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Chapter

Processes that Regulate the Ubiquitination of Chromatin and Chromatin-Associated Proteins

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Abstract

Ubiquitin is a post-translational modification important for many different processes in the cell, including antigen presentation and proteosomal degradation of proteins. It is heavily involved in the regulation of chromatin and the proteins that control chromatin-related processes. In this review, we will focus on ubiquitin-based chromatin regulation involved in four different processes. The first is DNA double strand break (DSB) repair and the role that ubiquitin plays in not just recruiting and stimulating DSB repair, but also the choice of pathway. The second is the PAF1 complex, which is involved in transcriptional elongation and interacts with RNAPII. The third is polycomb repressive complexes, specifically polycomb repressive complex 1, which utilizes ubiquitin to repress constitutively inactive genes. The last role of ubiquitin discussed is ubiquitin as a mitotic bookmark, which serves to provide a record of -active genes as cells transit mitosis. Each of these processes has independent pathways, but each is necessary for proper cellular function and organismal health.

Keywords: PRC1, RING1A, BMI1, bookmark ubiquitination

1. Introduction

Ubiquitin is most clearly associated with the process of targeted protein degradation, but it is involved in many cellular processes such as chromatin regulation, immune response, and antigen processing [1]. Proteosomal degradation is mediated through polyubiquitin chains linked via lysine-48 (K48) on the ubiquitin chain, interacting with the proteasome. Other processes utilize monoubiquitination or polymerization of ubiquitin molecules via another lysine. The ubiquitination system in humans is incredibly complex, with over 1000 known factors and over 10,000 known sites of ubiquitination, enabling its many and diverse roles in cellular biology.

In this review, we focus on four specific roles of ubiquitin in regulating chromatin: DNA repair, transcription elongation, epigenetic silencing via the polycomb repressive complex, and bookmark ubiquitination. In these processes, the ubiquitin moiety interfaces with many other epigenetic marks, such as acetylation, methylation, and histone modification to regulate a given process.

The process of ubiquitination (or ubiquitylation) is the attaching of one ubiquitin protein to a substrate and is performed by a cascade of three enzymes: E1 (activating), E2 (conjugating) and E3 (ligase) [2–5]. Substrates are proteins, and in the

context of chromatin, histones are the most common class of substrate [6]. Histones form octamers containing two each of the four core histones (H2A, H2B, H3, H4), and when a histone octamer is wrapped with two turns of DNA, it is called a nucleosome. Each histone has a tail that extends outside the core of the nucleosome, where it is more accessible to the modifying enzymes. In addition to ubiquitination, other modifications occur, such as acetylation or methylation. The primary role of these marks is governing the localization on the genome of specific epigenetic marks as well as the compaction and decompaction of chromatin, which regulates accessibility of the transcription machinery to chromatin. Histone ubiquitination also serves as signaling molecules for other downstream regulators of transcription, which modulates transcription both directly and indirectly. Part of this concept includes histone cross-talk, where those regulators of transcription integrate signals of multiple distinct histone modifications on the same or nearby histones to generate a phenotype due to the composite signals [7]. Therefore, nucleosome modification has a fundamental function in silencing and activation of transcription. Most histone ubiquitination occurs as a monoubiquitination, but polyubiquitin chains have also been observed. There are a number of small ubiquitin-like modifier (SUMO) proteins that share structural resemblance to ubiquitin and play some similar roles [8]. SUMO proteins come in a variety of isoforms with varying capacity for chain formation and are conjugated to substrates in a similar manner as ubiquitination.

2. Ubiquitin in DNA damage repair

Survival of organisms and their cells depends on stability and integrity of DNA, but maintaining this integrity is a challenge for cells because they are constantly subjected to DNA damage from a variety of sources [9, 10]. DNA damage can cause disease and prevent faithful transfer of genetic information from one generation to the next. DNA double strand breaks (DSB) present a difficult problem to correct since there may be no template to guide error-free repair. In the DNA damage response, cells arrest the cell cycle and activate repair machinery. There are two primary methods eukaryotic cells use to repair DSBs: nonhomologous end joining (NHEJ) and homologous recombination (HR) [11]. The NHEJ pathway occurs throughout the cell cycle (except during mitosis) and is performed more commonly, but it is error-prone since it does not utilize a template [12]. By contrast, HR is only active during S and G2 phases of the cell cycle and because it uses the template in a sister chromatid, the repair has higher fidelity. The faulty repair observed in NHEJ can cause chromosomal rearrangements and mutations, leading to cancer susceptibility. In the following paragraphs, we highlight the roles of a variety of ubiquitin ligases and ubiquitin binding proteins to regulate the DNA damage response.

2.1 Initiation of DSB repair

When DSBs occur, ionizing radiation-induced foci form from genome-localized high concentrations of repair machinery with a host of bound factors necessary for DSB repair. To form these ionizing radiation-induced foci, histones near the damage site become modified with K63-linked polyubiquitin chains via the action of the E2 Mms2-Ubc13 and the E3 ligases RNF168 and RNF8 [13]. These K63 chains serve as markers for recruitment of downstream repair proteins and as transcriptional repressors to prevent propagation of problems caused by broken DNA strands.

RNF8 has a forkhead-associated domain that binds to ionizing radiation induced foci following a cascade of events starting with the MRE11-RAD50-NBS1 (MRN) complex binding to the DSB end, followed by ATM phosphorylation of a variant

H2A histone called H2AX. This phosphorylated H2AX-serine139 is known as γH2AX [14]. Sequentially, MDC1 (mediator of DNA damage checkpoint 1) binds, [15–17], and MDC1 serves as scaffold protein near sites of DNA damage, which it localizes to by using its BRCT (BRCA carboxyl terminus) domains to recruit RNF8. Following RNF8 recruitment, RNF8 ubiquitinates the linker histone H1, and thereby recruits RNF168 via ubiquitin binding domains (UBDs) binding to the ubiquitin mark [13]. RNF168, in turn, ubiquitinates histone H2A (**Figure 1A**).

Once RNF168 has been recruited by RNF8-mediated H1 ubiquitination, it can recognize its own H2A ubiquitination mark due to the UBD on its C-terminus, allowing self-propagation of the DSB repair response [18]. RNF168-mediated H2A is capable of monoubiquitination of H2AK13–15 [19]. Chain elongation by RNF168 is atypical: as mentioned, most DSB-related ubiquitination is comprised of polyubiquitin chains linked by K63, but RNF168, when overexpressed, creates K27 linked chains instead of linkages via K63 [20]. H2AK15ub is important for the recruitment of downstream factors, most importantly 53BP1, which promotes NHEJ [21]. RNF8 and RNF168 recruit more E3 ubiquitin ligases through direct interaction with HERC2 and via their ubiquitin ligase activity, but these other E3 ligases stimulate DSB repair as scaffolds, rather than as ubiquitin ligases [22]. For example, BRCA1 and BARD1 have E3 ligase function, but their role in DSB repair is independent of their ubiquitination activity [23, 24].

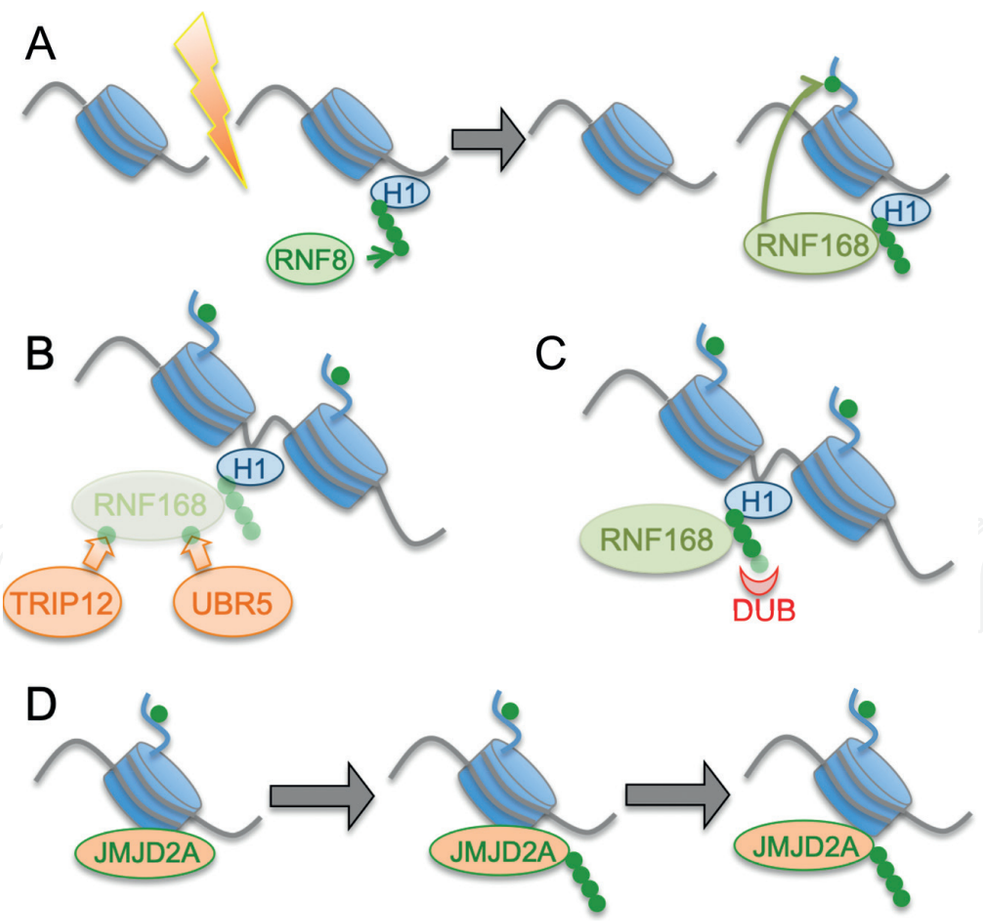


Figure 1.
Ubiquitination of chromatin regulates DSB repair. (A) Following RNF8 recruitment by ATM-phosphorylated MDC1, RNF8 ubiquitinates histone H1, which is necessary for RNF168 recruitment. (B) Ubiquitination regulates expression of DSB factors. UBR5 and TRIP12 are E3 ligases, which ubiquitinate RNF168 to target them for proteosomal degradation. (C) Deubiquitinases break down polyubiquitin chains, removing ubiquitin signals that recruit DSB repair factors. (D) Ubiquitination leads to factor removal from DSB sites. JMJD2A is recognized by 53BP1 and is involved in 53BP1 recruitment. When JMJD2A is ubiquitinated, segregase activity removes it from DSB sites.

In NHEJ, DNA broken strand ends are bound by the Ku70-Ku80 heterodimer, which in turns allows recruitment of the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) [25, 26]. Following phosphorylation of DNA-PKcs, the DNA ends are trimmed to make them ready for ligation. This trimming plays a major role in why NHEJ is error-prone and is more damaging to cells than HR. DNA ligase IV and its associated proteins are responsible for ligating these trimmed ends and finishing the NHEJ process.

HR is dependent on a series of posttranslational modifications, including ubiquitination, reviewed in [27]. These modifications control a carefully orchestrated system that recruits and displaces DNA repair factors at multiple different sites during the process of HR. The role of ubiquitin in regulating HR is predicated on the following well-established model of HR. When broken DNA strands are detected, the MRN complex begins end clipping via the endonuclease activity of MRE11, along with other proteins such as EXO1, CtIP, and DNA2, creating extensive ssDNA near the break site. In addition to end clipping, the MRN complex recruits ATM. Resection of these ends at the break site allows binding by single-strand DNA binding protein, replication protein A (RPA), which is then displaced by RAD51, which enables the important difference between HR and NHEJ. RAD51 searches for homology and locates a template with which to use to repair the damaged strand. Because HR depends on this template, it can only occur during the S and G2 phases of the cell cycle, when the damaged DNA has already been replicated and the copied DNA serves as a template strand. Choosing which repair pathway, NHEJ or HR, a cell uses for repair is an important determiner of genome stability and involves complex regulatory processes involving ubiquitin.

Regulation of DSB repair depends on a variety of ubiquitination writers, readers and erasers. The readers and erasers require specific UBDs to recognize their specific conformations of ubiquitination. There are more than 20 unique types UBDs that are found in mammalian proteins and a few of these are enriched in proteins associated with DNA damage repair: ubiquitin-interacting motif (UIM), ubiquitin-binding zinc finger (UBZ) and motif interacting with ubiquitin (MIU) [28]. These proteins possess multiple UBDs that each bind to the target cooperatively to increase specificity and affinity. In several ubiquitin ligases, including RNF168, RNF169, RAD18, and RAP80, specificity is increased further because they possess ligand-binding regions adjacent to their UBDs, allow cooperative binding at higher specificities and affinities than otherwise possible [29].

2.2 RNF8 and RNF168 regulation

RNF8 and RNF168 are important regulators of the entire DSB repair response, and require careful regulation themselves. Because these ubiquitin ligases can recognize the same mark they create, their action can cause over-recruitment and overproduction of ionizing radiation-induced foci without control mechanisms. Overproduction of these ionizing radiation-induced foci would cause widespread transcriptional repression across much larger portions of the genome than necessary. One mechanism of limitation is direct ubiquitination of RNF168 by TRIP12 and UBR5, ubiquitin ligases that recognize certain N-terminal domains and direct proteosomal protein degradation on those targets, causing a decrease in the amount of RNF168 in the cell (**Figure 1B**) [30].

Another method of RNF8 and RNF168 regulation occurs via deubiquitinating enzymes (DUBs) (**Figure 1C**). Ubiquitin-specific protease 3 (USP3) has been shown to increase genomic instability and lead to spontaneous tumors when depleted in mice. This finding was supported when it was shown that UPS3 depletion led to increased levels of H2A ubiquitination, indicating the role of properly

regulated H2A ubiquitination in DSB repair [31]. USP3, USP16 and USP44 and their family members also deubiquitinate H2A and thus downregulate the DSB response. One of the significant differences between these DUBs is their affinity for different lysine chains. For example, USP3 is known to target the H2A protein K13 and K15 sites that RNF168 targets as well as the K119 and K120 monoubiquitination sites of PRC1. In contrast, PSMD14 deubiqutinates K63-linked poly-ubiquitin, a different RNF8-RNF168 mediated target [32, 33]. Another DUB, USP14, downregulates DSB repair by decreasing RNF168 ubiquitination and RNF168-mediated ubiquitin signals in the setting of inhibited autophagy [34].

RNF8 and RNF168-mediated DSB repair can also be downregulated by phosphorylation. During mitosis, chromatin structure undergoes massive changes and most nuclear processes pause. Phosphorylation of RNF8 and MDC1 (a scaffold protein) prevents DSB repair from occurring during mitosis by blocking their interaction with 53BP1.

2.3 Pathway choice

As there are two primary pathways of DSB repair that function through entirely different mechanisms, NHEJ versus HR, cells must decide which pathway to activate. HR requires a perfect homolog to use as a template across the DSB, and for this reason HR should only function following replication of the DNA during S phase or in G2. Cells must have built-in mechanisms to suppress HR during G1, since during this stage of the cell cycle HR would use inappropriate nonhomologous DNAs as template and thus be mutagenic [35]. In addition, during mitosis, NHEJ is repressed by phosphorylation and inactivation of 53BP1 and RNF8 by the cyclin-dependent kinase CDK1 [36]. This inactivation of DNA repair is protective against chromosomal fusions at telomeres that would lead to aneuploidy. The structure of the DSB is also a factor in the decision of cells to engage in which pathway. In general, more complex DSB structures cannot be repaired via NHEJ and require the more time-consuming HR pathway [37, 38].

The RNF8-RNF168 ubiquitination pathway plays an important role in determining which DSB pathway will predominate in a cell. BRCA1 stimulates HR but antagonizes NHEJ, and conversely 53BP1 antagonizes HR and promotes NHEJ [39, 40]. Ubiquitination via RNF8 and RNF168 leads to retention of 53BP1 and BRCA1 at DSB sites and the balance between these two proteins is the primary decision point between NHEJ and HR. 53BP1 functions to inhibit end resection that is necessary for HR, allowing only NHEJ to be performed. 53BP1 binds H2AK15ub (catalyzed by RNF168) through its own ubiquitin-dependent recruitment motif and also possesses Tudor domains, which recognize H4K20me2 [21, 41]. It is proposed that H4K20me2 is the signal that 53BP1 recognizes to promote its recruitment at DSB sites. RNF8–168 ubiquitinates other proteins that impact the pathway selection, including JMJD2A, JMJD3A, and L3MBTL1. When these three factors are ubiquitinated, they are released from H4K20me2. Through the action of JMJD2A, JMJD3A, and L3MBTL1 vacating H4K20me2, 53BP1 can bind freely without competition (**Figure 1D**) [42, 43]. H4K20me2 is another mechanism supporting the cell cycle dependent decision point between pathways. As S phase continues and more DNA is replicated, H4K20me2 becomes diluted between the two replicated DNA strands, reducing 53BP1 capacity for binding through its Tudor domains, and shifting the balance away from NHEJ to HR [44]. While 53BP1 is bound, RIF1 (and other factors) are recruited to 53BP1 and inhibit resection of the DNA ends. These factors are responsible for replacing BRCA1 at DSB sites, inhibiting HR. BRCA1, in turn, inhibits RIF1 binding at these sites, inhibiting NHEJ.

The antagonist of 53BP1 is BRCA1, which promotes HR over NHEJ by way of supporting RAD51 activity. BRCA1 is a scaffold with activity that also depends on

RAP80, which has a ubiquitin binding domain suspected to recognize RNF8-RNF168-mediated H2A ubiquitination and is a part of the BRCA1-A complex, which it targets to these sites of RNF8-RNF168 ubiquitination [45, 46]. However, RAP80 depletion does not lead to the expected abolishment of HR, but to increased HR activity. To explain this, it has been proposed that RAP80 is functioning to sequester BRCA1 away from DSB sites, so when RAP80 is removed, BRCA1 recruitment to DSB sites is unregulated, leading to the over activity of HR [47]. This would suggest an unknown regulator of BRCA1 recruitment to sites requiring HR activity. BRCA1 also antagonizes 53BP1 by recruiting phosphorylated UHRF1, an E3 ligase that ubiquitinates RIF1, which is bound to 53BP1 at DSB sites. Ubiquitinated RIF1 becomes displaced, reducing 53BP1 mediated repression of DNA end resection [48]. Cockayne syndrome B (CSB) protein has been proposed as fulfilling this role because it seems to antagonize 53BP1 support of NHEJ [49, 50]. In addition, when CSB is removed, DNA damage responses have been limited and CSB has been found accumulating at DSB sites.

One mechanism of HR regulation is the proteasome-mediated degradation of factors important to HR, such as CtIP [51]. The decision point depends on the resection of broken DNA ends by factors such as CtIP in HR. During S and G2, when HR is stimulated, CtIP is ubiquitinated by RNF138 to promote CtIP localization to DSB sites [52]. RNF138 also ubiquitinates the NHEJ factor Ku80 during S phase, causing the Ku70/80 heterodimer to dissociate from DSB sites, and thus suppressing NHEJ during S and G2 phases [53].

HR is also inhibited during G1 via ubiquitination of PALB2, a factor involved in HR along with BRCA1 [54]. This ubiquitination by the E3 ligase complex CRL3 is in the BRCA1-binding domain of PALB2 and sterically blocks the two proteins from binding. The ubiquitination is antagonized by USP11, a DUB that is degraded during G1. Thus, while the balance of CRL3 to USP11 is heavily in favor of CRL3 in G1, the BRCA1-binding site on PALB2 is ubiquitinated, and so the PALB2-BRCA1 interaction is blocked, preventing BRCA1 activity, and therefore, HR.

3. Ubiquitin and transcription elongation

A second key process regulated by ubiquitination of chromatin is transcription. As mRNA is transcribed, one protein complex that associates with the elongating RNA Polymerase II (RNAPII) is the Polymerase Associated Factor 1 Complex (PAF1) (**Figure 2A**) [55–57]. The PAF1 complex regulates RNAPII related transcription elongation and posttranscriptional events and is conserved across many species.

The PAF1 complex in humans is comprised of six protein subunits: PAF1, CDC73, CTR9, LEO1, RTF1, and WDR61 [58]. WDR61 is not present in yeast, although it is present in humans. Cells without PAF1 or CTR9 have a global decrease in protein levels and exhibit growth defects [59, 60]. The complex is found on active genes, at levels directly relating to transcription [61–63]. PAF1 binds directly to the carboxy terminal domain (CTD) of RNAPII via the Cdc73 subunit when the RNAPII CTD becomes phosphorylated via CDK9, and via Rtf1 binding along with the elongation factor Spt5 [64–66]. The localization and recruitment of PAF1C to specific sites on active genes is dependent upon many factors; in humans, PAF1C recruitment is highest at the transcription start site (TSS) or immediately (~2 nucleosomes) following the TSS [62, 67].

The PAF1 complex regulates transcription and the chromatin template to ensure its readiness for transcription. The impact of PAF1C on human chromatin was first established from its role in the ubiquitination of histone H2B at K120 (**Figure 2B**) [68]. H2Bub is an important epigenetic mark that is associated with both activating and deactivating transcription, though its primary effect on chromatin is

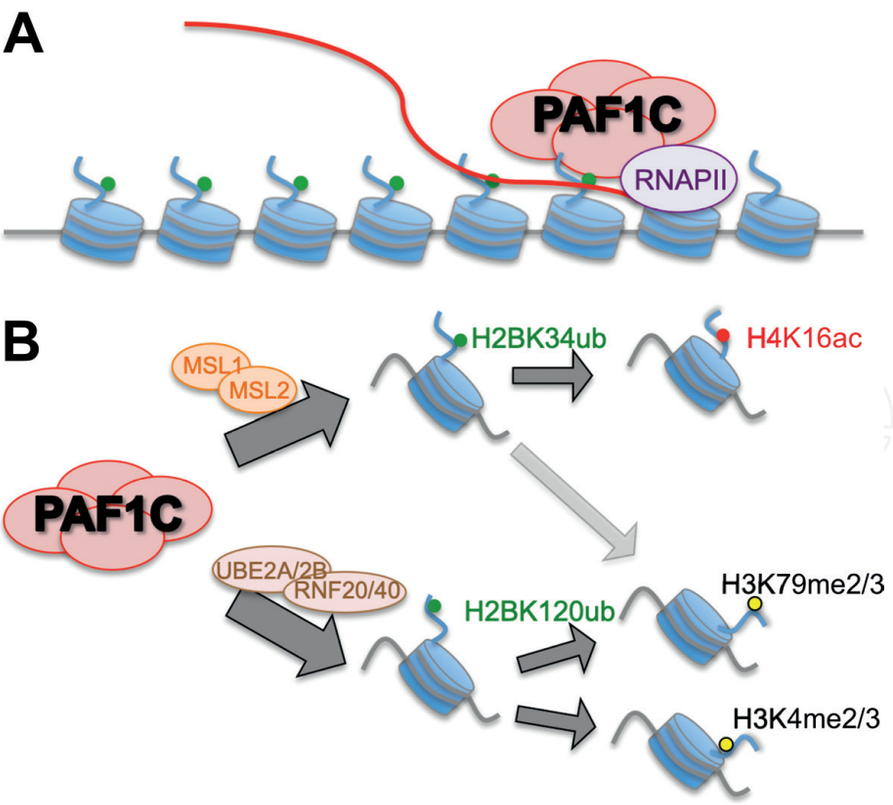


Figure 2.
The PAF1 complex ubiquitinates histone H2B during transcription elongation. (A) PAF1C ubiquitinates histones following transcription by RNAPII and elongation of mRNA. (B) The PAF1C controls multiple histone modifications, including non-ubiquitination events. The two histone ubiquitinations controlled by PAF1C are H2BK34ub and H2BK120ub. Ubiquitination of H2BK120ub is stimulated by PAF1C in concert with the UBE2A/2B and RNF20/40 heterodimers. H2BK120ub is necessary for H3K79me2/3 and H3K4me2/3, catalyzed by additional methyltransferases. The other histone ubiquitination, H2BK34ub, is created by PAF1C interaction with the MSL1/2 complex and promotes H4K16ac via MOF activity. The faded arrow represents crosstalk by which H2BK34ub regulates H3K4 and H3K79 methylation.

disrupting compaction [69]. The ubiquitination at H2BK120 is catalyzed by the E3 ligase complex containing RNF20/40, which interacts directly with the PAF1 complex, and is conjugated by the E2 UBE2A/2B. In addition to H2B ubiquitination at K120, monoubiquitination can also occur at K34 on H2B, a separate mark placed by the heterodimeric E3 ligase, MSL1/2 [70]. Both H2BK120ub and H2BK34ub stimulate histone methylation at H3K79 and H3K4, which has been demonstrated via decreases in both H2BK120ub and H2BK34ub following PAF1 depletion [71]. H2BK120ub is necessary for H3K4 and H3K79 trimethylation, while H2BK34ub functions through *trans*-tail crosstalk to regulate these methylations. The mechanism of this effect was revealed by experiments showing that depletion of PAF1 caused a decrease in RNF20/40 and MSL1/2 association to chromatin, indicating the role of PAF1C as promoting localization of these E3 ligases, the method by which PAF1C regulates H2Bub [71]. RNF20/40 and MSL1/2 each depend on the specific binding to chromatin by the other ligase and the corresponding histone mark, demonstrating how much interdependence exists between the two co-regulated ligases. This interaction has multiples sources. CDK9 is a kinase that promotes PAF1C association to chromatin, but is itself dependent on both PAF1C-mediated chromatin marks for its chromatin association [71].

Deregulation of this pathway and H2B monoubiquitination is commonly found in cancers. This can occur via multiple mechanisms, such as mutations in CDC73, one of the components of PAF1C, which has been observed in multiple cancers [72]. In addition, silencing of expression by methylation of the RNF20 promoter and RNF20 enhancers has also been observed in many breast cancers [73]. However,

it has also been observed that decreased levels of H2Bub have also been shown to be associated with decreased tumor growth, an apparent contradiction to H2Bub as a cancer-causing mutation [73]. Deregulation of a mark can also occur from overactive removal; there are several DUBs responsible for H2Bub deubiquitination, including USP3, USP7, USP12, USP22, USP44, USP46, USP49 [74–78]. Upregulation of these DUBs can cause similar phenotypes as RNF20 depletion. Errors in H2B monoubiquitination lead to errors in chromatin structure on scales larger than the aberrantly ubiquitinated nucleosome [69].

Dysregulation of RNF20 and concomitant H2B ubiquitination has been linked to a wide variety of cancer pathways. One method for H2Bub depletion leading to cancer occurs via H2Bub regulated inflammation and the interaction with NF-κB [79]. Inflammation involves the production of cytokines and chemokines that promote oncogenic activity and NF-κB is a key regulator of the inflammatory system. Reduction in H2Bub has been shown to lead to activated NF-κB, and thus its downstream regulation targets, leading to active inflammation in mice. This was indeed shown to lead to increased colorectal cancer in these animals. Ovarian cancers also display H2Bub dysfunction. One study found that the majority of high grade serous ovarian cancers show global decreases in H2Bub [80]. The most deadly cancer worldwide is lung cancer, and one of the more common forms of lung cancer is lung adenocarcinoma. In human lung adenocarcinomas, H2Bub decreases have been associated with increased cancer burden and a less differentiated carcinoma, a marker of poor prognosis [79].

Mixed lineage leukemia is a classification of cancers that depend on the MLL1 gene, and rearrangements of MLL1 have been shown to be dependent on RNF20 and its role in chromatin regulation [81]. Cells lacking RNF20 showed decreased tumor growth [82]. This role of RNF20 allowing cancer progression is contrary to its role in protecting against the above cancers, but does serve to highlight the fundamental role that H2B ubiquitination plays in maintenance of chromatin.

4. Polycomb repressive complex

While the preceding section described how histone H2B is ubiquitinated at multiples sites as a part of active transcription process, this section describes the ubiquitination of histone H2A, which has an opposite impact on gene expression. The polycomb repressive complex 1 (PRC1) monoubiquitinates H2A at lysine 119. H2AK119ub is a repressive mark, associated with inactive transcription by condensing chromatin, making it less accessible by transcription factors and associated machinery [83]. This repressive mark is only the most common role of PRC1, as it has also been shown to have diverse effects that have the overall impact of permanently silencing chromatin as part of the differentiation process. The two polycomb group (PcG) complexes, PRC1 and PRC2, modify chromatin to repress transcription and lead to the methylation of the promoter DNA to stably repress transcription at targeted genes. PRC2 contains the methyltransferase EZH2, which methylates histone H3 on lysine 27, H3K27me3. This review will focus on PRC1. PRC2 is necessary for targeted recruitment of PRC1, as experiments have shown that knockdown of PRC2 components also decrease PRC1-mediated H2A ubiquitination. The ubiquitination function of PRC1 is antagonized by the last form of polycomb repressive complex, polycomb repressive deubiquitinase (PR-DUB), which deubiquitinates H3K119 [84, 85]. The complimentary actions of PRC1 and PR-DUB to regulate H2AK119ub suggests the fundamental role it plays in repressing transcription.

PRC1 complexes exist in a number of different forms that have the same general structure, with different proteins occupying each position. The core of each complex contains a RING protein and a polycomb group RING finger protein, which bind via

their RING domains [86]. This core serves as the base for further PRC1 proteins to bind. There are two possible RING proteins, RING1A and RING1B, and six possible polycomb group ring finger (PCGF) proteins, PCGF1–6. All eight of these proteins possess a RAWUL (RING finger and WD40 Ubiquitin-like) domain somewhere in their structure, which bind additional proteins [87, 88]. These additional proteins include chromobox and human polyhomeotic homolog (HPH) proteins, which, when included in the PRC1, form what is known as canonical PRC1 [89]. It was previously assumed canonical PRC1 performed the H2Aub function that is associated with PRC1, but it is now known that noncanonical PRC1 also plays an important role in gene regulation [90]. Between the variability of chromobox, HPH, RING, and PCGF proteins, there are well over 100 unique combinations of canonical PRC1 complexes that can form. This diversity plays an important role in the diverse targeting and functions exhibited by PRC1. The PCGF member of the complex binds specifically to a variety of proteins, which are responsible for targeting and regulation of the PRC1 activity [91]. Accordingly, PCGF RAWUL domains exhibit more selective binding than their counterpart RAWUL domains on the RING1A or RING1B protein. The importance of the RING domains is that the RING proteins are E3 ubiquitin ligases, responsible for the primary activity of H2AK119 ubiquitination. However, PCGF-4 (also known as BMI-1) and RING1A both do not directly ubiquitinate H2A, as only RING1B directly ubiquitinates H2A. Instead complexes containing BMI-1 and RING1A serve to promote the RING1B E3 ligase activity [92].

4.1 PRC1 function

RING1B monoubiquitinates H2AK119 as part of the PRC1 activity following PRC2 methylation at H3K27 (**Figure 3**). PcG-regulated genes show aberrant transcriptional levels following removal of PRC1 via RING1B knockdown using shRNA [93]. PRC1-related ubiquitination and subsequent gene silencing is associated with multiple silencing contexts. PcG proteins are known to occupy and thus regulate, developmental genes, X-chromosome inactivation, and parent of origin imprinting. The most widely accepted model of the activity of PRC1-mediated inactivation of target genes is through chromatin compaction. Promoters of active genes become compacted in the setting of PRC1 action, preventing RNA polymerases from accessing the targeted gene, and therefore preventing transcription. This concept has been supported by *in vitro* experiments and *in vivo* experiments showing decreased nuclease digestion at genes with PRC1-mediated compaction of chromatin [94]. While the fact that PRC1-mediated ubiquitination of H2A leads to diminished transcription via chromatin compaction is indisputable, the mechanism is currently unclear. It has been shown that PRC1 does not have a role in regulating chromatin accessibility, only to nucleosome spacing and occupancy. Identifying the direct mechanism by which PRC1-mediated H2Aub inhibits transcription needs further elucidation.

Targeting of PcG complexes occurs via Polycomb Response Elements (PREs), which are DNA elements that cannot be recognized by any PcG protein because PcG proteins do not appear to possess any sequence specific DNA binding subunits [95]. While several proteins have been suggested to have a role in recognizing the PRE and enabling recruitment of PcG complexes, none have been confirmed to be sufficient to mediate PcG recruitment alone, suggesting the PcG recruitment is dependent on the interactions of several proteins coordinately creating a stable protein-DNA complex [96]. In addition to protein-DNA interactions to promote PcG recruitment, protein-protein interactions are important. PRC2 has histone methyltransferase function, methylating H3K27. PRC1 can directly recognize the H3K27me3 mark produced by PRC2 [97, 98]. Similarly, PRC2 can bind to H2Aub. This complementary interaction can serve to support preservation of PcG silencing

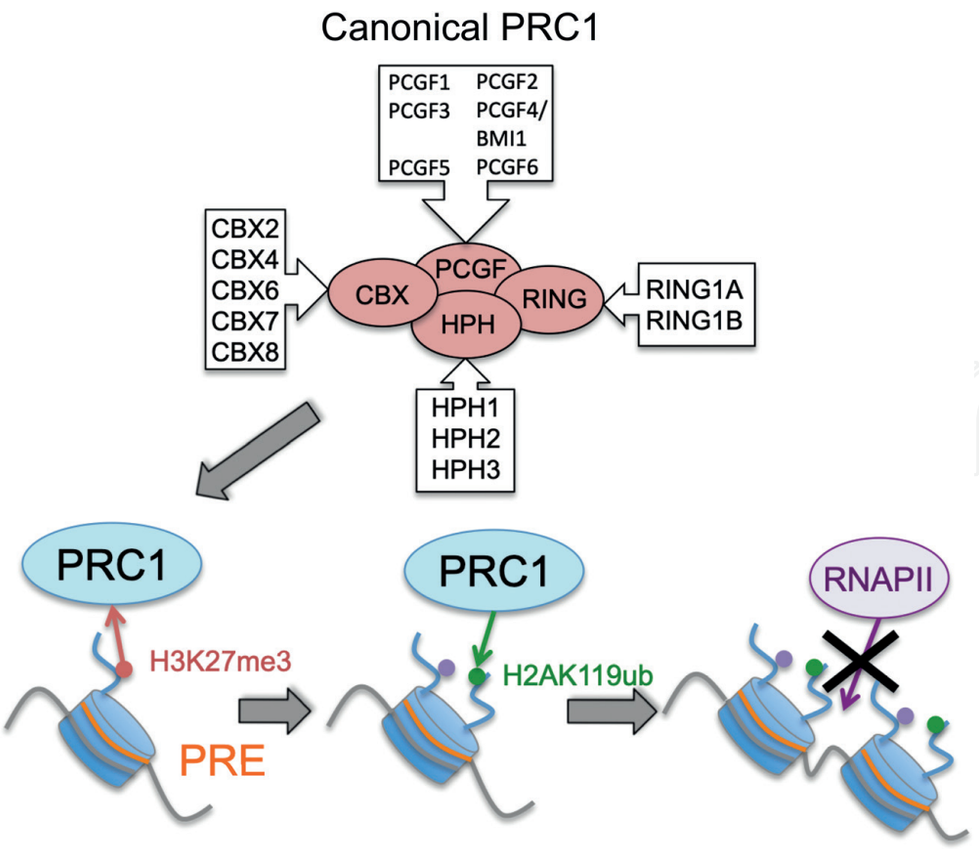


Figure 3.
Canonical PRC1 ubiquitinates H2AK119. Canonical PRC1 contains a RING protein and a PCGF protein, as defines PRC1, but is only called canonical in the presence of a Chromobox protein and a human polyhomeotic protein. This canonical PRC1 is responsible for the primary function of PRC1, ubiquitination of H2AK119. Recognition occurs at Polycomb Response Elements (PRE) containing specific DNA sequences that have been methylated by PRC2 at H3K27me3. PRC1-mediated H2AK119ub is a repressive mark, leading to decreased accessibility of targeted genes by transcription machinery, leading to inactivation of targeted genes.

across disruptive events to the genome, such as DNA synthesis, when histones are divided between the sister chromosomes [99]. Therefore, the most commonly accepted model of PcG recruitment is that PREs are recognized by adaptor proteins, which recruit PRC2 to promoters, which methylates H3K27. PRC1 recognizes H3K27me3 and is recruited to ubiquitinate H2AK119.

In addition to the repressive effect of PRC1, it has been shown to have activating effects on transcription. PcG proteins mediate their activity by regulating genome architecture [100]. It has been reported in mouse embryonic stem cells that RING1A and RING1B organize genes into three-dimensional interaction networks, which maintains interactions between promoters in the network. When PRC1 was removed, promoter-enhancer interactions were affected, leading to activation of affected promoters and increased transcription. This supports the compaction-based theory of PRC1 transcriptional repression and provides a mechanism for this activity. Deep sequencing of ChIP experiments against selected PRC1 proteins, including both RING1A and RING1B, has shown their enrichment at active transcriptional sites in human fibroblasts [101]. This experiment also showed cell-type specific binding of PRC1. RING1B, the primary ubiquitin ligase involved PRC1-mediated H2Aub, has been found associated with Aurora B kinase at active promoters in lymphocytes, while RING1B knockdown decreased transcription at these sites, suggesting an important activating function of RING1B [102]. Cells that have had conditionally-inactivated RING1A and RING1B, and thus inactivated PRC1, exhibit errors in DNA replication [103]. Slow elongation and even stalling of replication forks has been observed in these cells in specific pericentromeric regions.

These S phase errors were rescued by monoubiquitination events, suggesting the role of RING1A/B in S phase is dependent on their function as ubiquitin ligases. In breast cancer, RING1B has been found at oncogene promoters, playing an activating role and promoting cancer development and metastasis [104]. All these activating effects of PcG proteins suggest that there is much not known about the diverse array of proteins involved in PRC1 and that understanding PRC1 function may explain many previously unknown chromatin regulation events.

5. Ubiquitin as a mitotic bookmark

Mitotic bookmarks are a mechanism of dividing cells that maintain the epigenetic and transcriptional state despite the rigors demanded by the mitosis process [105]. Although epigenetic marks persist from mother cell to daughter cell, the compaction of the genome during mitosis requires many epigenetic marks to be temporarily erased. Every mitosis, the epigenome is bookmarked, erased, and reestablished as the cells reenter G1. Cells need a mechanism allowing them to reestablish cell specific chromatin marks after they have been erased during mitosis. The mechanism cells use for "remembering" chromatin architecture is mitotic bookmarking, whereby specific molecules or proteins are found on promoters of genes that enable memory of the chromatin state before mitosis. By definition, bookmarks must be deposited in association with active genes before or at the beginning of mitosis, persist throughout mitosis, and transmit gene expression memory to the cell after mitosis (**Figure 4**). These mitotic bookmarks involve multiple chromatin changes, including histone modifications and histone variants. Transcription factors also make up a large number of mitotic bookmarks. Many of those transcription factors bookmark specific subsets of genes. One example of a highly selective mitotic bookmark is Brd4, which is found only on the transcription start sites of genes that are expressed at the end of mitosis and beginning of G1 [106]. Mitotic bookmarks can also regulate a specific biological process, as in the case of GATA1, which occupies locations on key hematopoietic genes during mitosis [107]. Ubiquitin has also been found to play a role as a mitotic bookmark, but while many mitotic bookmarks are specific for certain genes or pathways, the mitotic bookmark ubiquitination appears to be generally acting at genes with high transcriptional activity.

This novel role of ubiquitin was first identified through a variant of ChIP-seq experiments that found ubiquitin present on certain sites during mitosis that were previously not described [108]. Those experiments showed that during interphase, ubiquitin was present on the chromatin of transcribed regions of transcriptionally active genes, consistent with the known function of PAF1C. The novel observation was that ubiquitinated chromatin associated proteins were bound to promoters during mitosis, contrasted to interphase, when ubiquitin localized to the promoter was absent. The fundamental difference between interphase and mitosis was a shift of the ubiquitin detected near promoters of the same genes that were previously ubiquitinated on their transcribed regions. For example [109], the GAPDH gene is heavily ubiquitinated over the gene body during G1 (**Figure 4A**, indicated in blue), while during mitosis that ubiquitination over the gene body is absent but ubiquitination is detected over the promoter (**Figure 4A**, gold) [108]. The ubiquitinated promoter sites were consistently ubiquitinated at 150 bp upstream of the transcription start sites, suggesting a specific function relating each of these promoters. The fact that this ubiquitin bookmark was identified on promoters of active genes further supported the conclusion that this novel finding of ubiquitin in mitosis was playing a role as a bookmark, not just an incidental observation. This conclusion was also supported by the fact that the promoter-associated, mitotic ubiquitin was found on the same genes as

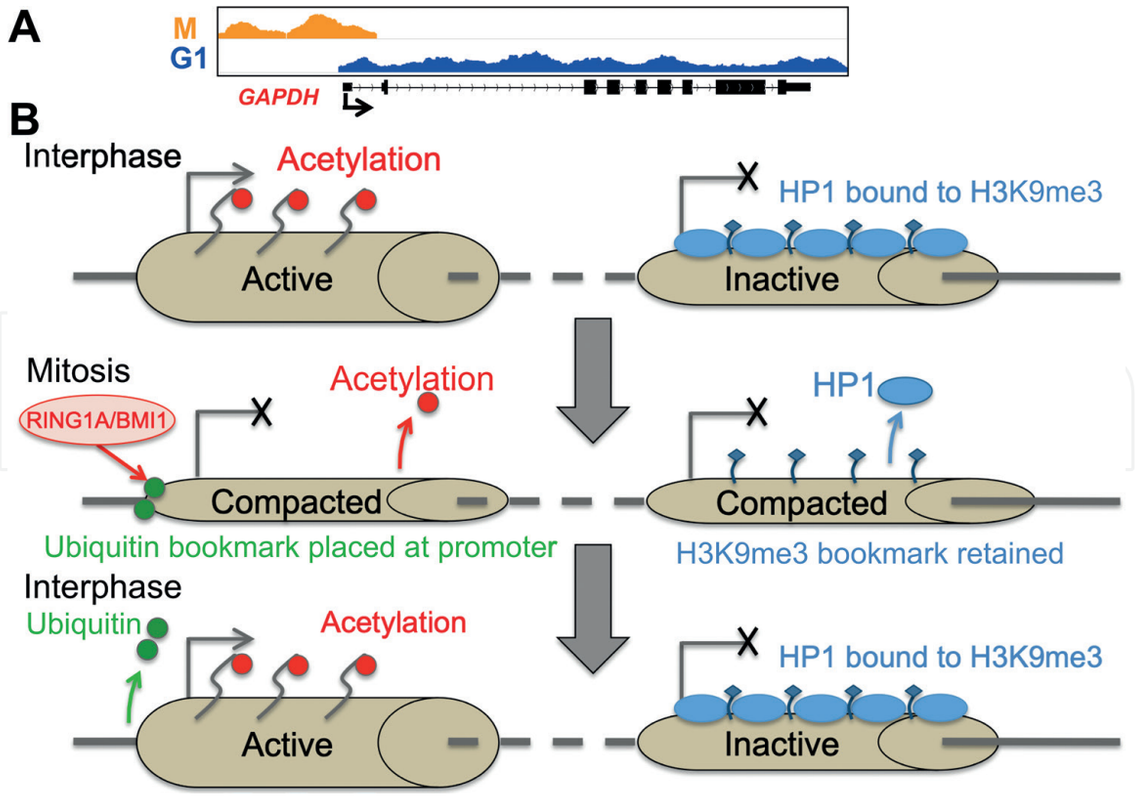


Figure 4.
A mitotic bookmark containing ubiquitin is necessary for maintaining the active chromatin state after completion of mitosis. (A) An example of measuring the ubiquitin density on an active gene (GAPDH) during mitosis (gold) and during G1 (blue) [108]. The localization of ubiquitin on the chromatin shift from over the gene body during G1 through G2 to over the promoter during mitosis. (B) Model for mitotic bookmarking. During interphase, active genes have active chromatin has associated epigenetic marks, such as acetylation, whereas repressive marks as heterochromatin protein 1 (HP1) are present on inactive genes. When cells transition into mitosis, all of those marks are removed. Instead, mitotic bookmarks are placed on the active genes to enable cells to "remember" which genes were active. Ubiquitin is found on promoters of a subset of active genes and is necessary to support transcription following completion of mitosis. This ubiquitin bookmark is dependent on the E3 ligases RING1A and BMI-1. The HP1 localization is bookmarked by H3K9me3.

those with PAF1C-associated transcriptional H2B-ubiquitin. The association between these two forms of ubiquitination suggests that the ubiquitin bookmark is dependent upon transcription, as is PAF1C-associated H2B-ubiquitin. However, the mechanisms underlying creation of these ubiquitination marks is different, suggesting that there is no direct relationship between these two transcription-associated marks.

The presence of the ubiquitin bookmark is dependent upon the E3 ligases RING1A and BMI-1, which are both parts of the polycomb repressive complex discussed previously [109]. Surprisingly, RING1B, the primary E3 ligase involved in the PRC1 primary function has no role in bookmark ubiquitination, suggesting that the role of RING1A and BMI-1 in creating the ubiquitin bookmark is independent of their role in the PRC1 complex. However, this remains untested and what factors interact with RING1A and BMI-1 when they are involved in bookmark ubiquitination is an open question. With the discovery of the ligases responsible for the ubiquitin bookmark, it was possible to test experimentally how the process is regulated. RING1A depletion caused a decrease in phosphorylated RNAPII at promoters; the phosphorylated RNAPII was used as a surrogate for transcriptional activity, indicating that the ubiquitin bookmark was necessary for the proper transcription of the bookmarked genes, one of the criteria for mitotic bookmark. So far, what is known about the ubiquitin bookmark is that is present during mitosis, responds to changes in gene expression, and that it impacts transcription. These are the basic requirements to satisfy the definition of a mitotic bookmark.

Beyond the basic outline of a bookmark, there are relatively few facts known about the ubiquitin bookmark. Its localization to promoters of active genes is notable, but only a subset of active genes is found to be bookmarked, suggesting the input of more factors than active transcription and ubiquitination via RNF20/40 with PAF1C. What these factors are, even what kind of signal they are, is still unknown. Only a little is also known about the mechanism by which bookmark ubiquitination affects transcription. H3K4me3 is a histone modification known to associate with sites of active transcription. H3K4me3 has been observed decreasing when RING1A has been depleted, suggesting that the lack of ubiquitin bookmarking has caused a decrease in transcription by decreasing H3K4me3 as a signal for transcription [109]. If this phenomenon is unique to H3K4me3 or if it is common to other histone modifications correlating to active transcription is unknown. Further studies to determine this mechanism will inform the importance of this bookmark and how broadly it affects cellular function and differentiation.

Currently the exact composition of the ubiquitin bookmark is undetermined. The ubiquitin bookmark must have a substrate protein that is directed to the sites identified to have ubiquitin bookmarks, and serves as the connection to chromatin. The signal has been detected via affinity-tagged ubiquitin molecules that do not discriminate between mono- or polyubiquitin, nor between the different lysine residues with which the polyubiquitin chain could be constructed. Ubiquitin is the only known component of the bookmark, but there must be other components yet to be identified. Given that most chromatin-associated proteins dissociate from the genome during mitosis, there are fewer candidates for the substrate than would be in interphase, though the possibility exists that a protein previously unknown to remain during mitosis exhibits that ability as part of the ubiquitin bookmark.

Another aspect for expanding our understanding of the ubiquitin bookmark is expanding the finding of the ubiquitin bookmark to other cell lines. Thus far, all the prior work done on the ubiquitin bookmark has been done in HeLa cells, a common model system. As the ubiquitin bookmark has not been demonstrated in any other cell lines, nor in tissue samples, questions of the ubiquity of the bookmark are raised. It is formally possible that the ubiquitin bookmark is unique to HeLa cells or just cancerous cell lines and is not apparent in tissues in organisms. Obviously, the role of the ubiquitin bookmark is only relevant in an actively dividing cell, although the majority of cells in living tissues are postmitotic. Detecting the presence or lack thereof of the ubiquitin bookmark in other cell lines should be one of the most pressing directions of current research. The significance of the ubiquitin bookmark as a relatively new and poorly understood process suggests a new field in epigenetics, or at least a significant evolution in our understanding of mitotic bookmarks as primarily transcription factors that control limited selections of genes to a much larger, potentially genome-wide scale.

6. Concluding remarks

Chromatin is dynamically modified as genes are silenced, as genes are expressed, as DNA damage is repaired, and as the genome is prepared for cell division. In this review, we highlighted the diverse roles of ubiquitin in each process. Understanding the complexity of the ubiquitin system is a monumental task of which the scientific community is only scratching the surface. Four important processes were reviewed here, and these processes are paramount to proper cellular functions and deregulation is generally implicated in cancers.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

[1] Eytan E, Ganoth D, Armon T, Hershko A. ATP-dependent incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(20):7751-7755

[2] Schulman BA, Harper JW. Ubiquitin-like protein activation by E1 enzymes: The apex for downstream signalling pathways. Molecular Cell. 2009;**10**(5):319-331

[3] Ye Y. Building ubiquitin chains: E2 enzymes at work. Nature Reviews. Molecular Cell Biology. 2011;**10**(11):755-764

[4] Smit JJ, Sixma TK. RBR E3-ligases at work. EMBO Reports. 2014;**15**(2):142-154

[5] Deshaies RJ, Joazeiro CAP. RING domain E3 ubiquitin ligases. Annual Review of Biochemistry. 2009;**78**(1):399-434. Available from: http://www.annualreviews.org/doi/10.1146/annurev.biochem.78.101807.093809

[6] Swatek KN, Komander D. Ubiquitin modifications. Cell Research. 2016;**26**(4):399-422

[7] Lee J, Smith E, Shilatifard A. The language of histone crosstalk. Cell. 2013;**142**(5):682-685

[8] Jürgen Dohmen R. SUMO protein modification. Biochimica et Biophysica Acta (BBA)—Molecular Cell Research. 2004;**1695**(1-3, 113):31

[9] Lindahl T. Instability and decay of the primary structure of DNA. Nature. 1993;**363**:709-715

[10] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2010;**461**(7267):1071-1078. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2906700&tool=pmcentrez&rendertype=abstract

[11] Kowalczykowski SC. An overview of the molecular mechanisms of recombinational DNA repair. Cold Spring Harbor Perspectives in Biology. 2015;**7**(11):a016410. Available from: http://cshperspectives.cshlp.org/lookup/doi/10.1101/cshperspect.a016410

[12] Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, et al. ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. The EMBO Journal. 2009;**28**(21):3413-3427

[13] Thorslund T, Ripplinger A, Hoffmann S, Wild T, Uckelmann M, Villumsen B, et al. Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature. 2015;**527**(7578):389-393

[14] Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Current Biology. 2000;**10**(15):886-895

[15] 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**(5):887-900

[16] Huen MS, Grant R, Manke I, Minn K, Yu X, Yaff MB, et al. The E3 ubiquitin ligase RNF8 transduces the DNA damage signal via an ubiquitin-dependent signaling pathway. Cell. 2007;**131**(5):901914

[17] 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. 2007;**318**(5856):1637-1640

[18] Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell. 2005;**123**(7):1213-1226

[19] Mattiroli F, Vissers JHA, Van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell. 2012;**150**(6):1182-1195. Available from: http://dx.doi.org/10.1016/j.cell.2012.08.005

[20] Gatti M, Pinato S, Maiolica A, Rocchio F, Prato MG, Aebersold R, et al. RNF168 promotes noncanonical K27ubiquitination to signal DNA damage. Cell Reports. 2015;**10**(2):226-238. Available from: http://dx.doi.org/10.1016/j.celrep.2014.12.021

[21] Fradet-Turcotte A, Canny MD, Escribano-Díaz C, Orthwein A, Leung CCY, Huang H, et al. 53BP1 is a reader of the DNA damage-induced H2A Lys15 ubiquitin mark. Nature. 2013;**499**(7456):50-54

[22] Bekker-Jensen S, Danielsen JR, Fugger K, Gromova I, Nerstedt A, Bartek J, et al. HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. Nature Cell Biology. 2010;**12**(1):80-86

[23] Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, et al. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. The Journal of Biological Chemistry. 2001;**276**(18):14537-14540

[24] Shakya R, Reid LJ, Reczek CR, Cole F, Egli D, Lin CS, et al. BRCA1 tumor suppression depends on BRCT phosphoprotein binding, but not its E3 ligase activity. Science. 2011;**334**(6055):525-528

[25] Fuentes L, Lebenkoff S, White K, Gerdts C, Hopkins K, Potter JE, et al. Phosphorylation of Ku dictates DNA double-strand break (DSB) repair pathway choice in S phase. Nucleic Acids Research. 2015;**93**(4):292-297

[26] Zhou Y, Lee J, Jiang W, Crowe J, Zha S, Paull T. Regulation of the DNA damage response by DNA-PKcs inhibitory phosphorylation of ATM. Molecular Cell. 2017;**65**(1):91-104

[27] Schwertman P, Bekker-Jensen S, Mailand N. Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. Nature Reviews Molecular Cell Biology. 2016;**17**(6):379-394. Available from: http://dx.doi.org/10.1038/nrm.2016.58

[28] Dikic I, Wakatsuki S, Walters KJ. Ubiquitin-binding domains from structures to functions. Nature Reviews Molecular Cell Biology. 2009;**10**(10):659-671. Available from: http://dx.doi.org/10.1038/nrm2767

[29] Panier S, Ichijima Y, Fradet-Turcotte A, Leung CCY, Kaustov L, Arrowsmith CH, et al. Tandem protein interaction modules organize the ubiquitin-dependent response to DNA double-strand breaks. Molecular Cell. 2012;**47**(3):383-395

[30] Gudjonsson T, Altmeyer M, Savic V, Toledo L, Dinant C, Grøfte M, et al. TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. Cell. 2012;**150**(4):697-709. Available from: http://dx.doi.org/10.1016/j.cell.2012.06.039

[31] Lancini C, van den Berk PCM, Vissers JHA, Gargiulo G, Song J-Y, Hulsman D, et al. Tight regulation of ubiquitin-mediated DNA damage

response by USP3 preserves the functional integrity of hematopoietic stem cells. Journal of Experimental Medicine. 2014;**211**(9):1759-1777. Available from: http://www.jem.org/lookup/doi/10.1084/jem.20131436

[32] Butler LR, Densham RM, Jia J, Garvin AJ, Stone HR, Shah V, et al. The proteasomal de-ubiquitinating enzyme POH1 promotes the double-strand DNA break response. EMBO Journal. 2012;**31**(19):3918-3934. Available from: http://dx.doi.org/10.1038/emboj.2012.232

[33] Kato K, Nakajima K, Ui A, Muto-Terao Y, Ogiwara H, Nakada S. Fine-tuning of DNA damage-dependent ubiquitination by OTUB2 supports the DNA repair pathway choice. Molecular Cell. 2014;**53**(4):617-630. Available from: http://dx.doi.org/10.1016/j.molcel.2014.01.030

[34] Sharma A, Alswillah T, Singh K, Chatterjee P, Willard B, Venere M, et al. USP14 regulates DNA damage repair by targeting RNF168-dependent ubiquitination. Autophagy. 2018;**14**(11):1976-1990. Available from: https://www.tandfonline.com/doi/full/10.1080/15548627.2018.1496877

[35] Karanam K, Kafri R, Loewer A, Lahav G. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Molecular Cell. 2012;**47**(2):320-329

[36] Orthwein A, Fradet-Turcotte A, Noordermeer SM, Canny MD, Brun CM, Strecker J, et al. Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. Science. 2014;**344**(6180):189-193

[37] Reynolds P, Anderson JA, Harper JV, Hill MA, Botchway SW, Parker AW, et al. The dynamics of Ku70/80 and DNA-PKcs at DSBs induced by ionizing radiation is dependent on the complexity of damage. Nucleic Acids Research. 2012;**40**(21):10821-10831

[38] Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A, et al. Factors determining DNA double-strand break repair pathway choice in G2 phase. The EMBO Journal. 2011;**30**(6):1079-1092

[39] Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, Van Der Gulden H, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nature Structural & Molecular Biology. 2010;**17**(6):688-695

[40] Bunting SF, Callén E, Wong N, Chen H, Polato F, Gunn A, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2011;**141**(2):243-254

[41] Mathias JR, Dodd ME, Walters KB, Yoo SK, Erik A, Huttenlocher A. Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in. DNA Repair. 2010;**33**(11):1212-1217

[42] Mallette FA, Mattiroli F, Cui G, Young LC, Hendzel MJ, Mer G, et al. RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. The EMBO Journal. 2012;**31**(8):1865-1878

[43] Acs K, Luijsterburg MS, Ackermann L, Salomons FA, Hoppe T, Dantuma NP. The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. Nature Structural & Molecular Biology. 2011;**18**(12):1345-1350. Available from: http://dx.doi.org/10.1038/nsmb.2188

[44] Pellegrino S, Michelena J, Teloni F, Imhof R, Altmeyer M. Replication-coupled dilution of H4K20me2 guides

53BP1 to pre-replicative chromatin. Cell Reports. 2017;**19**(9):1819-1831

[45] Hu Y, Scully R, Sobhian B, Xie A, Shestakova E, Livingston DM. RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. Genes & Development. 2011;**25**(7):685-700

[46] Leung JWC, Makharashvili N, Agarwal P, Chiu LY, Pourpre R, Cammarata MB, et al. ZMYM3 regulates BRCA1 localization at damaged chromatin to promote DNA repair. Genes & Development. 2017;**31**(3):260-274

[47] Lombardi PM, Matunis MJ, Wolberger C. RAP80, ubiquitin and SUMO in the DNA damage response. Journal of Molecular Medicine. 2017;**95**(8):799-807

[48] Zhang H, Liu H, Chen Y, Yang X, Wang P, Liu T, et al. A cell cycle-dependent BRCA1-UHRF1 cascade regulates DNA double-strand break repair pathway choice. Nature Communications. 2016;**7**:1-14. Available from: http://dx.doi.org/10.1038/ncomms10201

[49] Batenburg NL, Thompson EL, Hendrickson EA, Zhu X-D. Cockayne syndrome group B protein regulates DNA double-strand break repair and checkpoint activation. EMBO Journal. 2015;**34**(10):1399-1416. Available from: http://emboj.embopress.org/cgi/doi/10.15252/embj.201490041

[50] Pascucci B, Fragale A, Marabitti V, Leuzzi G, Salvatore Calcagnile A, Parlanti E, et al. CSA and CSB play a role in the response to DNA breaks. Oncotarget. 2018;**9**(14):11581-11591. Available from: http://www.oncotarget.com/fulltext/24342

[51] Ferretti LP, Himmels SF, Trenner A, Walker C, Von Aesch C, Eggenschwiler A, et al. Cullin3-KLHL15 ubiquitin ligase mediates CtIP protein turnover to fine-tune DNA-end resection. Nature Communications. 2016;**7**:1-16. Available from: http://dx.doi.org/10.1038/ncomms12628

[52] Schmidt CK, Galanty Y, Sczaniecka-Clift M, Coates J, Jhujh S, Demir M, et al. Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. Nature Cell Biology. 2015;**17**(11):1458-1470

[53] Ismail IH, Gagné JP, Genois MM, Strickfaden H, Mcdonald D, Xu Z, et al. The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. Nature Cell Biology. 2015;**17**(11):1446-1457

[54] Orthwein A, Noordermeer SM, Wilson MD, Landry S, Enchev RI, Sherker A, et al. A mechanism for the suppression of homologous recombination in G1 cells. 2016;**528**(7582):422-426

[55] Mueller CL, Jaehning JA. Ctr9 , Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Molecular and Cellular Biology. 2002;**22**(7):1971-1980

[56] Costa PJ, Arndt KM. Synthetic lethal interactions suggest a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. Genetics. 2000;**156**(2):535-547

[57] Squazzo SL, Costa PJ, Lindstrom DL, Kumer KE, Simic R, Jennings JL, et al. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. The EMBO Journal. 2002;**21**(7):1764-1774

[58] Zhu B, Mandal SS, Pham AD, Zheng Y, Erdjument-Bromage H, Batra SK, et al. The human PAF complex coordinates transcription with events downstream of RNA synthesis. Genes & Development. 2005;**19**(14):1668-1673

[59] Betz JL, Chang M, Washburn TM, Porter SE, Mueller CL, Jaehning JA. Phenotypic analysis of Paf1/RNA polymerase II complex mutations reveals connections to cell cycle regulation, protein synthesis, and lipid and nucleic acid metabolism. Molecular Genetics and Genomics. 2002;**268**(2):272-285

[60] Mueller CL, Porter SE, Hoffman MG, Jaehning JA. The Paf1 complex has functions independent of actively transcribing RNA polymerase II. Molecular Cell. 2004;**14**(4):447-456

[61] Chen FX, Woodfin AR, Gardini A, Rickels RA, Marshall SA, Smith ER, et al. PAF1, a molecular regulator of promoter-proximal pausing by RNA polymerase II. Cell. 2015;**162**(5):1003-1015. Available from: http://dx.doi.org/10.1016/j.cell.2015.07.042

[62] Van Oss SB, Shirra MK, Bataille AR, Wier AD, Yen K, Vinayachandran V, et al. The histone modification domain of Paf1 complex subunit Rtf1 directly stimulates H2B ubiquitylation through an interaction with Rad6. Molecular Cell. 2016;**64**(4):815-825

[63] Yu M, Yang W, Ni T, Tang Z, Nakadai T, Zhu J, et al. RNA polymerase II—Associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. Science. 2015;**350**(6266):1383-1386

[64] Zaborowska J, Egloff S, Murphy S. The pol II CTD: New twists in the tail. Nature Structural & Molecular Biology. 2016;**23**(9):771-777. Available from: http://dx.doi.org/10.1038/nsmb.3285

[65] Mbogning J, Nagy S, Pagé V, Schwer B, Shuman S, Fisher RP, et al. The PAF complex and Prf1/Rtf1 delineate distinct Cdk9-dependent pathways regulating transcription elongation in fission yeast. PLoS Genetics. 2013;**9**(12):29-31

[66] Liu Y, Warfield L, Zhang C, Luo J, Allen J, Lang WH, et al. Phosphorylation of the transcription elongation factor Spt5 by yeast Bur1 kinase stimulates recruitment of the PAF complex. Molecular and Cellular Biology. 2009;**29**(17):4852-4863. Available from: http://mcb.asm.org/cgi/doi/10.1128/MCB.00609-09

[67] Mayer A, Lidschreiber M, Siebert M, Leike K, Söding J, Cramer P. Uniform transitions of the general RNA polymerase II transcription complex. Nature Structural & Molecular Biology. 2010;**17**(10):1272-1278. Available from: http://www.nature.com/doifinder/10.1038/nsmb.1903

[68] Ng HH, Dole S, Struhl K. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. The Journal of Biological Chemistry. 2003;**278**(36):33625-33628

[69] Fierz B, Chatterjee C, McGinty RK, Bar-Dagan M, Raleigh DP, Muir TW. Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. Nature Chemical Biology. 2011;**7**(2):113-119. Available from: http://dx.doi.org/10.1038/nchembio.501

[70] Wu L, Zee BM, Wang Y, Garcia BA, Dou Y. The RING finger protein MSL2 in the MOF complex is an E3 ubiquitin ligase for H2B K34 and is involved in crosstalk with H3 K4 and K79 methylation. Molecular Cell. 2011;**43**(1):132-144. Available from: http://dx.doi.org/10.1016/j.molcel.2011.05.015

[71] Wu L, Li L, Zhou B, Qin Z, Dou Y. H2B ubiquitylation promotes RNA pol II processivity via PAF1 and pTEFb. Molecular Cell. 2014;**54**(6):920-931

[72] Wang P, Tan M, Zhang C, Morreau H, Teh BT. HRPT2, a tumor suppressor gene for hyperparathyroidism–jaw tumor syndrome. Hormone and Metabolic Research. 2005;**37**(6):380-383

[73] Prenzel T, Begus-Nahrmann Y, Kramer F, Hennion M, Hsu C, Gorsler T, et al. Estrogen-dependent gene transcription in human breast cancer cells relies upon proteasome-dependent monoubiquitination of histone H2B. Cancer Research. 2011;**71**(17):5739-5753

[74] Nicassio F, Corrado N, Vissers JHA, Areces LB, Bergink S, Marteijn JA, et al. Human USP3 is a chromatin modifier required for S phase progression and genome stability. Current Biology. 2007;**17**(22):1972-1977

[75] Van Der Knaap JA, Kumar BRP, Moshkin YM, Langenberg K, Krijgsveld J, Heck AJR, et al. GMP synthetase stimulates histone H2B deubiquitylation by the epigenetic silencer USP7. Molecular Cell. 2005;**17**(5):695-707

[76] Joo HY, Jones A, Yang C, Zhai L, Smith AD IV, Zhang Z, et al. Regulation of histone H2A and H2B deubiquitination and xenopus development by USP12 and USP46. The Journal of Biological Chemistry. 2011;**286**(9):7190-7201

[77] Fuchs G, Shema E, Vesterman R, Kotler E, Wolchinsky Z, Wilder S, et al. RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. Molecular Cell [Internet]. 2012;**46**(5):662-673. Available from: http://dx.doi.org/10.1016/j.molcel.2012.05.023

[78] Zhang Z, Jones A, Joo H-Y, Cao Y, Chen S, Erdjument-Bromage H, et al. USP49 deubiquitinates histone H2B and regulates pre-mRNA intron splicing. Genes & Development. 2013;**27**:1581-1595

[79] Tarcic O, Pateras IS, Cooks T, Shema E, Kanterman J, Ashkenazi H, et al. RNF20 links histone H2B ubiquitylation with inflammation and inflammation-associated cancer. Cell Reports. 2016;**14**(6):1462-1476. Available from: http://dx.doi.org/10.1016/j.celrep.2016.01.020

[80] Dickson KA, Cole AJ, Gill AJ, Clarkson A, Gard GB, Chou A, et al. The RING finger domain E3 ubiquitin ligases BRCA1 and the RNF20/RNF40 complex in global loss of the chromatin mark histone H2B monoubiquitination (H2Bub1) in cell line models and primary high-grade serous ovarian cancer. Human Molecular Genetics. 2016;**25**(24):5460-5471

[81] Wang E, Kawaoka S, Yu M, Shi J, Ni T, Yang W, et al. Histone H2B ubiquitin ligase RNF20 is required for MLL-rearranged leukemia. Proceedings of theNational Academy of Sciences. 2013;**110**(10):3901-3906. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1301045110

[82] Garrido Castro P, Van Roon EHJ, Pinhanços SS, Trentin L, Schneider P, Kerstjens M, et al. The HDAC inhibitor panobinostat (LBH589) exerts in vivo anti-leukaemic activity against MLL-rearranged acute lymphoblastic leukaemia and involves the RNF20/RNF40/WAC-H2B ubiquitination axis. Leukemia [Internet]. 2018;**32**(2):323-331. Available from: http://dx.doi.org/10.1038/leu.2017.216

[83] Aranda S, Mas G, Di Croce L. Regulation of gene transcription by polycomb proteins. Science Advances. 2015;**1**(11):1-15

[84] Bach SV, Hegde AN. The proteasome and epigenetics: Zooming in on histone modifications. Biomolecular Concepts. 2016;**7**(4):215-227

[85] Chittock EC, Latwiel S, Miller TCR, Müller CW. Molecular architecture of polycomb repressive complexes. Biochemical Society Transactions. 2017;**45**(1):193-205. Available from: http://biochemsoctrans.org/lookup/doi/10.1042/BST20160173

[86] Satijn DP, Otte AP. RING1 interacts with multiple polycomb-group proteins and displays tumorigenic activity. Molecular and Cellular Biology. 1999;**19**(1):57-68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9858531%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC83865

[87] Bezsonova I, Walker JR, Bacik JP, Duan S, Dhe-Paganon S, Arrowsmith CH. Ring1B contains a ubiquitin-like docking module for interaction with Cbx proteins. Biochemistry. 2009;**48**(44):10542-10548

[88] Sanchez-Pulido L, Devos D, Sung ZR, Calonje M. RAWUL: A new ubiquitin-like domain in PRC1 ring finger proteins that unveils putative plant and worm PRC1 orthologs. BMC Genomics. 2008;**9**:1-11

[89] Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisis F, et al. PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. Molecular Cell. 2012;**45**(3):344-356

[90] Morey L, Aloia L, Cozzuto L, Benitah SA, Di Croce L. RYBP and Cbx7 define specific biological functions of polycomb complexes in mouse embryonic stem cells. Cell Reports. 2013;**3**(1):60-69. Available from: http://dx.doi.org/10.1016/j.celrep.2012.11.026

[91] Junco S, Wang R, Gaipa J, Taylor A, Schirf V, Gearhart MD, et al. Structure of the polycomb group protein PCGF1 (NSPC1) in complex with BCOR reveals basis for binding selectivity of PCGF homologs. Structure. 2014;**21**(4):665-671

[92] Gray F, Cho HJ, Shukla S, He S, Harris A, Boytsov B, et al. BMI1 regulates PRC1 architecture and activity through homo- and hetero-oligomerization. Nature Communications. 2016;**7**:13343

[93] Yu M, Mazor T, Huang H, Huang HT, Kathrein KL, Woo AJ, et al. Direct recruitment of polycomb repressive complex 1 to chromatin by core binding transcription factors. Molecular Cell. 2012;**45**(3):330-343. Available from: http://dx.doi.org/10.1016/j.molcel.2011.11.032

[94] Bell O, Schwaiger M, Oakeley EJ, Lienert F, Beisel C, Stadler MB, et al. Accessibility of the Drosophila genome discriminates PcG repression, H4K16 acetylation and replication timing. Nature Structural & Molecular Biology. 2010;**17**(7):894-900

[95] Dorafshan E, Kahn TG, Schwartz YB. Hierarchical recruitment of polycomb complexes revisited. Nucleus. 2017;**8**(5):496-505. Available from: https://doi.org/10.1080/19491034.2017.1363136

[96] Kassis J, Brown J. Polycomb group response elements in Drosophila and vertebrates. Advances in Genetics. 2012;**81**:83-118. Available from: http://europepmc.org/abstract/MED/23419717

[97] Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by polycomb and HP1 chromodomains. Genes & Development. 2003;**17**(15):1870-1881

[98] Kalb R, Latwiel S, Baymaz HI, Jansen PWTC, Müller CW, Vermeulen M, et al. Histone H2A monoubiquitination promotes histone H3 methylation in polycomb repression. Nature Structural & Molecular Biology. 2014;**21**(6):569-571. Available from: http://dx.doi.org/10.1038/nsmb.2833

[99] Laprell F, Finkl K, Müller J. Propagation of polycomb-repressed chromatin requires sequence-specific recruitment to DNA. Science. 2017;**356**(6333):85-88

[100] Schoenfelder S, Sugar R, Dimond A, Javierre B, Furlan-magaril M, Segonds-pichon A, et al. Europe PMC funders group polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. 2016;**47**(10):1179-1186. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4847639/pdf/emss-67387.pdf

[101] Pemberton H, Anderton E, Patel H, Brookes S, Chandler H, Palermo R, et al. Genome-wide co-localization of polycomb orthologs and their effects on gene expression in human fibroblasts. Genome Biology. 2014;**15**(2):1-16

[102] Frangini A, Sjöberg M, Roman-Trufero M, Dharmalingam G, Haberle V, Bartke T, et al. The Aurora B kinase and the polycomb protein Ring1B combine to regulate active promoters in quiescent lymphocytes. Molecular Cell. 2013;**51**(5):647-661

[103] Bravo M, Nicolini F, Starowicz K, Barroso S, Cales C, Aguilera A, et al. Polycomb RING1A- and RING1B-dependent histone H2A monoubiquitylation at pericentromeric regions promotes S-phase progression. Journal of Cell Science. 2015;**128**(19):3660-3671. Available from: http://jcs.biologists.org/cgi/doi/10.1242/jcs.173021

[104] Chan HL, Beckedorff F, Zhang Y, Garcia-Huidobro J, Jiang H, Colaprico A, et al. Polycomb complexes associate with enhancers and promote oncogenic transcriptional programs in cancer through multiple mechanisms. Nature Communications. 2018;**9**(1):3377. Available from: http://www.nature.com/articles/s41467-018-05728-x

[105] Sarge K, Park-Sarge O. Mitotic bookmarking of formerly active genes: Keeping epigenetic memories from fading. Cell Cycle. 2009;**8**(6):818-823

[106] Dey A, Nishiyama A, Karpova T, McNally J, Ozato J. Brd4 marks select genes on mitotic chromatin and directs postmitotic transcription. Molecular Biology of the Cell. 2009;**20**(23):4899-4909

[107] Kadauke S, Udugama M, Pawlicki J, Achtman J, Jain D, Cheng Y, et al. Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. Cell. 2012;**150**(4):725-737

[108] Arora M, Zhang J, Heine GF, Ozer G, Liu HW, Huang K, et al. Promoters active in interphase are bookmarked during mitosis by ubiquitination. Nucleic Acids Research. 2012;**40**(20):10187-10202

[109] Arora M, Packard CZ, Banerjee T, Parvin JD. RING1A and BMI1 bookmark active genes via ubiquitination of chromatin-associated proteins. Nucleic Acids Research. 2015;**44**(5):2136-2144