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Molecules in focus

Histone H2A ubiquitination in transcriptional regulation and DNA damage repair

Wenlai Zhou, Xiangting Wang, Michael G. Rosenfeld*

Howard Hughes Medical Institute, Department of Molecular Medicine, School of Medicine, University of California, San Diego, La Jolla, CA, USA

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ABSTRACT

The precise molecular strategies that coordinate patterns of transcriptional response to specific signals is central for understanding normal development and disease. Precise control of transcriptional programs underlying metazoan development is modulated by enzymatically active coregulatory complexes, coupled with epigenetic strategies. Epigenetic modifications, particularly DNA methylation and covalent histone modifications, for instance acetylation, methylation, phosphorylation and ubiquitination, play an essential role in transcription regulation, chromatin remodeling, genome instability and X chromosome inactivation. Recently, the ubiquitinases and deubiquitinases responsible for histone H2A ubiquitination and deubiquitination have been identified and characterized. These studies suggest that histone H2A ubiquitination play important roles in many cellular events, such as transcription initiation and elongation, silencing, and DNA repair. Alteration of histone H2A ubiquitination modifications may contribute human diseases, such as cancer. In this review, we discuss enzymes involved in H2A ubiquitination/deubiquitination and that possible molecular mechanisms underlying histone H2A ubiquitination/deubiquitination in transcriptional regulation and DNA damage repair.

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1. Introduction

Packaging of genomic DNA into chromatin appears to inhibit all stages of transcription, including initiation and elongation (Li et al., 2007). The nucleosome is the basic unit of chromatin and is composed of 147 base pairs (bp) of DNA wrapped 1.65 turns around the histone octamer of the four core histones (H2A, H2B, H3 and H4) (Luger et al., 1997). The histone code model (Jenuwein and Allis, 2001; Strahl and Allis, 2000) suggests that post-translational modifications such as acetylation, methylation, phosphorylation, sumolytion and ubiquitination of histone tail represent a major mechanism by which cells control the structure and function of chromatin (Kouzarides, 2007). Diverse histone modifications have been linked to the regulation of cellular activities such as transcription, repair, and replication. Most chromatin-modulating enzymes have been identified recently. Although the precise mechanisms of histone modifications involved in the transcription process are not fully understood, increasing recent evidence indicates that they work together in the form of a histone code to regulate the recruitment of chromatin-modulating factors (Berger, 2002; Mellor, 2005).

Protein mono- or polyubiquitination modification plays a critical role in a variety of cellular processes including protein degradation, cell cycle regulation, protein trafficking, stress response, signal transduction and transcriptional regulation. H2A ubiquitination was mapped to the highly conserved residue Lys 119 (Goldknopf et al., 1975) and comprise between 5% and 15% of the available H2A and is a relatively abundant modification (Goldknopf et al., 1975). The role of histone ubiquitination in gene expression and chromatin remodeling remains the least understood although three decades have passed since the discovery that the core histone H2A is monoubiquitinated. However, recent studies indicate that H2A ubiquitination play critical roles in transcriptional regulation and DNA repair.

2. The role of histone H2A ubiquitination on transcriptional silencing and repression

The role of histone ubiquitination in gene expression and chromatin remodeling has been studied extensively lately. This leads to identify many of enzymes responsible for the addition and removal of ubiquitin for the histone H2A (Fig. 1) (Joo et al., 2007; Nakagawa et al., 2008; Zhao et al., 2008; Zhou et al., 2008; Zhu et al., 2007).

Abbreviations: AR, androgen receptor; NER, nucleotide excision repair; FACT, facilitates chromatin transcription.

* Corresponding author at: Howard Hughes Medical Institute, Department of Molecular Medicine, School of Medicine, University of California, San Diego, 9500 Gilman Drive, Rm 345, La Jolla, CA 92093-0648, USA. Tel.: +1 858 534 5858; fax: +1 858 534 8180.
E-mail address: mrosenfeld@ucsd.edu (M.G. Rosenfeld).

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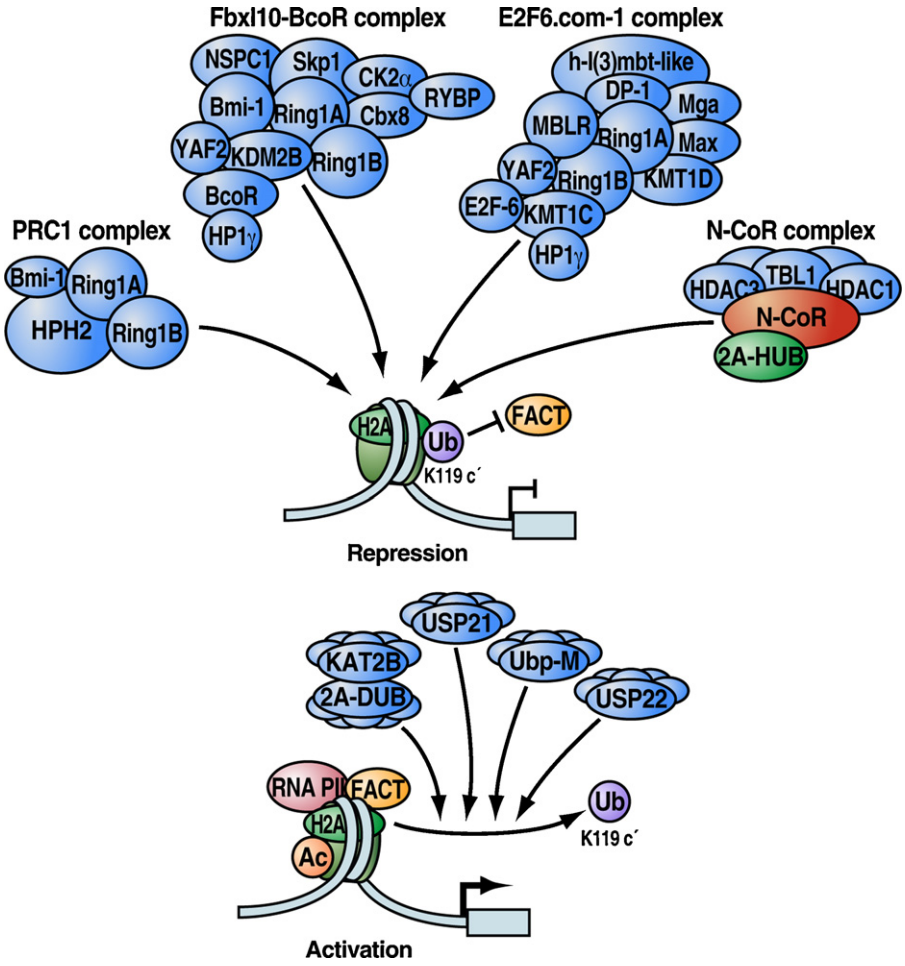

Fig. 1. Histone H2A ubiquitination regulates transcriptional activation and repression. The H2A ubiquitination mediated by Ring 1B, a componet of three different repressive complexes: PRC1, E2F6.com-1 and FBXL10-BcoR complex, and 2A-HUB, associated with the NCoR/HDAC1/3 corepressor complex, is required for the repression of gene expression. Conversely, the uH2A deubiquitination mediated by the 2A-DUB/PCAF coactivator complex, Usp-M, Usp21 and Usp22, is required for gene activation.

Previous studies show that RING2/Ring1a, one component of the polycomb repressive complex (-like hPRC1L complex) mediates H2A ubiquitination, at lysine 119 and links H2A ubiquitination to polycomb silencing (Fig. 1) (Wang et al., 2004) and X chromosome inactivation (de Napoles et al., 2004; Fang et al., 2004). Although there are three RING domain containing proteins, Ring1B (RING2/RNF2), Ring 1A (RING1) and Bmi-1, in PRC1 complex, only Ring 1B has E3 ubiquitin ligase activity specific to histone H2A (Cao et al., 2005; Wang et al., 2004). Recent studies suggests that both Ring1A and Bmi-1 strongly stimulate E3 ubiquitin ligase activity of Ring 1B (Cao et al., 2005). *In vivo* study (de Napoles et al., 2004) indicates that Ring 1A can substitute for Ring 1B in term of its function in X chromosome inactivation. uH2A co-localizes with PRC1 on the inactive X chromosome (Xi) in mouse and it no longer recruited to Xi in cells depleted both Ring 1A and Ring 1B by siRNAs specific to them (de Napoles et al., 2004; Fang et al., 2004). Moreover, histone H2A ubiquitination mediated by Ring 1B is required for polycomb-target genes, such as HOX gene silencing (Cao et al., 2005).

In addition, Ring 1A and Ring 1B were also found in other repressor complexes, such as BCoR complex and E2F6.com-1 repressive complex (Fig. 1) (Gearhart et al., 2006; Ogawa et al., 2002). H2A ubiquitination in these setting may play a role in repression of BCL6 target genes or E2F- and Myc-responsive genes in quiescent cells (Gearhart et al., 2006; Ogawa et al., 2002). Interestingly, while the E2F6.com-1 repression complex contains the Eu-HMTase1 (KMT1D) and NG36/G9a (KMT1C) lys-9 H3 histone methyltransferases (Ogawa et al., 2002), the Fbx110-BCoR complex contains FBXL10/JHDH1B which is a nucleolar protein and demethylates tri-methylated lys-4 H3. These findings suggest that H2A ubiquitination may have a crosstalk to other histone modifications. Indeed, recently study indicates that H2A ubiquitination inhibits MLL3-mediated di- and tri-methylation of lys-4 H3 and represses transcriptional initiation *in vitro* (Nakagawa et al., 2008). On the other hand, loss of H2A ubiquitination did not affect on other histone repression markers, such as lys-27 H3 or lys-9 H3 methylation (Cao et al., 2005; Nakagawa et al., 2008; Wang et al., 2004).

We have recently identified another H2A ubiquitin E3 ligase 2A-HUB/hRUL138 (Zhou et al., 2008), which was recruited by N-CoR/HDAC1/3 corepressor complex to a subset of chemokine gene promoters. H2A ubiquitination mediated by 2A-HUB acts to prevent the Spt16 subunit of FACT recruitment at the promoter region to block RNA polymerase II releasing at the early stage of elongation. In summary, distinct H2A ubiquitin ligases, each recruited based on interactions with different corepressor complexes, contribute to specific transcriptional repression programs (Fig. 1).

3. The role of histone H2A deubiquitination on transcriptional activation

At least four histone H2A deubiquitinases, including Ubp-M, 2A-DUB, Usp21 and USP22, were identified so far (Fig. 1) (Joo et al., 2007; Nakagawa et al., 2008; Zhao et al., 2008; Zhu et al., 2007).

Early studies suggest that Ubp-M is able to deubiquitinate histone H2A *in vitro* and play roles in regulating mitotic chromatin, possibly by deubiquitinating histones (Cai et al., 1999). Interestingly, Ubp-M is sequentially phosphorylated and dephosphorylated during cell cycle and an enzymatically inactive form of Ubp-M associates with mitotic chromosomes and blocks cell division (Cai et al., 1999). Recently, using biochemical purification method, Joo and colleagues identified that the major deubiquitinase for histone H2A is Ubp-M (Usp16) in Hela cells (Joo et al., 2007). Ubp-M specifically deubiquitinates histone H2A but not H2B *in vitro* and *in vivo*. Consistent with previous observation, Ubp-M plays an important role in the mitotic phase of the cell cycle and H2A deubiquitination by Ubp-M is a prerequisite for phosphorylation of Ser10 of H3 by the Aurora B kinase (Joo et al., 2007). Histone H2A deubiquitination mediated by Ubp-M is required for HOX gene expression and posterior development in *Xenopus laevis* (Joo et al., 2007). Another H2A deubiquitinase, 2A-DUB (KIAA1915/MYSM1) contains a JAMN/MPN+ domain, which has an intrinsic metalloprotease-like activity that is able to catalyze hydrolysis of the isopeptide bonds of nedd-8 and/or ubiquitin chains (Zhu et al., 2007). H2A deubiquitination by 2A-DUB is required for the activation of several transcriptional events, including androgen receptor (AR)-regulated target gene activation in prostate cancer cells (Zhu et al., 2007). 2A-DUB associates with histone acetyltransferase p300/CBP-associated factor (PCAF/KAT2B) in a coregulatory protein complex and regulates transcription by coordinating histone acetylating, deubiquitination, and destabilizing the association of linker histone H1 from core nucleosomes (Zhu et al., 2007). Interestingly, Usp22, one component of the GCNs HAT-containing TFTC/STAGA complex, is able to deubiquitinate histone H2A and H2B *in vitro* and required for full transcriptional activation by the androgen receptor (AR) (Zhao et al., 2008). These findings suggest that there are non-redundant roles of histone deubiquitinase and histone acetyltransferase (HAT) activities mediated by 2A-DUB/PCAF complex and the TFTC/STAGA complex in the dynamic turnover of histone modifications during the transcription regulation by nuclear receptors. Lastly, Usp21 was identified as an ubiquitin-specific protease that catalyzes the hydrolysis of uH2A in hepatocyte regeneration (Nakagawa et al., 2008). Usp21 is required for transcription initiation and deubiquitination of histone H2A by Usp21 is prerequisite for subsequent di- and tri-methylation of H3K4 (Nakagawa et al., 2008).

4. Histone H2A ubiquitination and DNA repair

Recently studies suggest that ubiquitination of H2A emerges as a general histone modification induced by DNA damage and plays an important role of DNA repair-induced chromatin remodeling. Monoubiquitination of histone H2A by Ring2 is induced by DNA damage in the vicinity of DNA lesions and required for functional nucleotide excision repair (Bergink et al., 2006). Ultraviolet (UV)-induced H2A ubiquitination relies on the DNA damage signaling kinase ATR (ATM- and Rad3-related) but not the ATM (ataxia telangiectasia mutated) (Bergink et al., 2006). However, phosphorylation of histone H2AX induced by UV damage was not required for H2A ubiquitination (Bergink et al., 2006). DDB1-CUL4A^{DDB2} ubiquitin ligase and monoubiquitinated histone H2A interacts in response to UV irradiation (Kapetanaki et al., 2006). Although, ubiquitination of H2A after UV irradiation is impaired in cells with mutation of DDB2 gene comparing normal cells, it is not clear whether DDB1-CUL4A^{DDB2} ubiquitin ligase can promote H2A monoubiquitination directly. However, this study indicates that H2A ubiquitination modulated by DDB1-CUL4A^{DDB2} complex facilitates the initiation of nucleotide excision repair (NER) (Kapetanaki et al., 2006). H2AX is phosphorylated after the induction of DNA double-strand breaks

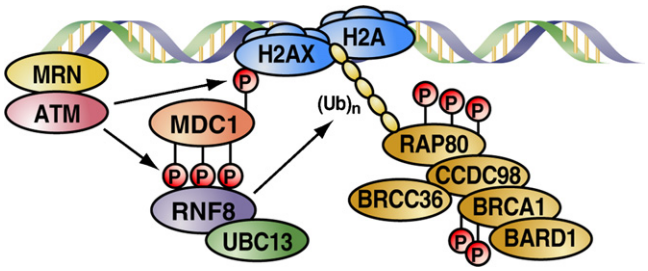

Fig. 2. A proposed model of the DNA-damage-responsive pathway involving H2A ubiquitination. ATM is activated and recruited by the MRN sensor complex upon DSBs. ATM then catalyzes the phosphorylation of H2AX and MDC1. Phosphorylated MDC1 is recognized by the RNF8, which catalyzes K63-linked polyubiquitination of histone H2A and γ-H2AX. These polyubiquitin chains are recognized by RAP80, thereby facilitating the recruitment of the BRCA1/BARD1/CCDC98/RAP80/BRCC36 protein complex to DSB sites.

(DSBs), and phosphorylated H2AX (γ-H2AX) participates in foci formation at sites of DNA damage. H2AX also can be ubiquitinated *via* the ubiquitin-conjugating enzyme UBC13 induced by DNA damage (Ikura et al., 2007). The acetylation of H2AX by TIP60 is prerequisite for its ubiquitination (Ikura et al., 2007). The function of H2AX ubiquitination is to facilitate the release of H2AX from damaged chromatin (Ikura et al., 2007). Recently, a novel H2A or H2AX E3 ubiquitin ligase, RNF8, was identified to function as a bridge between the protein phosphorylation and protein ubiquitination pathways that are important for the activation and maintenance of the DNA-damage response (Fig. 2) (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). H2A and H2AX ubiquitination catalyzed by RNF8 might serve as docking sites for damage repair machinery during the transduction of the DNA-damage signal (Huen et al., 2007; Mailand et al., 2007).

5. Future prospects

Distinct H2A ubiquitinases and deubiquitinases recruited based on interactions with different corepressor complexes or coactivator complexes indicates that they may have different functions on transcriptional repression or activation programs, and act on different sets of target genes. Is the mechanism of transcription repression by H2A ubiquitination the same for all these different H2A ubiquitin ligase complexes? Is the mechanism of transcription activation by H2A deubiquitination the same for these different H2A deubiquitinases complex? As many distinct classes of histone-modifying enzymes, such as histone deacetylases, histone ubiquitin ligases, histone demethylases or histone acetylases, histone deubiquitinases, histone methyltransferases were found in the same complexes, it will be interesting to learn how these histone-modifying enzymes activities act coordinately to regulate transcriptional activation or repression. Another interesting question to be addressed is whether there are multiple rounds of H2A ubiquitination and deubiquitination during transcription initiation and elongation? Furthermore, investigation of post-transcriptional modification of histone ubiquitin ligases and deubiquitinases, in response to upstream signals, should be helpful to better understand the molecular mechanisms of their functions in transcriptional regulation and DNA repair.

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References

Berger SL. Histone modifications in transcriptional regulation. Current Opinion in Genetics & Development 2002;12:142–8.

Bergink S, Salomons FA, Hoogstraten D, Groothuis TA, de Waard H, Wu J, et al. DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A. Genes & Development 2006;20:1343–52.

Cai SY, Babbitt RW, Marchesi VT. A mutant deubiquitinating enzyme (Ubp-M) associates with mitotic chromosomes and blocks cell division. Proceedings of the National Academy of Sciences of the United States of America 1999;96:2828–33.

Cao R, Tsukada Y, Zhang Y. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. Molecular Cell 2005;20:845–54.

de Napoles M, Mermoud JE, Wakao R, Tang YA, Endoh M, Appanah R, et al. Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. Developmental Cell 2004;7:663–76.

Fang J, Chen T, Chadwick B, Li E, Zhang Y. Ring1b-mediated H2A ubiquitination associates with inactive X chromosomes and is involved in initiation of X inactivation. Journal of Biological Chemistry 2004;279:52812–5.

Gearhart MD, Corcoran CM, Wamstad JA, Bardwell VJ. Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. Molecular and Cellular Biology 2006;26:6880–9.

Goldknopf IL, Taylor CW, Baum RM, Yeoman LC, Olson MO, Prestayko AW, et al. Isolation and characterization of protein A24, a "histone-like" non-histone chromosomal protein. Journal of Biological Chemistry 1975;250:7182–7.

Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, et al. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 2007;131:901–14.

Ikura T, Tashiro S, Kakino A, Shima H, Jacob N, Amunugama R, et al. DNA damage-dependent acetylation and ubiquitination of H2AX enhances chromatin dynamics. Molecular and Cellular Biology 2007;27:7028–40.

Jenuwein T, Allis CD. Translating the histone code. Science (New York, NY) 2001;293:1074–80.

Joo HY, Zhai L, Yang C, Nie S, Erdjument-Bromage H, Tempst P, et al. Regulation of cell cycle progression and gene expression by H2A deubiquitination. Nature 2007;449:1068–72.

Kapetanaki MG, Guerrero-Santoro J, Bisi DC, Hsieh CL, Rapic-Otrin V, Levine AS. The DDB1-CUL4A^{DDB2} ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. Proceedings of the National Academy of Sciences of the United States of America 2006;103:2588–93.

Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science (New York, NY) 2007;318:1637–40.

Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705.

Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128:707–19.

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 1997;389:251–60.

Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell 2007;131:887–900.

Mellor J. The dynamics of chromatin remodeling at promoters. Molecular Cell 2005;19:147–57.

Nakagawa T, Kajitani T, Togo S, Masuko N, Ohdan H, Hishikawa Y, et al. Deubiquitylation of histone H2A activates transcriptional initiation via trans-histone cross-talk with H3K4 di- and trimethylation. Genes & Development 2008;22:37–49.

Ogawa H, Ishiguro K, Gaubatz S, Livingston DM, Nakatani Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. Science (New York, NY) 2002;296:1132–6.

Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–5.

Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, et al. Role of histone H2A ubiquitination in polycomb silencing. Nature 2004;431:873–8.

Zhao Y, Lang G, Ito S, Bonnet J, Metzger E, Sawatsubashi S, et al. A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Molecular Cell 2008;29:92–101.

Zhou W, Zhu P, Wang J, Pascual G, Ohgi KA, Lozach J, et al. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. Molecular Cell 2008;29:69–80.

Zhu P, Zhou W, Wang J, Puc J, Ohgi KA, Erdjument-Bromage H, et al. A Histone H2A deubiquitinase complex coordinating histone acetylation and H1 dissociation in transcriptional regulation. Molecular Cell 2007;27:609–21.