RNA & DISEASE 2014; 1: e422. doi: 10.14800/rd.422; © 2014 by Peng Mao, *et al*.
http://www.smartscitech.com/index.php/rd

REVIEW

Rescue of DNA damage-stalled RNA Pol II: histone H2B in action

Peng Mao, Michael J. Smerdon

School of Molecular Biosciences, Washington State University Pullman, WA 99164-7520, USA

Correspondence: Michael J. Smerdon
E-mail: smerdon@wsu.edu
Received: March 13, 2014
Published: December 21, 2014

RNA Pol II elongation in eukaryotes is coupled with a series of histone modifications. Elongating RNA Pol II can be strongly stalled by lesions on the DNA template. However, it is unclear whether RNA Pol II stalling affects elongation-associated histone modifications. We have explored this important question by investigating the function of histone H2B mono-ubiquitylation (H2Bub), a well-characterized epigenetic mark associated with RNA Pol II elongation, in the cellular response to DNA lesions induced by ultraviolet (UV) radiation. We found that, in contrast to transcription elongation, RNA Pol II stalling induced by UV lesions triggers rapid and significant H2B deubiquitylation that removes ubiquitin from H2B. Interestingly, in yeast mutant cells that lack H2B deubiquitylation enzymes, rescue of the stalled RNA Pol II by transcription-coupled repair (TCR) is significantly impaired. Thus, our study has established a direct connection between RNA Pol II stalling and a histone modification response.

Keywords: Chromatin; deubiquitylation; DNA repair; nucleosome

To cite this article: Peng Mao, *et al*. Rescue of DNA damage-stalled RNA Pol II: histone H2B in action. RNA Dis 2014; 1: e422. doi: 10.14800/rd.422.

At the primary level of packaging, genomic DNA in eukaryotic cells is wrapped around histone proteins to form the nucleosome, a modular assembly of two stable heterodimers of H2A/H2B and one H3/H4 tetramer associated with 147 bp of DNA [1]. Nucleosomes are further packaged into a structural hierarchy to form the compact structure of chromatin [2]. Histones can be extensively modified by a wide variety of enzymes [3]. The epigenetic marks on histones play important roles in processes occurring on chromatin, such as transcription, DNA replication, and DNA repair [4]. Histone H2B mono-ubiquitylation (H2Bub) is a conserved modification from yeast to mammals. Ubiquitin can be attached to the C-terminal lysine residue (K123 in yeast and K120 in mammals) by the E2 conjugating enzyme Rad6 and the E3 ligase Bre1 [5, 6]. Conversely, H2B ubiquitylation can be reversed by the deubiquitylases Ubp8 and Ubp10 in yeast [7, 8]. Previous studies have demonstrated that RNA Pol II elongation is essential for ubiquitylation of H2B, with the Rad6-Bre1 complex traveling with the elongating form of RNA Pol II and adding ubiquitin to H2B during the elongation process (reviewed in [9]). Since H2Bub predominantly occurs in transcription elongation, it suggests that H2Bub may play an important role in RNA Pol II elongation. Indeed, mutation of the yeast H2B K123 residue renders the cells hypersensitive to the transcription elongation inhibitor 6-azauracil (6-AU) [10]. Detailed mechanistic studies show that ubiquitylation of H2B facilitates transcription elongation by promoting nucleosome reassembly in the wake of RNA Pol II transcription [11-13]. Additionally, H2Bub plays an essential role in mediating H3 K4 and K79 tri-methylation [14], two epigenetic marks that are associated with active RNA Pol II transcription [15].

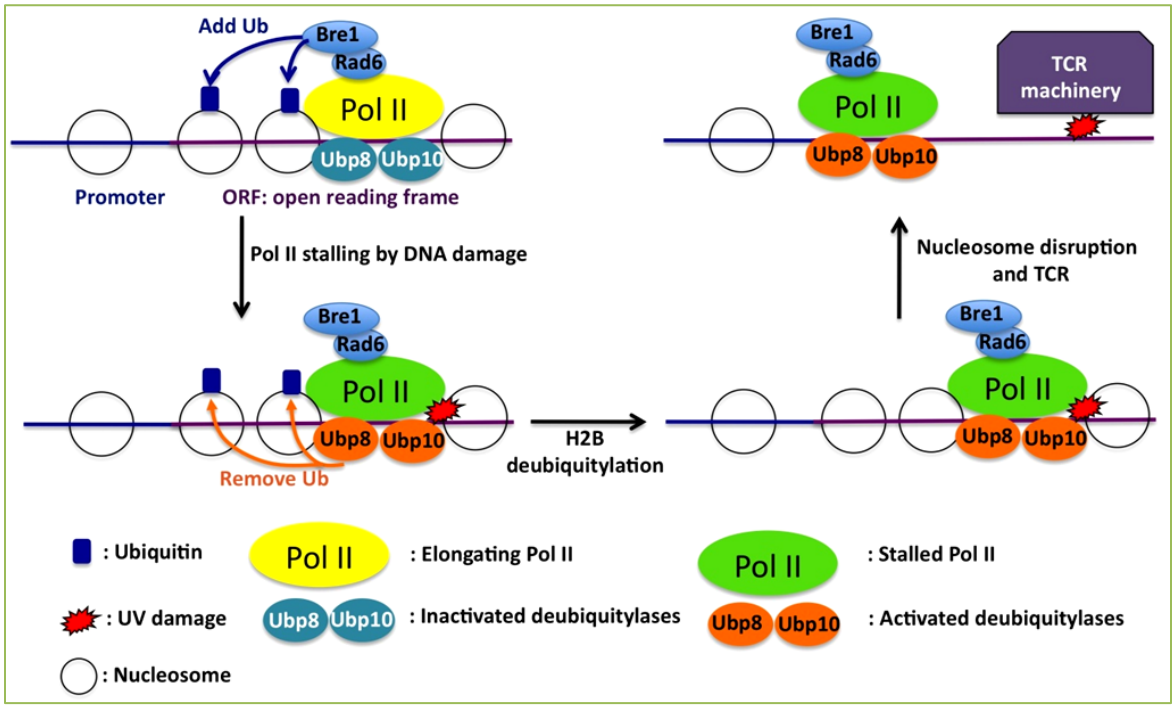

Figure 1. **Model for H2B deubiquitylation in response to UV damage-induced RNA Pol II stalling**. Ubiquitin is added to H2B by the Rad6-Bre1 enzymes associated with the elongating Pol II during transcription elongation. When the elongating Pol II is arrested by UV damage, the stalled RNA Pol II acts as a signal to activate its-associated Ubp8 and Ubp10 to deubiquitylate H2B. H2B deubiquitylation facilitates disruption of nucleosomes adjacent to the stalled RNA Pol II, which is necessary for the subsequent recruitment/assembly of TCR machinery to reversely translocate RNA Pol II and repair the UV DNA damage.

Elongation of RNA Pol II can be strongly stalled by DNA lesions such as UV-induced helix-distorting DNA damage [16]. Presumably, the stalled RNA Pol II prevents its-associated Rad6-Bre1 complex from adding ubiquitin to new H2B proteins. Whether the pre-existing ubiquitylation is maintained or reversed upon UV damage-arrested RNA Pol II needs to be experimentally tested. We addressed this question by studying cellular H2Bub levels at different times after UV radiation. Our data indicate that UV light stimulates a rapid and significant decrease in H2Bub levels in both yeast and human cells [17]. H2Bub is gradually recovered after a significant amount of UV damage in the genome has been repaired and transcription is resumed. In contrast to the H2B deubiquitylation response, H3 K4 and K79 trimethylation is not altered by UV radiation, indicating an uncoupling of the mechanism between H2B deubiquitylation and H3 demethylation. We further showed that the H2B deubiquitylation is triggered by UV-induced RNA Pol II stalling, as revealed by a significantly weaker H2B deubiquitylation response in a UV damage-bypassing RNA Pol II mutant.

As mentioned above, H2B deubiquitylation in yeast is catalyzed by two enzymes, Ubp8 and Ubp10. Our data showed that the UV-induced H2Bub decrease is slightly compromised in *ubp8Δ* or *ubp10Δ* single mutants; but is completely abolished in the *ubp8Δubp10Δ* double mutant, indicating a partially redundant function between Ubp8 and Ubp10 in deubiquitylating H2B in response to UV damage. The deubiquitylase Ubp8 has been shown to physically interact with elongating RNA Pol II to prevent excess H2B ubiquitylation during transcription elongation [18]. We found that Ubp10 also interacts with RNA Pol II, in a UV damage-independent manner. These observations identify RNA Pol II-associated Ubp8 and Ubp10 as a key link between H2B deubiquitylation and RNA Pol II stalling, indicating that the RNA Pol II stalling functions as a signal to activate its-associated H2B deubiquitylases.

The stalled RNA Pol II is extremely toxic to cell survival, as it blocks the passage of subsequent DNA or RNA polymerases, and also inhibits the access of DNA repair proteins to the damaged site. A specialized DNA repair pathway, transcription-coupled repair (TCR), has evolved to rescue DNA damage-arrested RNA polymerases [19]. Although much of the enzymology of TCR has been elucidated, the mechanism(s) of how this repair pathway interacts with chromatin remains unclear. Previous studies have revealed that the histone acetyltransferase p300 is recruited to UV damage-arrested RNA Pol II, suggesting a potential role of histone acetylation in TCR [20]. As we observed the induction of H2B deubiquitylation by

transcription stalling, we further explored the impact of H2B deubiquitylation on TCR. Importantly, UV damage repair by TCR in the transcribed strand of an actively transcribed gene (*Rpb2*) is significantly impaired in yeast cells lacking H2B deubiquitylases. Consistent with the TCR deficiency, the nucleosome occupancy is higher in the *ubp8Δubp10Δ* double mutant, and UV-induced nucleosome disruption is less efficient in the mutant. Taken together, the data suggest that H2B deubiquitylation is an important mechanism to 'loosen' nucleosome structure adjacent to UV damage-stalled RNA Pol II, thus allowing access of the TCR machinery to the damaged sites.

In summary, as shown in Figure 1, we have characterized the response and function of histone H2B deubiquitylation upon RNA Pol II stalling induced by UV damage to DNA. Our data demonstrate that H2B undergoes a rapid and significant deubiquitylation process, catalyzed by the deubiquitylases associated with RNA Pol II. This deubiquitylation mechanism appears to be important for destabilizing nucleosomes near the stalled RNA Pol II to increase the accessibility of UV damaged DNA to TCR proteins. We are currently investigating the mechanism by which the H2B deubiquitylases are activated by stalled RNA Pol II. It is interesting to note that activation of the yeast TCR protein Rad26 (an otholog to mammalian Cockayne syndrome group B protein, CSB) by UV damage requires its phosphorylation [21]. A similar mechanism may also apply to the activation of H2B deubiquitylases upon RNA Pol II stalling.

Acknowledgements

This study was supported by NIH grant ES002614 from the National Institute of Environmental Health Sciences (NIEHS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS or NIH.

References

1. Luger K, Mader AW, Richmond RK, Sargent, DF, Richmond, TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 1997; 389:251-260.
2. Luger K, Dechassa ML, Tremethick DJ. New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? Nat Rev Mol Cell Biol 2012; 13:436-447.
3. Suganuma T, Workman JL. Signals and combinatorial functions of histone modifications. Annu Rev Biochem 2011; 80:473-499.
4. Zentner GE, Henikoff S. Regulation of nucleosome dynamics by histone modifications. Nat Struct Mol Biol 2012; 20:259-266.
5. Robzyk K, Recht J, Osley MA. Rad6-dependent ubiquitination of histone H2B in yeast. Science 2000; 287:501-504.
6. Wood A, Krogan NJ, Dover J, Schneider J, Heidt J, Boateng MA, *et al*. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol Cell 2003; 11: 267-274.
7. Henry KW, Wyce A, Lo WS, Duggan LJ, Emre NC, Kao CF, *et al*. Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Dev 2003; 17:2648-2663.
8. Emre NC, Ingvarsdottir K, Wyce A, Wood A, Krogan NJ, Henry KW, *et al*. Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. Mol Cell 2005; 17:585-594.
9. Weake VM, Workman JL. Histone ubiquitination: triggering gene activity. Mol Cell 2008; 29:653-663.
10. Xiao T, Kao CF, Krogan NJ, Sun ZW, Greenblatt JF, Osley MA, *et al*. Histone H2B ubiquitylation is associated with elongating RNA polymerase II. Mol Cell Biol 2005; 25:637-651.
11. Fleming AB, Kao CF, Hillyer C, Pikaart M, Osley MA. H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. Mol Cell 2008; 31:57-66.
12. Batta K, Zhang Z, Yen K, Goffman DB, Pugh BF. Genome-wide function of H2B ubiquitylation in promoter and genic regions. Genes Dev 2011; 25:2254-2265.
13. Chandrasekharan MB, Huang F, Sun ZW. Ubiquitination of histone H2B regulates chromatin dynamics by enhancing nucleosome stability. Proc Natl Acad Sci U S A 2009; 106:16686-16691.
14. Nakanishi S, Lee JS, Gardner KE, Gardner JM, Takahashi YH, Chandrasekharan MB, *et al*. Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1. J Cell Biol 2009; 186:371-377.
15. Schulze JM, Hentrich T, Nakanishi S, Gupta A, Emberly E, Shilatifard A, *et al*. Splitting the task: Ubp8 and Ubp10 deubiquitinate different cellular pools of H2BK123. Genes Dev 2011; 25:2242-2247.
16. Brueckner F, Hennecke U, Carell T, Cramer P. CPD damage recognition by transcribing RNA polymerase II. Science 2007; 315:859-862.
17. Mao P, Meas R, Dorgan KM, Smerdon MJ. UV damage-induced RNA polymerase II stalling stimulates H2B deubiquitylation. Proc Natl Acad Sci U S A 2014; 111:12811-12816.
18. Wyce A, Xiao T, Whelan KA, Kosman C, Walter W, Eick D, *et al*. H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. Mol Cell 2007; 27:275-288.
19. Mellon I, Spivak G, Hanawalt PC. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell 1987; 51:241-249.
20. Fousteri M, Vermeulen W, van Zeeland AA, Mullenders L. Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. Mol Cell 2006; 23:471-482.
21. Taschner M, Harreman M, Teng Y, Gill H, Anindya R, Maslen SL, *et al*. A role for checkpoint kinase-dependent Rad26 phosphorylation in transcription-coupled DNA repair in Saccharomyces cerevisiae. Mol Cell Biol 2010; 30:436-446.