Regulation of histone H2A and H2B ubiquitylation

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Abstract

Histone ubiquitylation has emerged as an important chromatin modification with roles in transcription and trans-histone methylation. In the past several years, there has been dramatic progress in the identification of factors that control ubiquitin attachment to the core histones H2A and H2B. Recent advances in this area will be reviewed, and outstanding questions relating to the cellular functions of these modified histones will be discussed.

Keywords: histones H2A and H2B; ubiquitin conjugation; transcription activation and silencing

OVERVIEW OF PROTEIN UBIQUITYLATION

The 76 amino acid protein, ubiquitin, is attached to proteins through a series of enzymatic reactions that start with the ATP-dependent activation of ubiquitin by a ubiquitin activating enzyme (E1) (Figure 1) [1]. Activated ubiquitin is then conjugated via a thioester bond to a cysteine residue in an ubiquitin-conjugating enzyme (E2), and finally transferred to a lysine residue in a particular substrate with the help of a ubiquitin ligase (E3). Proteins can remain monoubiquitylated, or the monoubiquitin moiety can act as a ‘seed’ for the subsequent conjugation of additional ubiquitin residues, resulting in a polyubiquitin chain. The cellular fate of monoubiquitylated and polyubiquitylated proteins is very different [2]. Polyubiquitylated proteins are most commonly targeted for degradation via the 26S proteosome, although some alternative forms of polyubiquitylation are associated with DNA damage repair [3]. In contrast, monoubiquitylated proteins are stable and a common feature of proteins that traverse intracellular compartments [4, 5]. A wide variety of proteins become ubiquitylated and ubiquitylation itself is highly regulated, with E3 enzymes playing key roles in targeting the proteins that become modified. Both E2 and E3 enzymatic activities comprise large families, and their associations with each other, cellular localization, and substrate interactions all contribute to targeting a particular protein for either monoubiquitylation or polyubiquitylation.

Histones belong to the class of proteins that are predominantly monoubiquitylated. Core histones, linker histones and several histone variants have all been reported to be ubiquitylated, although the site of ubiquitin conjugation is known for only a few of the histone species [6]. In this review, I will focus on recent progress in the regulation of histone H2A and H2B monoubiquitylation, where historically most studies have been performed. Surprisingly, the factors that control ubiquitylation of these histones were identified only in the past 5 years, despite the fact that ubiquitylated H2A was first detected 30 years ago [7, 8]. Two features stand out: (i) ubiquitylation of H2A and H2B is regulated by a number of factors in addition to ubiquitin-conjugating enzymes and ubiquitin ligases; (ii) many of these factors play important roles in transcriptional regulation. This has led to the view that ubiquitin is conjugated to H2A and H2B primarily by co-transcriptional mechanisms, which has also emerged as an important regulatory pathway for several forms of histone methylation.
H2B [9, 10]. The C-terminal tail of H2B lies on the outside of the nucleosome core particle, and thus the lysine residue that resides there is potentially accessible to modifying activities [11]. The discovery that H2B is monoubiquitylated (ub-H2B) in budding yeast led to rapid advances in the identification of factors that control this modification. At the same time that ub-H2B was discovered in this organism, the cellular E2 responsible for H2B monoubiquitylation was identified as the product of the RAD6 gene, which encodes a known ubiquitin-conjugating enzyme [10, 12]. It was recently shown that enzymes with homology to Rad6 also target H2B for ubiquitylation in organisms other than yeast. Specifically, UbcH6 monoubiquitylates nucleosomal H2B in vitro in the context of a ub-H2B specific E3 complex [13]. Although Rad6 can modify both H2B and H2A in vivo in the absence of an E3 ligase [12, 14], it is not known if Rad6 has broad histone specificity in vivo or if each histone is targeted by a different E2.

Rad6 is an evolutionarily conserved protein with a catalytic site that contains an essential cysteine residue to which ubiquitin becomes conjugated [15–20]. Yeast Rad6 also has a unique C-terminal extension comprising a stretch of acidic amino acids that is specifically important for its activity in histone ubiquitylation [10, 14, 21–23]. The significance of this domain in H2B ubiquitylation is not understood. One possibility is that the acidic residues interact with the basic histone and help to stabilize Rad6’s association with chromatin. Alternatively, this region could play a unique role in the activity of yeast Rad6. Two other regions of Rad6 are also involved in its activity. As discussed below, the N-terminus of yeast Rad6 binds two different E3s that target Rad6 to different proteins and thus play a role in substrate specificity [24]. In addition, the conserved serine 120 residue is phosphorylated by two different cyclin-dependent kinases, which results in the stimulation of Rad6’s histone ubiquitylation activity. CDK2 increases the activity of vertebrate Rad6 towards H2A in vitro, and the BUR kinase stimulates the H2B ubiquitylation activity of yeast Rad6 in vivo [25, 26].

Rad6 is a multifunctional ubiquitin-conjugating enzyme that modifies other substrates besides H2B. Rad6 plays a prominent role in post-replication DNA damage repair (PRR), and in this role it monoubiquitylates the proliferating cell nuclear antigen (PCNA) at replication forks stalled at the sites of DNA lesions [27–30]. As the result of PCNA ubiquitylation, the replicative DNA polymerase is exchanged for translesion synthesis polymerases. Rad6 also targets several short-lived regulatory proteins for polyubiquitylation and subsequent turnover by the 26S proteasome [31, 32]. Target specificity is brought about by different E3 ubiquitin ligases that bind Rad6 and direct it to a particular substrate. The E3 Rad18 targets Rad6 to PCNA and Ubr1 targets Rad6 to proteins destined for degradation via the 26S proteasome. Some forms of polyubiquitin chains, however, can perform non-proteolytic signalling roles, for example, during DNA damage repair.

**Figure 1:** Ubiquitin-conjugating pathway. Ubiquitin is activated by a ubiquitin-activating enzyme (E1) and then transferred to one of the many different ubiquitin-conjugating enzymes (E2). Ubiquitin is conjugated to the correct substrate through the intermediary of a large family of ubiquitin ligases (E3), which bind both the E2-ub complex and substrate. For simplicity, a RING domain E3 is shown. Proteins can be monoubiquitylated, whereby a single ubiquitin is attached, or polyubiquitylated, in which multiple ubiquitin moieties are attached to each other. Whereas monoubiquitylation performs a non-proteolytic signalling role, polyubiquitylation typically targets proteins for degradation via the 26S proteasome. Some forms of polyubiquitin chains, however, can perform non-proteolytic signalling roles, for example, during DNA damage repair.
proteasomal degradation [15, 33, 34]. A third protein, Bre1, localizes yeast Rad6 to its nucleosomal target, H2B. Bre1 was identified in both proteomics and genetic screens for regulators of H2B ubiquitylation and subsequently found to be associated in a complex with Rad6 [35, 36]. All three of the Rad6-interacting E3s contain a RING domain, a zinc-binding motif that characterizes many different E3 enzymes [37]. The RING domain is essential for Bre1’s ligase activity and could play a role in its interaction with Rad6 and/or H2B [35]. Interestingly, Rad18 and Ubr1 both associate with the N-terminus of Rad6, and a rad6 mutant in which this region is deleted (rad6Δ1–9) is deficient in yeast sporulation, a phenotype shared by a mutant in which H2B cannot be ubiquitylated (hht1–K123R) [10, 24, 32]. This raises the possibility that all three E3s interact with the same region of Rad6 and that additional factors are involved in substrate selection. This appears to be the case for H2B ubiquitylation as discussed below.

Like Rad6, Bre1 is an evolutionarily conserved protein with structural and functional homologues in other eukaryotic species. Its human homologues are RNF20 and RNF40, two interacting RING domain proteins that associate in vitro with a human Rad6 homologue, UbcH6, to form an E2–E3 complex [13, 36]. Rad6–Bre1 interactions also appear to be functionally conserved as well-based on the observation that the human E2–E3 complex is required for monoubiquitylation of H2B in vitro and in vivo [13]. A Drosophila homologue of Bre1 (dBre1) has also been identified. Although dBre1 has not been shown to possess ubiquitin ligase activity towards H2B, it is presumed to be a functional homologue of yeast Bre1 because mutant cell lines show decreased levels of H3 lysine 4 methylation, which is regulated in trans by ub-H2B (see subsequently) [38].

As discussed above, Rad6 targets a variety of substrates in yeast through its interaction with different E3 ligases that potentially bind the same surface of Rad6. In the case of H2B ubiquitylation, additional factors besides Bre1 are required to target yeast Rad6 to its nucleosomal substrate in vitro and to activate its enzymatic activity. These factors all play a role in transcription, and in some cases their dual roles in transcription and ubiquitylation have been evolutionarily conserved. The overall picture that emerges is that ubiquitin is conjugated to H2B through a co-transcriptional mechanism that involves the association of Rad6–Bre1 with components of the transcription initiation and elongation machinery (Figure 2A). Interestingly, this mechanism was first hinted at 15 years ago through the observation that ub-H2B levels are coupled with ongoing transcription in human cells [39]. Rad6 is recruited to chromatin at a number of active gene promoters in an activator- and Bre1-dependent manner [35, 40]. This implies that Bre1 (and/or Rad6) might interact with a wide variety of transcriptional activators, although this has not been explicitly tested. While Bre1 is required to initially localize Rad6 to chromatin, this event is not sufficient to activate the ubiquitin-conjugating activity of Rad6 towards H2B. This occurs only after Rad6–Bre1 associates with the elongating form of RNA polymerase II (Pol II) that has been phosphorylated on serine 5 of the C-terminal domain (CTD) by Kin28, a subunit of the general transcription factor TFIIH [41, 42]. A key transcription elongation/processing factor, PAF, is required for the interaction of Rad6–Bre1 with elongating Pol II, and the association of PAF with the chromatin of gene coding regions is, in turn, dependent on the activity of another elongation factor, BUR kinase [42–47]. This regulatory cascade appears to be generally conserved in human cells: the human PAF complex and RNF20/RNF40 are localized to transcriptionally active genes in vitro, and human PAF is required for the optimal activity of the trimeric RNF20/RNF40–UbcH6 complex towards nucleosomal H2B in vitro [13]. Together, the data indicate that H2B ubiquitylation is dependent on at least two events: the initial localization of Rad6–Bre1 to its substrate, chromatin, and the secondary activation of chromatin bound Rad6–Bre1 by factors that are intimately involved in transcription elongation.

This model for Rad6-dependent H2B ubiquitylation raises a number of questions. Arguably, one of the most important is the mechanism by which Rad6 is activated upon its association with elongating Pol II. Several scenarios can be envisioned. In the first, Rad6 could be activated by factors that also associate with Pol II. In this regard, it is significant that the BUR kinase, a transcription elongation factor, participates in the activation of yeast Rad6 at two points. First, it regulates PAF association with chromatin, and second, it phosphorylates Rad6 in vitro and increases its H2B ubiquitylation activity in vivo [26, 47]. This raises the exciting possibility that BUR plays a major role in converting chromatin-bound Rad6 to a catalytically active form.
second scenario, the PAF complex, by bridging the association of Rad6–Bre1 and Pol II, might cause an allosteric change in the E2–E3 complex that stimulates its ubiquitin conjugation activity. Finally, some combination of these and other mechanisms might come into play. Regardless of the mechanism employed, the net result is that H2B becomes ubiquitylated throughout the promoters and coding regions of a number of actively transcribed genes and is generally unmodified in regions of silent chromatin [40]. The presence of ub-H2B at expressed genes appears to be important for both the activation and elongation phases of transcription. In the absence of ub-H2B, highly inducible yeast genes such as GAL1 and SUC2 are transcribed at lower levels than in wild-type cells, while knock-down of RNF20/ RNF40 or hPAF causes a similar negative effect on the transcription of Hox genes in human cells [13, 40, 48]. Additionally, mutants in the pathway to H2B ubiquitylation (e.g. rad6Δ, bre1Δ or htb1–K123R)

Figure 2: Co-transcriptional regulation of H2B and H2A ubiquitylation. A. H2B ubiquitylation. The sequence of events that is presumed to occur at transcriptionally active yeast genes is outlined. Rad6–Bre1 is recruited to the upstream activation site in the promoter by its interaction with a DNA sequence-specific activator (I). The E2–E3 complex is inactive in ubiquitin conjugation until the following events take place. First, RNA Pol II and general transcription factors are recruited to the core promoter, and the C-terminal domain (CTD) of Pol II is phosphorylated on serine 5 (S5) by Kin28 (II). This leads to the recruitment of the Set1 HMT. Next, the elongation factors PAF and BUR associate with Pol II (III). The BUR kinase assists PAF recruitment to chromatin, and the PAF complex, in turn, bridges Rad6–Bre1 interaction with Pol II. This may bring the E2–E3 complex into proximity of BUR, which phosphorylates Rad6 on serine 120 and converts it into an active ubiquitin-conjugating enzyme. Rad6–Bre1 ubiquitylates nucleosomal H2B on lysine 123 (IV). The presence of ubiquitylated H2B allows Set1-mediated di-and tri-methylation of lysine 4 on histone H3 by unknown mechanisms, and may occur in part through the ub-H2B-dependent recruitment of the 19S proteasomal subunit (not shown) (V). The ubiquitin mark is subsequently removed at promoters by the targeted recruitment of the SAGA histone acetyltransferase complex, which contains a ub-H2B specific ubiquitin protease, Ubp8 (not shown). This sequence of events occurs throughout the promoter and coding regions of actively transcribed genes through the association of Rad6–Bre1 with elongating Pol II. B. H2A ubiquitylation. The sequence of events at the Drosophila Ubx gene or human Hox genes is outlined. The PcG complex PRC2 interacts with PRE in gene promoters (I). PRC2 contains a methyltransferase activity that modifies histone H3 on lysine 27 (II). Lysine 27 methylation is a prerequisite for the chromatin association of a second Polycomb group complex, PRC1L, which contains a RING domain ubiquitin ligase (Ring1B/dRing) that targets nucleosomal H2A for ubiquitylation on lysine 119 (III). Unlike H2B ubiquitylation, H2A ubiquitylation is apparently restricted to promoter regions.
are sensitive to the drug 5-fluorouracil, which is correlated with a defect in transcription elongation [42]. However, ub-H2B has also been associated with transcriptional repression: in its absence, basal repression of the yeast ARG1, GAL1 and PHO5 genes is relieved [49, 50]. We still do not understand how the presence of ub-H2B affects either transcription activation or repression. Possible mechanisms include structural effects of the ubiquitin moiety on chromatin folding; a role for the ubiquitin moiety in assembling complexes important for transcriptional activation or repression; trans-histone regulation of histone H3 lysine 4 and lysine 79 methylation, both of which are associated with actively transcribed chromatin (see below), or some combination of these effects.

REGULATORS OF H2B DEUBIQUITYLATION
The ubiquitin moiety on H2B is dynamically regulated during gene expression in yeast, turning over at promoters and coding regions during both the initiation and elongation phases of transcription [40, 42, 48]. In addition, ubiquitin is globally removed from histones during metaphase and then reattached to histones at anaphase [51–53]. Global deubiquitylation of H2B also occurs during yeast stationary phase in response to the depletion of glucose from the culture medium [54]. It has recently become clear that protein deubiquitylation is as highly regulated as protein ubiquitylation. Large families of ubiquitin proteases (Ubps) have been identified in both human and yeast cells, and some of these Ubps have been shown to target ubiquitylated histones [55–57]. Two Ubps in yeast remove the ubiquitin moiety from H2B that is present in different chromatin compartments. The first, Ubp8, is a stoichiometric subunit of the SAGA histone acetyltransferase complex and deubiquitylates H2B at gene promoters through the targeted recruitment of SAGA to many actively transcribed genes [3, 48]. Thus, both the H2B ubiquitin-conjugating and deconjugating activities have been linked to steps in transcription. Moreover, the similar effects of ubp8Δ and htb1–K123R mutations on gene activation suggest that the sequential attachment and removal of ubiquitin on H2B is a prerequisite for optimal levels of gene expression [48]. A second Ubp, Ubp10, preferentially targets H2B for deubiquitylation in regions associated with transcriptional silencing, such as telomere-proximal regions [58, 59]. The maintenance of low levels of ub-H2B in silent chromatin has been hypothesized to provide a buffer to the spread of silencing factors such as Sir proteins, which preferentially bind unmodified histones [58–60]. In addition, Ubp10 also acts at some repressed euchromatic genes [59]. Here, Ubp10 might perform a similar function by removing the potentially activating ubiquitin mark. In contrast to Ubp8, little is known about the mechanisms targeting Ubp10 to silenced or repressed genes. However, some evidence suggests that its localization to silent chromatin occurs through a mutually reinforcing mechanism that involves the silencing factor Sir2 [58].

An H2B-specific Ubp, Usp7, has also been identified in Drosophila, and this deubiquitylating activity appears to be functionally similar to yeast Ubp10 in helping to maintain transcriptional silencing, in this case, at Polycomb-group-regulated genes [61]. Interestingly, Usp7 is stably associated with a metabolic enzyme, guanosine 5′-monophosphate synthetase (GMPS), which also contributes to H2B deubiquitylation. The significance of this association or the role of GMPS in ubiquitin deconjugation is not known.

TRANS-HISTONE METHYLATION BY UB-H2B
As the repertoire of histone modifications is examined in greater detail, examples of ‘crosstalk’ between various modifications have emerged. A dramatic example of such crosstalk is seen in the unidirectional regulation of histone H3 methylation on lysines 4 (K4) and 79 (K79) by H2B ubiquitylation [62–65]. Mutations in the H2B ubiquitylation site (htb1–K123R) or in components of the ubiquitin-conjugating machinery (e.g. rad6Δ, bre1Δ) globally reduce the cellular levels of both H3 modifications in yeast, while the absence of either H3K4 or H3K79 methylation has no effect on the levels of H2B ubiquitylation. This relationship appears to be evolutionarily conserved in humans, where siRNA knock-down of the RNF20/RNF40 E3s also reduces K4 and K79 methylation levels [13]. Like ub-H2B, H3K4 and H3K79 methylation are associated with transcriptionally active euchromatin and present at only very low levels in silent chromatin [66–71]. Moreover, H3K4 methylation is regulated by many of the same factors that control
H2B ubiquitylation, e.g. PAF and Kin28, with the H3K4 methyltransferase (HMT) Set1 also associating with elongating Pol II [46, 72–74]. The striking parallels between the co-transcriptional regulation of H2B ubiquitylation and H3K4 methylation have led to two broad models for how ub-H2B might control this particular methylation mark. In the first, the ubiquitin moiety on H2B is proposed to act as a ‘wedge’ to open up a domain of chromatin (e.g. a gene-coding region) [75]. In the second, the ubiquitin moiety is proposed to disrupt individual nucleosomes throughout promoters and coding regions [42]. Both models suggest that the ubiquitin moiety directly or indirectly disrupts or unfolds chromatin, allowing the Set1 HMT, which associates with chromatin independently of H2B ubiquitylation, to have access to the H3 N terminal tail [72, 76]. This access is postulated to occur, in turn, through the non-proteolytic activity of the 19S proteasomal subunit, which depends on ub-H2B for its association with actively transcribed genes [76]. However, Set1 access is likely to be transient, as ub-H2B turns over during transcription initiation and elongation. While there is no evidence specifically favouring either model, the observation that H2B is quite uniformly ubiquitylated across the coding regions of a number of transcriptionally active genes has lent support to the local nucleosome disruption model [42].

This picture is complicated by the reports that the methylation states of H3K4 and H3K79 are differentially regulated by H2B ubiquitylation. Both lysine residues are mono-, di- and tri-methylated, and all three states of methylation are under the control of either the Set1 (K4) or Dot1 (K79) HMT [77, 78]. However, only K4 and K79 di- and tri-methylation are fully dependent on H2B ubiquitylation, as shown by the selective absence of these two forms of methylation in an *htb1-K123R* mutant [79]. K4 tri-methylation itself also appears to be selectively regulated by the BUR kinase as well as by a domain of the Set1 HMT [47, 80, 81]. *bur* mutants, for example, have reduced levels of H2B ubiquitylation and K4 tri-methylation but approximately wild-type levels of K4 mono- and di-methylation [47].

Moreover, the observation that very low levels of ub-H2B are sufficient to trigger K4 di-methylation and K79 di- and tri-methylation suggests that the ubiquitin moiety might not regulate H3 methylation by directly altering the chromatin structure [47]. Thus, an alternative model is that the ubiquitin moiety provides a binding surface for the Set1 and/or Dot1 HMT complexes, triggering their activity in di- and tri-methylation. Interestingly, Dot1 has been reported to contain two UBA-like domains that are found in many factors that bind ubiquitin, and a subunit of the Set1 HMT complex called Spp1 is required for K4 tri-methylation [79, 81, 82]. Whether Dot1 or Spp1 directly interacts with the ubiquitylated tail of H2B has not been reported.

**REGULATORS OF HISTONE H2A UBIQUITYLATION**

Histone H2A is monoubiquitylated on lysine 119 in its C-terminal tail except in budding yeast, where the modified histone has not been found [83, 84]. As is the case for H2B, the region of the H2A tail that is ubiquitylated is located on the surface of the histone octamer and thus potentially available to the ubiquitin-conjugating machinery [11]. Significantly more ub-H2A than ub-H2B is present in cells (10–15% ub-H2A versus 1–5% ub-H2B), and unlike ub-H2B, whose distribution appears to be generally restricted to euchromatin, ub-H2A is concentrated in regions of heterochromatin. These regions include the sex body or XY body of meiotic prophase cells, unpaired autosomal regions in male meioses and the inactive X chromosome [85–87]. However, ub-H2A is also present globally throughout euchromatin, where it may be localized to regions of transcriptional silencing [88].

A fairly detailed picture of how H2A ubiquitylation is regulated has emerged in the past year. As with ub-H2B, the data point to a role for transcriptional regulators in controlling the attachment of ubiquitin to H2A. However, these regulators play an intimate role in gene silencing rather than in gene activation. The regulators in question are members of the Pcg PRC1L (PRC1-like) complex, an evolutionarily conserved factor required for the heritable silencing of homeotic genes in flies and vertebrates [89, 90]. Several lines of evidence have shown that this factor contains a ubiquitin ligase activity that specifically targets H2A.
for ubiquitylation. First, biochemical fractionation of the human PRC1L complex revealed that it contains three RING domain subunits, Ring1B, Ring1A and Bmi1 [91, 92]. Ring1B was found to have E3 ligase activity in vitro in a reconstituted E1–E2–E3 assay system, and ub-H2A was identified as the relevant substrate. The two other RING domain proteins stimulate Ring1B’s activity in the context of the PRC1 complex, but do not possess intrinsic E3 activity [92]. Second, PRC1 and ub-H2A were shown to co-localize on the inactive X chromosome (Xi) in mouse, while RNAi-mediated knock-down of Ring1B and Ring1A resulted in depletion of ub-H2A from Xi [88, 93]. Finally, ub-H2A was shown to be present at the promoter of the fly homeotic gene, Ubx, as well as at the promoters of a number of human Hox genes, and its localization to these promoters was found to be Ring1B-dependent [91, 92].

These observations have led to a model for how ub-H2A is localized to regions of PRC1-mediated gene silencing, such as the fly Ubx gene or vertebrate Hox gene clusters (Figure 2B). Two different PcG complexes are involved in transcriptional silencing of homeotic genes [94]. The first, PRC2, binds to polycomb response element (PRE) sites in the promoters of these genes [95]. This complex also contains a histone modifying activity, in this case an HMT that methylates H3 on lysine 27 [96, 97]. K27 methylation is a prerequisite for the localization of the PRC1 complex to the PRE, and this presumably occurs through the chromodomain-containing Pc subunit [95, 97]. A twist in the story is that while ub-H2B controls H3 K4 and K79 methylation, H3 K27 methylation is a prerequisite for H2A ubiquitylation: knock-down of the PRC2 K27 HMT activity eliminates not only the methylation mark but ub-H2A as well [92]. Thus, a regulatory cascade that begins with the binding of a silencing complex with histone-methyltransferase activity to upstream regulatory sites leads to the recruitment of a second silencing complex with histone ubiquitylation activity, and ultimately to localized ubiquitylation of H2A.

Despite these advances, there are a number of important gaps in this picture. The first has to do with the identity of the ubiquitin-conjugating enzyme that functions with the Ring1B ligase in vivo. Although Rad6 is an attractive candidate based on reports of its co-localization with ub-H2A in mouse spermatocytes, ub-H2A is still present in these meiotic cells when HR6B, the gene encoding the spermatocyte-specific version of Rad6, is knocked out [85]. However, UbcH5 was found to be the only E2 enzyme that functions in concert with RING1B to ubiquitylate H2A in vitro, suggesting that this activity may also be relevant in vivo [91]. The second has to do with the stability of ub-H2A at regions of gene silencing. As discussed above, ub-H2B appears to turn over continuously during the process of transcription initiation and elongation. A priori it might be assumed that ub-H2A would be a stable constituent of silent chromatin. However, heritable epigenetic states such as those associated with PcG silencing can be divided into two phases—an initiation phase and a maintenance phase [98]. Thus, if ub-H2A is required only to initiate heterochromatin formation, it might be present transiently at regions of gene silencing. Evidence in this regard is still inconclusive. On one hand, PRC1 and ub-H2A have been reported to be present on the inactive X only during the early stages of X inactivation [88, 93]. In addition, the silent X is not reactivated in Ring1B/Ring1A knockdown cells [88]. These data suggest that ub-H2A is a transient mark associated with the initiation of silent chromatin. On the other hand, knock-down of Ring1B in flies and humans leads to derepression of homeotic genes, which is more consistent with a role for ub-H2A in the maintenance of transcriptional silencing [91, 92]. Regardless of the role of ub-H2A in gene silencing, the ubiquitin moiety is removed from H2A at metaphase during each cell division. The ubiquitin protease that targets ub-H2A during mitosis is not known. The UbpM protease acts on ub-H2A in vitro and is associated with mitotic chromosomes in vivo, but there is as yet no evidence that it deubiquitylates H2A during cell division [99].

How does the presence of ub-H2A contribute to gene silencing? This is the same question raised for ub-H2B, and unfortunately we do not yet have an answer. Several possibilities can be considered, however. The ubiquitin moiety might perform a structural role by helping to form a condensed chromatin structure that is less accessible to the transcription machinery, to ATP-dependent nucleosome remodelling complexes or even to the PRC1L complex itself at regions where ub-H2B plays an activating role (e.g. at Hox genes). Based on the data showing that ub-H2A is present in a restricted
region of the fly Ubx gene and human Hox gene promoters [91, 92], this condensation might be localized, or the local presence of ub–H2A could set up a broader domain of condensed chromatin. Candidates for factors that might assist ub–H2A in chromatin compaction include histone deacetylases (HDACs), the linker histone H1 or the variant histone macroH2A, all of which have been associated with gene silencing at many of the same regions where ub–H2A is found [94, 100–103]. Alternatively, the ubiquitin moiety on H2A might indirectly prevent the association of the transcription machinery or nucleosome remodelers with chromatin by providing a binding surface for an inhibitor [104].

SUMMARY

After a long lag period, there has been rapid progress in the identification of factors that regulate mono-ubiquitylation of histones H2A and H2B. The picture that emerges is one in which components of the basic ubiquitylation machinery are either subunits of factors with known roles in transcription [e.g. Ring1B (E3) of the PRC1L silencing complex] or are regulated by their association with the transcription apparatus [e.g. Rad6–Bre1 (E2–E3) association with elongating Pol II]. These associations provide mechanisms not only to target ubiquitylation to the correct histone substrate but also to localize the modified histones to regions of gene activity (ub–H2B) or gene silencing (ub–H2A). In turn, the localization of ub–H2B and ub–H2A contributes in still-unknown ways to the regulation of transcriptional activation or silencing. A number of key questions are yet to be answered. For H2B ubiquitylation: how is the ubiquitylation machinery activated by its association with elongating Pol II and what is the mechanism by which ub–H2B regulates the trans-histone methylation of histone H3 on lysines 4 and 79? For H2A ubiquitylation: what is the identity of the cellular ubiquitin-conjugating enzyme that functions with Ring1B in the PRC1 complex? For both histones: how does the ubiquitin moiety function in the context of chromatin to either enhance or inhibit transcription—does it alter higher-order chromatin structure or provide an interaction surface for transcriptional regulators? These questions are part of an exciting period in which new information is being rapidly uncovered about an ‘old’ modification.

Key Points

- Histones H2A and H2B are monoubiquitylated on conserved C-terminal lysine residues.
- H2A and H2B are ubiquitylated primarily by co-transcriptional mechanisms.
- Regulators of H2A and H2B ubiquitylation play roles in gene silencing (ub–H2A) or in transcription initiation and elongation (ub–H2B).
- Histone H3 lysine methylation is connected to H2A and H2B ubiquitylation.

Acknowledgements

Yi Zhang is thanked for communicating unpublished data, and members of the Osley laboratory are thanked for their comments. Research in the author’s laboratory was supported by NIH grant GM40118.

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