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The Interplay of Histone H2B Ubiquitination with Budding and Fission Yeast Heterochromatin

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Abstract

Mono-ubiquitinated histone H2B (H2B-Ub) is important for chromatin regulation of transcription, chromatin assembly, and also influences heterochromatin. In this review, we discuss the effects of H2B-Ub from nucleosome to higher-order chromatin structure. We then assess what is currently known of the role of H2B-Ub in heterochromatic silencing in budding and fission yeasts (*S. cerevisiae* and *S. pombe*), which have distinct silencing mechanisms. In budding yeast, the SIR complex initiates heterochromatin assembly with the aid of a H2B-Ub deubiquitinase, Ubp10. In fission yeast, the RNAi-dependent pathway initiates heterochromatin in the context of low H2B-Ub. We examine how the different silencing machineries overcome the challenge of H2BUb chromatin and highlight the importance of using these microorganisms to further our understanding of H2BUb in heterochromatic silencing pathways.

Keywords

SIR complex; Ubp10; ubiquitination; silencing; heterochromatin; epigenetics

Introduction

The “Silent” histone code

Euchromatin has an open structure and contains active genes that are permissive for transcription. In contrast, heterochromatin is maintained in a compact structure to prevent transcription and silence genes. The molecular details of heterochromatic silencing have been determined using yeasts (*S. cerevisiae* and *S. pombe*) due to the streamlined versions of heterochromatin found in these organisms. The histone “code” of post-translational modifications (PTMs) on histones act as binding sites for chromatin-regulators to drive transcriptional activation or promote silencing of local chromatin (Strahl and Allis 2000; Suganuma and Workman 2011). The silent code in budding yeast requires an absence of PTMs. Marks associated with active chromatin, specifically acetylation of H4 and methylation of H3K4 and H3K79, antagonize silencing whereas the unmodified residues act

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as binding sites for the silencing machinery (Armache et al. 2011; Behrouzi et al. 2016; Hecht et al. 1995; Johnson et al. 2009; Onishi et al. 2007; Wang et al. 2013). This differs from H3K9me3 (Lys9 tri-methylation)-decorated heterochromatin found in *S. pombe* and metazoan cells.

Roles of Mono-ubiquitinated H2B

Mono-ubiquitination of chromatin involves the formation of an isopeptide bond between the ϵ -amino group of lysine on a histone (11–14 kDa) and the C-terminal glycine of ubiquitin (~8.5 kDa). In contrast, most PTMs are small chemical groups covalently bonded to histone residues. The bulky addition of mono-ubiquitin to chromatin modulates chromatin-dependent processes rather than involving the proteasomal degradation pathway as seen with poly-ubiquitinated proteins. In budding yeast, H2B is mono-ubiquitinated (H2B-Ub) at Lys123 (Lys119 in *S. pombe* and Lys120 in humans). The Rad6/Bre1 complex is the E2 ubiquitin conjugating enzyme and E3 ubiquitin ligase that catalyzes the ubiquitination of H2B (Fig. 1a) (Robzyk et al. 2000). As with most other PTMs, histone mono-ubiquitination is reversible through the actions of deubiquitinases (DUBs) (Komander et al. 2009) (Fig. 1b). In budding yeast, Ubp8 and Ubp10 are the known DUBs that regulate levels of H2B-Ub during transcription activation or heterochromatic silencing, respectively (Emre et al. 2005; Gardner et al. 2005; Henry et al. 2003; Schulze et al. 2011).

Biochemistry of H2B-Ub in chromatin structure and nucleosome dynamics

Due to ubiquitin being roughly half the size/mass as a histone protein, this modification is hypothesized to have some structural impact on chromatin, specifically that H2B-Ub chromatin promotes open chromatin. A number of biochemical studies concluded that H2B-Ub chromatin indeed resists compaction and this is primarily driven by electrostatic interactions of an acidic surface of ubiquitin between ubiquitin modifications and with histones (Debelouchina et al. 2017; Fierz et al. 2011; Machida et al. 2016). *In vivo*, lack of H2B-Ub leads to decreased nucleosome occupancy possibly due to challenges in nucleosome re-assembly and/or stability, whereas the presence of H2B-Ub stabilizes nucleosomes (Fig. 1a and 1b) (Batta et al. 2011; Chandrasekharan et al. 2009; Fierz et al. 2011; Machida et al. 2016; Segala et al. 2016). In the context of transcription and in DNA replication, the transient nature of H2B-Ub appears to facilitate these chromatin-dependent processes (Batta et al. 2011; Fleming et al. 2008; Pavri et al. 2006; Trujillo and Osley 2012; Wu et al. 2017). The ability for H2B-Ub to influence the behavior of chromatin and at the basic folding level of nucleosomes has important implications for heterochromatin establishment.

The epigenetic nature of heterochromatin requires that this structure be re-assembled after a disruptive process, such as DNA replication. Similarly, regions that transition from active to heterochromatin or vice versa must undergo chromatin re-structuring. Heterochromatic loci have low H2B-Ub levels and presumably nucleosomes are stable in a compact structure. Yet little is known about whether the transience of H2B-Ub is important during the initiation of heterochromatin establishment (Fig. 1c). In a heterochromatin re-establishment study, *htb-K123R* cells re-establish heterochromatic silencing with a quicker initial response than wild-

type (WT) cells but fail to reach complete silencing as seen in WT cells (Wu et al. 2017). It is possible that the inability to ubiquitinate H2B in the mutant cells leads to challenges in histone chaperone-mediated re-assembly and/or intrinsic stability of nucleosomes resulting in decreased nucleosome occupancy. This would negatively affect SIR complex assembly and spreading, and ultimately, silencing.

H2B-Ub is a prerequisite for anti-silencing H3 methylation

Trans-histone crosstalk

H2B-Ub participates in trans-histone crosstalk as it is required for subsequent H3 methylation at K79 and K4 (Briggs et al. 2002; Ng et al. 2003b; Shahbazian et al. 2005; Sun and Allis 2002; Vlaming et al. 2014). Methylation of H3K4 and H3K79 are strongly associated with active regions of the genome and are catalyzed by SET1 and DOT1, respectively (D'Urso and Brickner 2017; Ng et al. 2003a; Ng et al. 2003b). H3K4me and H3K79me antagonize SIR complex silencing by inhibiting full SIR complex engagement with the nucleosome (Armache et al. 2011; Behrouzi et al. 2016; Ehrentraut et al. 2011; Johnson et al. 2009; Kitada et al. 2012; Onishi et al. 2007; Santos-Rosa et al. 2004; van Leeuwen et al. 2002; Wang et al. 2013). *In vivo*, the importance of these marks is exemplified by faster heterochromatin assembly kinetics observed in *set1^Δ* and *dot1^Δ* strains, and slower assembly kinetics when the H3K4 demethylase (*jhd2^Δ*) is deleted (Katan-Khaykovich and Struhl 2005; Larin et al. 2015; Osborne et al. 2009). To date, there remains no identified demethylase of H3K79. Thus, H2B-Ub indirectly affects silencing by promoting anti-silencing H3 methylation.

Ubp10, a histone deubiquitinase, erases H2B-Ub in *S. cerevisiae* to promote heterochromatin formation

SIR complex-mediated heterochromatin establishment

Budding yeast heterochromatin is mediated by the silent information regulator (SIR) complex at the silent-mating type loci and the telomeres. The SIR complex is comprised of Sir2, Sir3, and Sir4, each with essential roles in heterochromatin assembly (Kueng et al. 2013; Oppikofer et al. 2013). Sir2, is the founding member of the Sirtuin family of histone deacetylases (HDACs), and deacetylates H4K16 in the presence of NAD. Sir3 is the main histone-binding protein and is considered the key architectural component that promotes spreading. Sir4 is a scaffolding protein that mediates interactions within the complex and with other proteins. Sir3 and Sir4 exhibit homo-dimerization, a property that facilitates SIR complex spreading.

The SIR complex challenge

The transition of an active region into a silent heterochromatic region requires the loss of histone acetylation and H3 methylation, particularly H3K79me (Miles and Breeden 2017; Oppikofer et al. 2011). This presents a challenge for the SIR complex as it only contains Sir2-dependent HDAC activity (Fig. 2a). Several possibilities would allow for SIR complex to overcome active H3 methylation marks: 1) Recruitment of demethylases, 2) Slow assembly kinetics to allow the eventual dilution of H3 methylation by cell division, or 3)

prevention of H3 methylation. It is unclear if Jhd2 can be recruited by SIR complex and there is no known H3K79 demethylase. Cells require multiple cell cycles to establish complete silencing by SIR complex, which may be one mechanism to dilute H3K79 methylation (Katan-Khaykovich and Struhl 2005; Larin et al. 2015; Osborne et al. 2009). With the identification of Ubp10, an H2B-Ub DUB implicated in silencing, the prevention of H3 methylation by disrupting the upstream requirement for H2B-Ub was suggested as an active way for SIR complex to overcome the challenge of silencing an active region (Emre et al. 2005; Gardner et al. 2005; Kahana and Gottschling 1999; Singer et al. 1998).

Ubp10 in silencing

Ubp10 plays a role in telomeric silencing by maintaining low levels of H2B-Ub. Ubp10 was originally identified as Dot4 in a telomeric silencing screen (Singer et al. 1998). The Dot1 H3K79 methyltransferase was also identified in this same screen, suggesting overlap in the activities of the two proteins. Sir4 and Ubp10 were subsequently shown to interact based on genetic and yeast two-hybrid analyses (Kahana and Gottschling 1999; Reed et al. 2015). Reciprocal deletion experiments further supported the idea of mutual recruitment between SIR complex and Ubp10 at the sub-telomeres. In addition, H2B ubiquitination appears to positively regulate H4K16 acetylation levels near the telomeres (Emre et al. 2005; Gardner et al. 2005; Rhie et al. 2013; Wan et al. 2010). Despite these studies, basic molecular questions remain, such as whether chromatin is required for the Ubp10•Sir4 interaction and how this interaction may modulate Ubp10 DUB activity. Determining how Ubp10 activity is regulated is important because Ubp10 has non-histone ubiquitinated targets, including Rpa190, a subunit of RNA polymerase I, and PCNA, the replication sliding clamp protein (Gallego-Sanchez et al. 2012; Richardson et al. 2012).

The role of Ubp10 in silencing has since been challenged due to the absence of certain changes one would predict in *ubp10*• cells at all heterochromatic regions. In the absence of Ubp10, increases in gene expression occur mostly at the sub-telomeric regions, as do modest increases in levels of H2B-Ub, and H3K4 and H3K79 methylation (Gardner et al. 2005; Orlandi et al. 2004; Rhie et al. 2013). Importantly, these changes were specific to *ubp10*• cells and not *ubp8*• cells. Yet, there were little detectable changes at the silent mating-type loci, which are regions of strong silencing due to flanking DNA silencer sequences (Fig. 2c) (Gardner et al. 2005; Orlandi et al. 2004; Schulze et al. 2011; Singer et al. 1998). It appears that regions with a strongly-reinforced heterochromatin structure maintain low H2B-Ub levels even when Ubp10 is absent, but through what mechanism?

SIR complex co-opts Ubp10 DUB activity to facilitate heterochromatin assembly

The findings from a recent study published in our lab emphasize the important role of Ubp10 in heterochromatin formation (Zukowski et al. 2017). Using a variety of biochemical techniques, our observations provide details in understanding how Ubp10 contributes to SIR complex-mediated heterochromatic silencing. Ubp10 is preferentially recruited to chromatin during assembly of the SIR complex onto chromatin rather than to an established heterochromatin structure (Fig. 2b and 2c). This provides an explanation for why most

changes in gene expression, H2B-Ub, and H3 methylation in a *ubp10*⁺ strain occur at sub-telomeric regions, which can undergo transcriptional events, as opposed to the constitutively silenced mating-type loci (Gardner et al. 2005; Orlandi et al. 2004). For example, the Pol II-dependent transcription of a non-coding RNA involved in telomere regulation, Telomeric Repeat containing RNA (TERRA), occurs at the sub-telomeres in yeast (Luke and Lingner 2009), potentially increasing the frequency of conversion of sub-telomeric chromatin from active to heterochromatic.

Interestingly, Ubp10 DUB activity is hindered when its substrate, H2B-Ub, is assembled into a nucleosome (Zukowski et al. 2017). Prevalent in DUB biology is the theme that DUBs are incorporated into larger complexes to achieve specific targeting and increased DUB activity (Lee et al. 2005; Morgan et al. 2016; Sahtoe and Sixma 2015). This is also true for Ubp10, as its DUB activity is enhanced on H2B-Ub nucleosomes when Ubp10 is in complex with Sir2/4. Our work demonstrates that even as a coupled epigenetic reader-erasure complex, the SIR complex must further recruit other erasure proteins to overcome the challenge of transitioning an active region to a silent one. The removal of H2B-Ub, the upstream prerequisite for H3 methylation, is an elegant and simple way to prevent the methylation marks. In light of this additional step in the SIR complex-heterochromatin pathway, an open and intriguing question is whether H2B-Ub directly affects SIR complex activities on chromatin. By comparison, SIR complex can bind acetylated and methylated chromatin, but cannot achieve transcriptional repression (Johnson et al. 2009; Kitada et al. 2012).

***S. pombe* heterochromatin and H2B-Ub**

Heterochromatin in *S. pombe* is found at the silent mating-type loci, the telomeres, and at the pericentromeric regions. In contrast to budding yeast, *S. pombe* can use the RNAi pathway to initiate heterochromatin (Fig. 2d), though RNAi-independent initiation also occurs. The core silencing machinery involves the RITS (RNA-induced transcriptional silencing) complex, the RDRC (RNA-directed RNA polymerase complex), the Ctr4 (a H3K9 methyltransferase) complex, SHREC (Snf2/Hdac – containing Repressor Complex), and Swi6 (a heterochromatin protein 1 homolog that binds H3K9me3) (Martienssen and Moazed 2015). The silent histone code in *S. pombe* involves H3K9me3 and deacetylation of histones by HDACs, including SHREC and Sir2. Although there is less known about H2B-Ub and its role in heterochromatin dynamics in fission yeast, there is evidence that reducing H2B-Ub is important for the transition of euchromatin into heterochromatin.

HULC, or the histone H2B ubiquitin ligase complex, regulates H2B-Ub levels, promotes H3K4 methylation, and is comprised of multiple subunits that include the Rad6 homolog, Rhp6, and the Bre1 homolog, Brl1 (Choi et al. 2002; Racine et al. 2012; Tanny et al. 2007; Zofall and Grewal 2007). Regulation of HULC subunits has important outcomes for heterochromatin formation and maintenance. Overexpression of Rhp6 results in disrupted silencing due to increases in RNAPII occupancy and decreases in H3K9me3 at centromeric repeats, whereas *rhp6*⁺ enhances silencing at regions of heterochromatin (Choi et al. 2002; Zofall and Grewal 2007). Brl1 ubiquitin ligase activity appears to be regulated by acetylation driven by Mst2, a member of the MYST family of histone acetyltransferases (Flury et al.

2017). When Brl1 cannot be acetylated (*brl1-K242R* genotype), H2B-Ub levels are reduced, and combined with *mst2^Δ*, an increase in the initiation and maintenance of silencing is observed. This phenotype is concomitant with an increase in siRNA abundance mapping to the reporter *ade6⁺* gene. In contrast, a Brl1-acetyl mimic mutant strain (*brl1-K242Q*) antagonizes the initiation of silencing.

The conserved Paf1c (RNA Polymerase II Associated Factor 1 complex) acts upstream in the H2B-Ub pathway (Fig. 2d). Paf1c has been implicated in recruiting and/or stimulating Rad6/Bre1 ubiquitin ligase activity on H2B in order to facilitate transcription elongation (Mueller and Jaehning 2002; Pavri et al. 2006; Van Oss et al. 2016; Wood et al. 2003). Similar to H2B-Ub disruption, deletions of Paf1c subunits enhance de novo silencing and its maintenance in fission yeast due to impaired transcriptional kinetics, the inability to release a nascent transcript, and transcription termination defects (Kowalik et al. 2015; Sadeghi et al. 2015; Shimada et al. 2016). Taken together, reducing levels of H2B-Ub slows transcriptional kinetics and allows the silencing machinery to target nascent transcripts. This promotes the RNAi-dependent silencing pathway to generate small interfering RNA for heterochromatin initiation.

The only DUB known to regulate H2B-Ub in *S. pombe* is Ubp8, a conserved DUB of the SAGA DUB module involved in transcription (Henry et al. 2003; Lee et al. 2005; Morgan et al. 2016). Pombe Ubp16 is proposed to be a homolog for budding yeast Ubp10, as it shares a similar ubiquitin specific protease (USP) catalytic domain and regulates ubiquitinated-PCNA during DNA replication (Alvarez et al. 2016; Kouranti et al. 2010). However, no known roles for Ubp16 in heterochromatic silencing in pombe have been determined. This may be due to the lack of H3K79 methylation, there is no known Dot1 homolog in fission yeast, and that disruption of heterochromatin by HULC is independent of H3K4 methylation (Zofall and Grewal 2007).

Conclusion

The initiation of heterochromatin establishment is promoted by different silencing machineries in budding and fission yeast, and thus, there are inherently different hurdles to overcome. In budding yeast, methylated H3 lysines act as anti-silencing marks by directly antagonizing SIR complex engagement with nucleosomes. SIR complex recruitment of Ubp10 enables the erasure of H2B-Ub, the pre-requisite mark for H3 methylation. In fission yeast, an RNAi system is integral to heterochromatin formation and promotes H3K9me3-driven assembly and maintenance of silent structures. The challenge of H2B-Ub in *S. pombe* may be overcome by preventing HULC recruitment/activation. How H2B-Ub is depleted from active regions that will undergo silencing in fission yeast requires more biochemical analyses. In both yeast systems, H2B-Ub appears to act at the crossroads of chromatin fate. Recent advances in semi-synthetic generation of mono-ubiquitinated histone substrates will allow us to further probe and characterize regulators of H2B-Ub. It is also increasingly important to reconstitute chromatin-based silencing systems *in vitro* to detail how H2B-Ub directly affects silencing machinery. Discerning the molecular mechanisms of H2B-Ub regulation in heterochromatin assembly will remain heavily dependent on using the tractable heterochromatin systems found in yeasts.

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Figure 1. The influence of H2B-Ub on chromatin structure and higher-order folding

a. Ubiquitination and deubiquitination regulates H2B-Ub on nucleosomes. (Top) Rad6/Bre1 conjugates ubiquitin onto H2B. (Bottom) *In vitro* H2B-Ub nucleosomes are stable and resist interactions within a chromatin array (sideways arrows) and between chromatin arrays (up and down arrows). H2B-Ub chromatin exhibits characteristics of an open chromatin structure. Bulk ubiquitinated chromatin in *ubp10*^Δ and *ubp8*^Δ cells resists salt-dependent solubilization and exhibits disrupted telomeric silencing. **b.** (Top) Deubiquitinases, Ubp10 or Ubp8 in budding yeast, cleave H2B-Ub. (Bottom) Lack of ubiquitination results in decreased

nucleosome occupancy on chromatin. This phenotype is observed in *htb-K123R* (budding yeast) or *htb-K119R* (fission yeast) cells. *In vitro* unmodified nucleosomes tend to interact within a chromatin array (sideways arrows) and between chromatin arrays (up and down arrows). **c.** It is unclear if H2B-Ub chromatin can directly influence silencing machinery binding or if Rad6/Bre1 is specifically excluded from silent chromatin.

Figure 2. The role of H2B-Ub in yeast heterochromatic silencing

a. In budding yeast, SIR complex can deacetylate H4K16 but it cannot silence chromatin with H3 methylation present. H2B-Ub promotes downstream methylation of H3K4 and H3K79, thereby indirectly antagonizing SIR complex-dependent silencing. This presents a problem for SIR complex *de novo* heterochromatin formation of an active region. **b.** At sub-telomeric regions SIR complex nucleates near the telomeres from recruitment factors (RF). The sub-telomeric regions experience more dynamic forces than at the silent mating-type loci, including occasional transcription by Pol II. Competition between factors that promote

silencing (ex. Ubp10) and those that promote transcription (ex. Rad6/Bre1) are constantly at play. **c.** The silent mating-type is a region of natural or “strong” silencing due to flanking silencer sequences that promote nucleation of SIR complex towards each other. This is a region of established heterochromatin that prevents transcriptional events, limiting the need to recruit Ubp10. **D.** A schematic of the pathways involved in RNAi-dependent silencing in fission yeast and the interplay with H2B-Ub. Complexes and activities are color-coded to match functional analogs/homologs found in budding yeast. These pathways are described in more detail in the text. Panels A-C adapted from (Zukowski et al. 2017).