demonstrated that the abolishment of E2 activity promoted early flowering. Schmitz et al. (2009) revealed that an increase in H2Bub1 downregulated the expression of *FLC*/*MAF2*,*3* and accelerated flowering time. Therefore, both H2B ubiquitination and deubiquitination are required for the transcriptional activation of *FLC*/*MAFs*, and H2Bub1 is involved in flowering time control in *Arabidopsis* (Cao et al., 2008; Gu et al., 2009; Schmitz et al., 2009; Xu et al., 2009).

In addition, deletion of the E3s responsible for H2B monoubiquitination (HUB1 and HUB2) leads to reduced seed dormancy, pale-colored leaves, a modified leaf shape, reduced primary root growth, and a bushy appearance, suggesting that H2Bub1 is involved in seed dormancy, cell cycle regulation, and early leaf and root development (Fleury et al., 2007; Liu et al., 2007). A recent study showed that H2Bub1 regulates plant defenses against necrotrophic fungal pathogens via the interaction of HUB1 with a subunit of the mediator MED21 (Dhawan et al., 2009). Moreover, the loss of the H2B deubiquitinase SUP32/UBP26 leads to decreased H3K9me2, suppressed siRNA-directed DNA methylation, and increased numbers of aborted seeds (Sridhar et al., 2007; Schmitz et al., 2009). These results suggest that H2Bub1 is involved in multiple developmental processes and responses to biotic stress.

The mechanisms that enable H2Bub1 to perform its functions

H2Bub1 is closely connected to gene activation, as it promotes transcription elongation in most eukaryotes, including yeast, humans, and *Arabidopsis* (Kao et al., 2004; Cao et al., 2008; Minsky et al., 2008; Shema et al., 2008). The H2Bub1 state is dynamic during transcription, and H2B deubiquitination is also required to activate transcription (Henry et al., 2003; Wyce et al., 2007). In yeast, the efficient initiation of transcription requires sequential ubiquitination and deubiquitination (Wyce et al., 2004; Osley, 2006; Osley et al., 2006; Weake and Workman, 2008).

H2Bub1 is required for H3K4 methylation

H3K4 methylation is a well-known marker of active transcription. In yeast, a lack of H2Bub1 prevents the di- and tri-methylation of H3K4 and H3K79, which is catalyzed by the Set1-containing COMPASS and Dot1 methyltransferases, respectively (Briggs et al., 2002; Dover et al., 2002; Sun and Allis, 2002; Lee et al., 2007; Nakanishi et al., 2009), suggesting that the methylation of H3K4 and H3K79 depends on H2Bub1, and that the regulation of gene expression by H2Bub1 is coupled with H3 methylation. A regulator of the H2Bub1-PAF complex is required for H3K4 and H3K79 methylation in yeast, and the deletion of PAF components results in undetectable levels of H3K4 and H3K79 methylation (Krogan et al., 2003; Wood et al., 2003b).

The molecular mechanism through which H2Bub1 regulates COMPASS activity was recently uncovered: H2Bub1 controls the binding of the Cps35 subunit of COMPASS to the target, which is essential for the catalytic activity of COMPASS *in vivo* (Lee et al., 2007). COMPASS associates with RNA Pol II through an interaction with the PAF complex. Without H2B monoubiquitination, COMPASS lacks the Cps35 subunit necessary for its activity, and can only mono-methylate H3K4. Rad6-Bre1 mediated H2Bub1 recruits Cps35, which activates COMPASS for the di- and tri-methylation of Lys-4 on histone H3, thereby promoting transcription elongation and gene expression (Fig. 1A; Weake and Workman, 2008). Other factors, including the 19S proteasome and Ccr4-Not mRNA processing complex, also contribute to the regulation of H3K4 methylation by H2Bub1 (Ezhkova and Tansey, 2004; Laribee et al., 2007b).

H2B monoubiquitination directly stimulates H3K4 methylation in human cells (Kim et al., 2005, 2009; Zhu et al., 2005b). Impaired H2Bub1 decreases the global levels of H3K4me2, H3K4me3, and H3K79me2 (Kim et al., 2005, 2009), while the accumulation of H2Bub1 elevates global H3K4me3 and H3K79me2 levels (Zhu et al., 2005b). The knockdown of human PAF components leads to reduced levels of H3K4me1, H3K4me3, and H3K79me2, indicating a conserved transcriptional regulatory cascade involving the PAF complex, H2Bub1, and H3K4 and H3K79 methylation in yeast and humans (Fig. 1B; Zhu et al., 2005a, 2005b).

In *Arabidopsis*, we found that a lack of H2B ubiquitination did not affect global H3K4 mono-, di-, and tri-methylation; H3K36 di-methylation; or H3K9 di-methylation (Cao et al.,

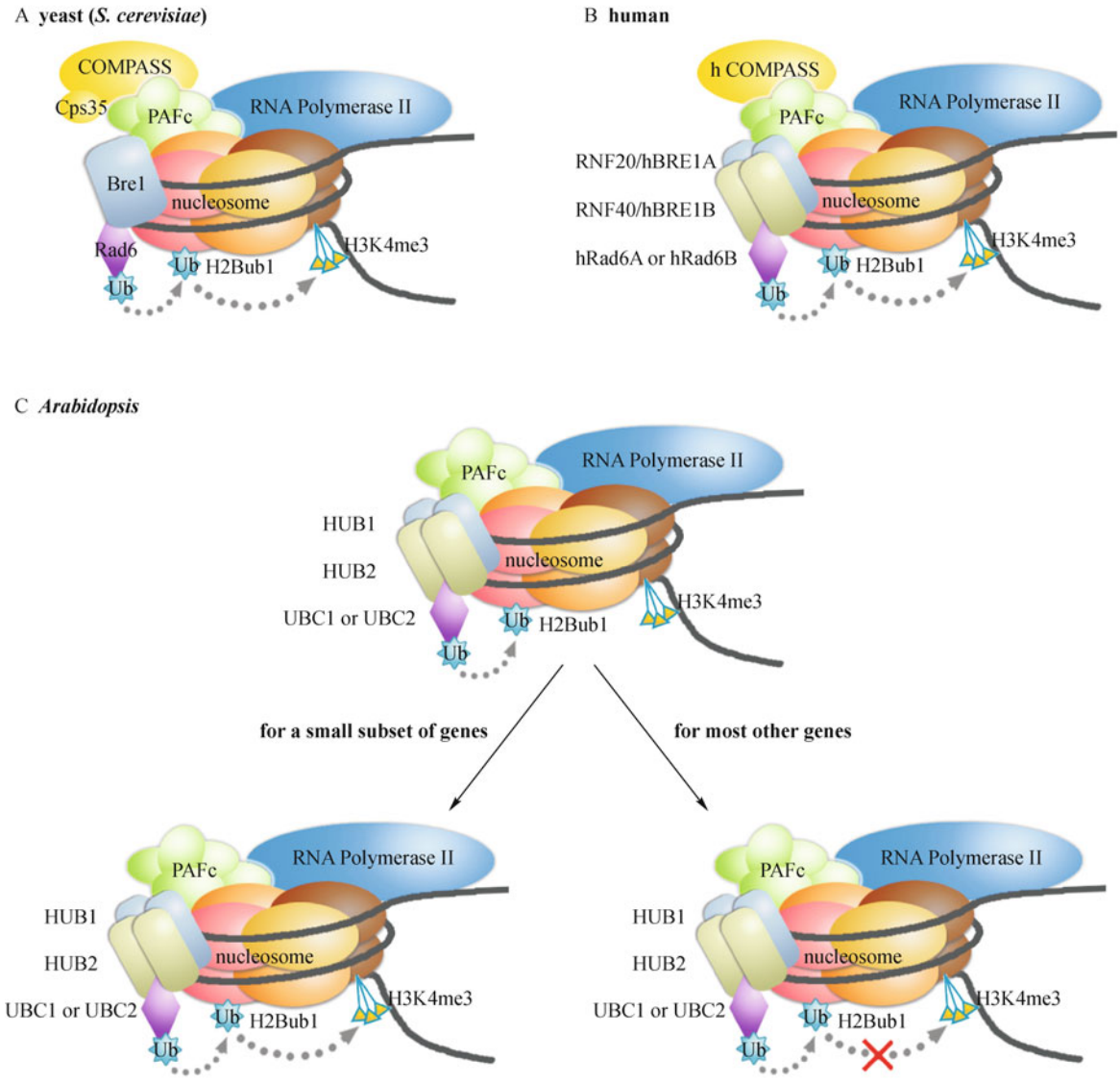

Figure 1 H2Bub1 regulates H3K4 methylation in yeast, humans, and *Arabidopsis*. The production of H2Bub1 is conserved among yeast, humans, and *Arabidopsis*. Rad6-Bre1 and their homologs are responsible for H2B monoubiquitination; and the PAF complex is required for H2Bub1 formation. H2Bub1 activates gene expression by promoting H3K4 methylation in different organisms, but the dependency of H3K4 methylation on H2Bub1 is divergent. (A) Yeast H2Bub1 controls the binding of Cps35 (a subunit of the H3K4 methyltransferase COMPASS) to the target chromatin, which is essential for the catalytic activity of COMPASS; thus, H2B monoubiquitination is required for H3K4 methylation in yeast. (B) Human H2Bub1 is also necessary for H3K4 methylation, although the regulatory mechanism is unclear. (C) In *Arabidopsis*, H2B monoubiquitination is required for H3K4 tri-methylation for only a small subset of genes (e.g., *FLC*, *MAF4*, and *MAF5*).

2008). Similarly, Gu et al. (2009) and Dhawan et al. (2009) found that global H3K4 di- and tri-methylation levels were unchanged by a lack of H2Bub1. In addition, elevated H2Bub1 was found not to affect global H3K4 tri-methylation or H3K36 mono-, di-, and tri-methylation (Schmitz et al., 2009), suggesting that the methylation of histone H3 for most *Arabidopsis* genes is not dependent on H2Bub1. Notably, although *Arabidopsis* PAF1c is essential for H2B mono-ubiquitination, it has no effect on global H3 methylation, as genome-wide levels of H3K4 di- and tri-methylation, H3K36 di-methylation, and H3K79 di-methylation were unchanged when PAF1c was mutated (Oh et al., 2004). Evidence from various species suggests that the biochemical pathway underlying H2Bub1 and the dependency of H2B monoubiquitination on the PAF complex are conserved among yeast, humans, and *Arabidopsis*, whereas the dependency of H3 methylation on H2Bub1 is not (global H3K4 and H3K79 methylation is totally dependent on

H2Bub1 in yeast and humans, but not in *Arabidopsis*).

Although H2Bub1 does not regulate global H3K4 methylation in *Arabidopsis*, we discovered that in *Arabidopsis* H2Bub1 activates gene expression through H3K4 tri-methylation in a gene-specific manner (Cao et al., 2008). Our finding indicates that the deletion of H2Bub1 in *FLC*/*MAF4*,*5* chromatin downregulates H3K4 tri-methylation and H3K36 di-methylation, resulting in repressed expression of those genes (Fig. 1C, Cao et al., 2008). Gu et al. (2009) also reported that H2B monoubiquitination in *FLC*/*MAF4* chromatin is required for H3K4 tri-methylation and expression of those genes. In addition, the elevated H2Bub1 in *FLC* chromatin downregulates H3K36 tri-methylation and upregulates H3K27 tri-methylation, thereby repressing *FLC* expression (Schmitz et al., 2009). These data suggest that H2Bub1 affects gene expression by regulating H3 methylation in *Arabidopsis*, although the regulatory effect of H2Bub1 on H3 methylation is much lower than that in yeast and humans; moreover, H2B monoubiquitination and deubiquitination in *FLC* chromatin are required for its expression.

We also found that in the chromatin of certain genes (e.g., *MAF2*, *MAF3*, and *ACTIN*), H3K4 tri-methylation and gene expression were unchanged even when H2Bub1 was deleted (Cao et al., 2008). Given that no global change in the level of H3 methylation was detected after H2Bub1 depletion in *Arabidopsis*, the abolishment of H2Bub1 does not affect H3K4 methylation in most loci marked by H2Bub1 (Fig. 1C, Cao et al., 2008). The mechanism underlying the coupling and uncoupling of H2Bub1 to H3 methylation and transcription is unclear; however, the dynamics of H2Bub1 may help explain the connection. We found that the H2Bub1 level in *FLC*/*MAF4*,*5* chromatin (which assists in the regulation of H3 methylation, and, therefore, in gene expression activation) was very low and in a much dynamic state, whereas H2Bub1 in *MAF2*, *MAF3*, and *ACTIN* chromatin (which uncouples H3 methylation from gene expression) was higher and more stable. The dynamic character of H2Bub1 may be essential for the hyper-methylation of H3K4 in the activation of gene transcription, while stable H2Bub1 is not (Cao et al., 2008). Consistent with this observation, it has been reported that H2B monoubiquitination is highly transient in yeast and humans, and that a dynamic H2Bub1 status is important for transcriptional regulation (Henry et al., 2003; Minsky et al., 2008).

H2Bub1 directly promotes Pol II elongation

Besides being coupled to H3 methylation, H2Bub1 is directly required for transcription elongation by RNA Pol II (Pavri et al., 2006; Laribee et al., 2007a; Shukla and Bhaumik, 2007; Tanny et al., 2007). Yeast H2Bub1 enhances the recruitment of elongating RNA Pol II to the coding region of genes, and it promotes efficient RNA Pol II elongation through chromatin, independent of H3K4 methylation (Laribee et al., 2007a; Shukla and Bhaumik, 2007; Tanny et al., 2007). Recent studies have suggested that H2Bub1 acts synergistically with the histone chaperone FACT to directly stimulate transcription elongation (Pavri et al., 2006; Fleming et al., 2008; Hartzog and Quan, 2008). There is a regulatory interplay between H2Bub1 and FACT: Spt16 (a FACT subunit) promotes H2Bub1, and H2Bub1 in turn regulates the association of FACT with transcribed chromatin (Fleming et al., 2008). H2Bub1 cooperates with Spt16 to reassemble nucleosomes and restore chromatin structure during transcription elongation, thereby promoting efficient and accurate RNA Pol II function (Fleming et al., 2008). In humans, H2Bub1 also cooperates with FACT to directly stimulate transcription elongation (Pavri et al., 2006). Recent work in *Arabidopsis* indicates that FACT interacts genetically with HUB1/2 to regulate multiple developmental processes (Lolas et al., 2010), suggesting that there is a similar regulatory mechanism between H2Bub1 and FACT in transcription elongation in *Arabidopsis*.

H2Bub1 blocks the recruitment of Ctk1

Yeast Ctk1 mediates phosphorylation of the RNA Pol II C-terminal domain (CTD) on Ser-2, which plays an important role in the later stages of transcriptional elongation. Rad6-Bre1 interacts with the CTD of RNA Pol II via the PAF complex. Wyce et al. (2007) showed that H2B monoubiquitination blocked the association of Ctk1 with actively transcribed genes, while the deubiquitination of H2Bub1 by Ubp8 was required for the recruitment of Ctk1, subsequent phosphorylation of the CTD at Ser-2, and recruitment of the Set2 methyltransferase and certain RNA 3′-end processing factors, resulting in H3K36 methylation and several RNA processing events. In humans, the RNA Pol II Ser-2-specific kinase CDK9 is essential for H2B monoubiquitination (Pirngruber et al., 2009). Furthermore, CDK9 and H2Bub1 control replication-dependent histone mRNA 3′-end processing, indicating the existence of a different regulatory mechanism in humans (Pirngruber et al., 2009).

H2Bub1 regulates nucleosome stability

Recently, Chandrasekharan et al. (2009, 2010a) demonstrated that instead of opening up chromatin, H2B monoubiquitination was important for nucleosome stability. The replacement of ubiquitin with a bulkier SUMO molecule resulted in a lack of H2Bub1 function, suggesting that ubiquitin does not serve as a "wedge" to loosen nucleosomes for factor access. Instead, H2Bub1 stabilized nucleosomes by preventing H2A-H2B eviction. Moreover, Chandrasekharan et al. (2010b) demonstrated that in the presence of H2Bub1, nucleosomes retained a "docking site" for the binding of Set1-COMPASS with the chromatin.

Conclusions

H2Bub1 is a transient and co-transcriptional process that has been detected in a number of eukaryotes, including yeast, humans, and *Arabidopsis*. The production of H2Bub1 is highly conserved among these species as described below. First, not only are the E2 and E3 responsible for H2Bub1 conserved (Rad6-Bre1 and their homologs), but the way they perform their functions is similar among divergent species (Robzyk et al., 2000; Hwang et al., 2003; Wood et al., 2003a; Zhu et al., 2005b; Cao et al., 2008; Kim et al., 2009). For example, *RAD6*, which encodes the sole E2 and functions in a single-moiety manner in *S. cerevisiae* (Robzyk et al., 2000), has two functional homologs in humans and *Arabidopsis*, which redundantly ubiquitylate H2B in a single-moiety manner (Cao et al., 2008; Gu et al., 2009; Kim et al., 2009; Xu et al., 2009), whereas Bre1 functions as part of an E3 complex in *S. cerevisiae* (our unpublished data), while the two Bre1 homologs in humans and *Arabidopsis* are both required for H2B monoubiquitination as part of a hetero-tetrameric E3 (Zhu et al., 2005b; Cao et al., 2008; Kim et al., 2009). Moreover, the E2 physically interacts with the E3 in all three species (Wood et al., 2003a; Cao et al., 2008; Kim et al., 2009). Second, the specific site in H2B to which ubiquitin is attached is conserved (the "K" in the H2B C-terminal sequence "AVT**K**FTSS") (Robzyk et al., 2000; Zhu et al., 2005b; Sridhar et al., 2007; Tanny et al., 2007; Cao et al., 2008). Third, Rad6-Bre1 (or their functional homologs) controls genome-wide H2B ubiquitination, but not in a gene-specific manner. In addition, H2Bub1 is associated with the coding region of genes, which is consistent with its function in transcriptional elongation (Xiao et al., 2005; Cao et al., 2008; Minsky et al., 2008). Fourth, H2B monoubiquitination is highly connected to gene activation, whereas the deubiquitination of monoubiquitinated H2B leads to the downregulation of target genes (Kao et al., 2004; Zhu et al., 2005b; Cao et al., 2008; Gu et al., 2009; Xu et al., 2009). Fifth, H2Bub1 activates gene expression by promoting H3K4 methylation (Lee et al., 2007; Cao et al., 2008; Gu et al., 2009; Kim et al., 2009). Sixth, the PAF complex is required for H2B monoubiquitination (Wood et al., 2003b; Kim et al., 2009; our unpublished data). Thus, H2B monoubiquitination mediated by Rad6-Bre1 (or their functional homologs) has been strongly conserved during evolution.

The dependency of H3K4 methylation on H2Bub1 varies in different eukaryotic organisms, suggesting that the mechanism of action for H2Bub1 is not as well conserved as its production pathway. In yeast and humans, H3K4 methylation is entirely dependent on H2Bub1 (Lee et al., 2007; Kim et al., 2009). In contrast, in *Arabidopsis*, global H3K4 methylation is unaffected by the deletion of H2Bub1; only gene-specific H3K4 methylation has been found to be downregulated in a small subset of genes (Cao et al., 2008; Gu et al., 2009; Dhawan et al., 2009), suggesting that H2Bub1 is required for H3K4 methylation only for a portion of the genes in the *Arabidopsis* genome. In *Arabidopsis*, impaired H3K4 methylation leads to a much more severe phenotype than the deletion of H2Bub1, indicating that compared to H2Bub1, H3K4 methylation plays more important roles in gene expression and plant development, which further suggests that the importance of H2Bub1 appears to have been progressively weakened, while that of H3K4 methylation has become strengthened, during plant evolution. Because of the unmovable feature, plant may use this simple regulatory mechanism to response quickly to the environmental stimulus. The molecular mechanism of gene-specific H2Bub1 in regulating H3K4 methylation in *Arabidopsis* is currently unclear.

In addition, at least 1%–2% of the H2B is monoubiquitinated in most organisms, and H2Bub1 is associated with actively transcribed genes, while the expression of only a small subset of genes is altered by H2Bub1 elimination. Thus, key questions remain to be answered: First, what is the significance of H2Bub1 in the chromatin of genes in plants in which H3K4 methylation is uncoupled from H2Bub1? Second, how the H2Bub1 pathway links to PAFc in chromatin modification and gene transcription? And third, how dose H2Bub1 cross-talk to H3 or DNA methylation?

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