



## Review

## RNF20–RNF40: A ubiquitin-driven link between gene expression and the DNA damage response

Yosef Shiloh<sup>a,\*</sup>, Efrat Shema<sup>b</sup>, Lilach Moyal<sup>a</sup>, Moshe Oren<sup>b</sup><sup>a</sup>The David and Inez Myers Laboratory for Cancer Genetics, Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel<sup>b</sup>Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel

## ARTICLE INFO

## Article history:

Received 28 June 2011

Revised 25 July 2011

Accepted 25 July 2011

Available online 4 August 2011

Edited by Ashok Venkitaraman and Wilhelm Just

## Keywords:

Histone H2B

Ubiquitylation

Gene regulation

DNA damage response

Chromatin organization

## ABSTRACT

The DNA damage response (DDR) is emerging as a vast signaling network that temporarily modulates numerous aspects of cellular metabolism in the face of DNA lesions, especially critical ones such as the double strand break (DSB). The DDR involves extensive dynamics of protein post-translational modifications, most notably phosphorylation and ubiquitylation. The DSB response is mobilized primarily by the ATM protein kinase, which phosphorylates a plethora of key players in its various branches. It is based on a core of proteins dedicated to the damage response, and a cadre of proteins borrowed temporarily from other cellular processes to help meet the challenge. A recently identified novel component of the DDR pathway – histone H2B monoubiquitylation – exemplifies this principle. In mammalian cells, H2B monoubiquitylation is driven primarily by an E3 ubiquitin ligase composed of the two RING finger proteins RNF20 and RNF40. Generation of monoubiquitylated histone H2B (H2Bub) has been known to be coupled to gene transcription, presumably modulating chromatin decondensation at transcribed regions. New evidence indicates that the regulatory function of H2Bub on gene expression can selectively enhance or suppress the expression of distinct subsets of genes through a mechanism involving the hPAF1 complex and the TFIIIS protein. This delicate regulatory process specifically affects genes that control cell growth and genome stability, and places RNF20 and RNF40 in the realm of tumor suppressor proteins. In parallel, it was found that following DSB induction, the H2B monoubiquitylation module is recruited to damage sites where it induces local H2Bub, which in turn is required for timely recruitment of DSB repair protein and, subsequently, timely DSB repair. This pathway represents a crossroads of the DDR and chromatin organization, and is a typical example of how the DDR calls to action functional modules that in unprovoked cells regulate other processes.

© 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

### 1. The DNA damage response: borrowing functional modules in an emergency

Cellular metabolism is controlled by numerous, interlocked signaling networks. These networks are constantly responding to internal and external stimuli related to the cell's normal life cycle and functions, and to threats to its well being. A major threat to cellular homeostasis is subversion of its genomic stability, which may lead to undue cell death or to neoplasia [1,2]. DNA damage caused by internal or external damaging agents is a major danger to the integrity of the cellular genome. The cellular defense system against this threat is the DNA damage response (DDR) – an elaborate signaling network activated by DNA damage, which swiftly modulates many physiological processes [3,4]. A strong trigger of the DDR is the DNA double-strand break (DSB) [5,6]. DSBs are

induced by ionizing radiation, radiomimetic chemicals, reactive oxygen species formed in the course of normal metabolism, and can also result from replication fork stalling. DSBs also accompany normal genomic transactions such as meiotic recombination and the rearrangement of the antigen receptor genes via V(D)J recombination. The major DSB repair pathways in eukaryotic cells are error-prone nonhomologous end-joining (NHEJ) [7], and a high-fidelity process based on homologous recombination (HR) between sister chromatids [8,9]. The overall cellular response to DSBs goes far beyond repair, however: this broad, powerful signaling network activates special cell cycle checkpoints, and swiftly and vigorously affects many cellular systems [3,4,10]. Thus, the DSB response is emerging as one of the cell's strongest and most comprehensive responses to stimuli.

The DSB response is a hierarchical process executed through a series of highly controlled steps [3,6]. It is based on a signal transduction mechanism that begins with *sensor* proteins, whose function is to sense the damage and/or chromatin alterations

\* Corresponding author. Fax: +972 3 6407471.

E-mail address: [yossih@post.tau.ac.il](mailto:yossih@post.tau.ac.il) (Y. Shiloh).

following damage induction, and transmit a signal to *transducers* [11]. The transducers are robust protein kinases which relay the signal to numerous downstream effectors involved in specific pathways. This initial response at the DSB sites and subsequent spreading of the DNA damage alarm, are characterized by extensive protein post-translational modifications (PTMs) [12] such as phosphorylation [13,14], ubiquitylation and SUMOylation [15], and acetylation [16]. One of the first steps in the DSB response is the recognition of the damage by the sensor complex MRE11-RAD50-NBS1 (MRN) [17]. The MRN complex binds to exposed DNA ends, unwinds the DNA molecule, and plays a major role in DNA end resection and in the activation and recruitment of the ATM kinase [17,18], which is the primary transducer of the DSB response [19]. In response to DSBs, ATM is rapidly activated and phosphorylates a plethora of key players in various damage response pathways, some of which are themselves protein kinases that phosphorylate additional substrates [13,14,20]. ATM belongs to a conserved family of PI3K-like protein kinases (PIKKs) [21] that includes, among others, the two DDR transducers: DNA-dependent protein kinase (DNA-PK) [22] and ataxia-telangiectasia- and Rad3-related (ATR) [23,24]. The three PIKKs share substrates in the DSB response but exhibit selective substrate specificities in response to different genotoxic stresses and to different DSB inducers [21,25].

In humans, germ-line mutations in genes encoding damage response proteins can lead to inherited genomic instability syndromes that usually involve some degree of tissue degeneration (most notably the nervous and immune systems), sensitivity to specific genotoxic stresses, cancer predisposition, and occasionally premature aging [2,26]. A prototype genomic instability syndrome is ataxia-telangiectasia (A-T), which is caused by *ATM* mutations that eliminate or inactivate the human ATM protein. The hallmarks of A-T are cerebellar atrophy, immunodeficiency, and marked cancer predisposition [27,28].

The DDR must deal with a serious threat by quickly fine tuning a vast array of cellular processes. This response cannot be dependent on the production of new proteins – a time consuming process that is out of the question in face of the danger to genome integrity. Instead, it must turn to existing resources and recruit them to the cause. It is becoming evident that while the cellular response to DNA damage relies on a core of DDR-dedicated proteins, many of its arms are based on temporary recruitment of proteins or functional modules that normally operate in other processes. Indeed, it has been shown that the DDR temporarily calls into action proteins that normally act in other contexts, such as gene expression [29–35], or RNA metabolism [36]. This means that the DDR pulls players from various cellular processes out of their regular context and assigns them temporary tasks under its command. In many cases this means physical relocation of these proteins to the DNA damage sites for a period of time. Here we summarize recent data from our and other labs on a functional module that is involved in a histone modification associated with chromatin reorganization in the transcription context – monoubiquitylation of histone H2B. In unprovoked cells, monoubiquitylated histone H2B (H2Bub) is an important regulator of gene expression. Our work recently showed that this regulation is confined to specific gene subsets, with direct implications for tumor suppression. On the other hand, we and others recently found that upon DSB induction, H2Bub becomes an important histone modification at the damage sites that is required for timely damage repair.

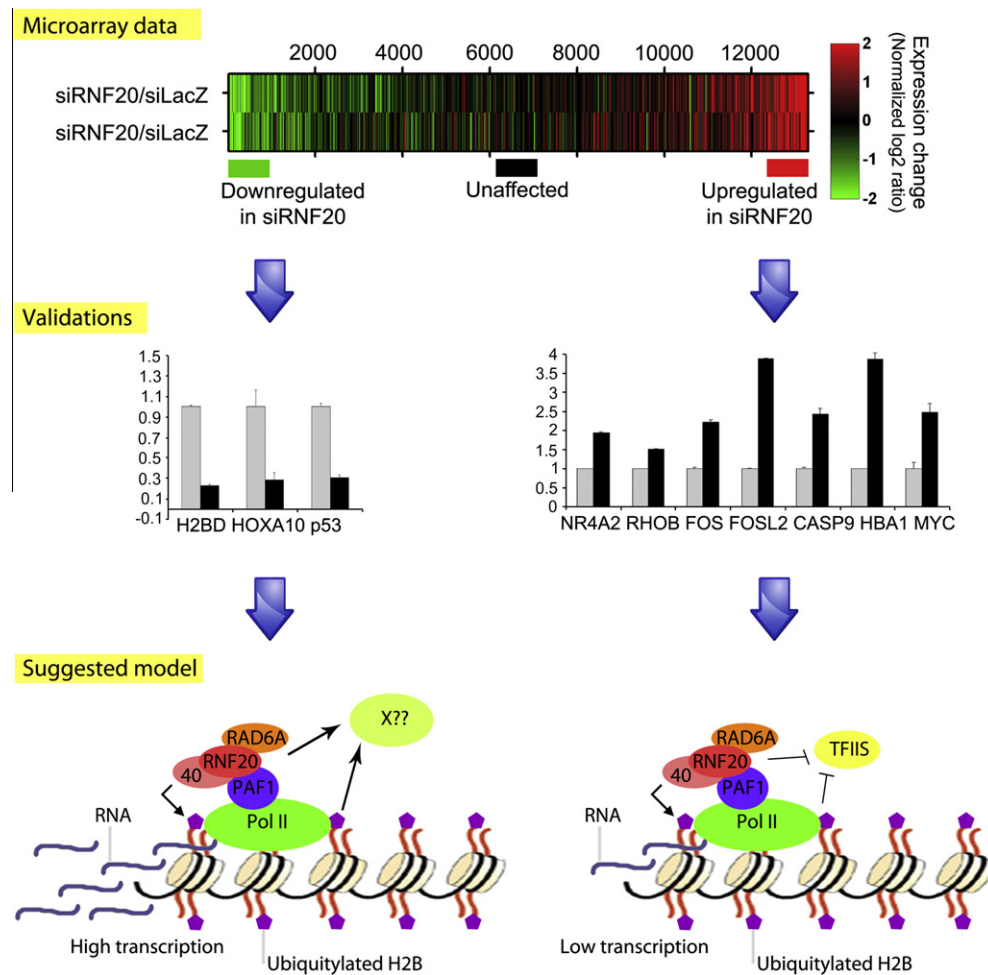
## 2. Monoubiquitylated histone H2B: from general to highly specific transcription regulator

Ubiquitylation of histone H2B in mammalian cells was identified over three decades ago [37], but more than two decades passed before the function of this modification in regulating

mammalian chromatin-associated processes was deciphered. The first breakthrough came with the identification of the budding yeast protein Bre1 [38,39], which, together with the ubiquitin-conjugating enzyme Rad6, serves as the E3 ligase in the monoubiquitylation of the yeast histone H2B on lysine 123 (K123) within transcribed chromatin [38–41]. Notably, H2B monoubiquitylation was subsequently found to be required for di- and trimethylation of lysine 4 and lysine 79 of histone H3 at transcribed chromatin [42–48]. This pathway is conserved from yeast to mammals, and is dependent on a host of additional proteins that converge at the elongating RNA Pol II [41,49–54]. Subsequently, the mammalian orthologs of the yeast Bre1, RNF20 and RNF40, were identified [55,56]. These two proteins form a tight heterodimer that acts as the major E3 ligase responsible for histone H2B monoubiquitylation on K120 in mammalian cells. Accordingly, depletion of either RNF20 or RNF40 leads to almost complete loss of H2Bub.

Another technical breakthrough came with the generation of antibodies that specifically recognize H2Bub [57]. These antibodies enabled the determination of H2Bub genomic distribution by chromatin immunoprecipitation (ChIP), followed by genomic DNA microarray analysis (ChIP/chip) or DNA sequencing (ChIP/Seq). This approach revealed a genome-wide positive correlation between expression levels and H2Bub levels within the transcribed region [53]. Moreover, Minsky et al. [53] demonstrated that H2Bub is selectively associated with the transcribed regions, but not the promoters, of expressed genes. These observations do not necessarily imply an active role for H2Bub in mammalian transcription regulation; nevertheless, such a role can be deduced from earlier studies in the budding yeast, where the use of strains harboring a point mutation in the ubiquitylation site of H2B revealed the direct involvement of H2Bub in transcriptional regulation [40,58,59].

While genetic manipulation of H2B is relatively easy in the yeast, which harbors only two H2B genes and can survive with even a single copy, this is impractical in mammals, whose genomes contain at least 17 H2B genes [60]. A more feasible strategy to investigate the role of H2Bub in mammalian transcription regulation is to deplete the cells of the responsible E3 ligase, RNF20, via RNAi [61]. Surprisingly, expression microarray analysis of human HeLa cells depleted of RNF20 revealed that, despite the positive correlation between H2Bub and expression levels [57], the expression of most genes was essentially independent on H2Bub levels and was unaffected by RNF20 depletion [57]. Thus, although H2Bub is positively correlated with the transcription process in general, it is not rate-limiting for the transcription of most genes. Nevertheless, a relatively modest subset of genes did show significant altered expression upon RNF20 depletion. Intriguingly, this subset comprised two distinct groups of genes that behaved in opposite ways: while approximately 3% of the HeLa cell genes exhibited reduced expression upon RNF20 depletion, and were thus termed “RNF20-dependent genes”, a similar percentage of genes were significantly upregulated, suggesting that for those genes RNF20 actually played a suppressive role (Fig. 1). There is still no direct evidence that the regulation of mammalian gene expression by RNF20 is due to modulation of H2B ubiquitylation; as mentioned above, obtaining such evidence is hindered by the large number of H2B genes. Nevertheless, several lines of evidence strongly suggest that this is indeed the case. For example, depletion of hRAD6A, the E2 enzyme that serves in RNF20-mediated H2B monoubiquitylation [52], leads to a strong drop in H2Bub levels and reproduces the transcriptional effects of RNF20 depletion on both gene groups [62]. A similar effect is seen upon knockdown of WAC1, a functional partner of RNF20–RNF40 and regulator of H2B ubiquitylation levels [63] (data not shown). These observations, together with the finding that the E3 ligase activity of RNF20 is essential for its ability to regulate gene expression [62],



**Fig. 1.** Transcription regulation by RNF20. Microarray data: Expression microarray analysis of the impact of RNF20 knockdown [61]. RNA was extracted from HeLa cells 48 hours after transfection with either RNF20 siRNA or LacZ siRNA, and hybridized to Affymetrix HG-U133A oligonucleotide arrays. Colors indicate the normalized  $\log_2$  expression ratio (siRNF20/siLacZ) for each gene. The two rows represent two independent biological repeats. Validations: qRT-PCR validation of selected genes from the microarray analysis. Shown are the RNF20-dependent genes *HIST1H2BD*, *HOXA10* and *p53*, and the RNF20-suppressed genes *NR4A2*, *RHOB*, *FOS*, *FOSL2*, *CASP9*, *HBA1* and *cMYC*. Suggested model: The complex of RNF20/RNF40/hRAD6A binds the elongating RNA polymerase II through the hPAF1 complex and ubiquitylates histone H2B. For RNF20-dependent genes, this enzymatic activity either leads to the recruitment of a positive elongation factor that facilitates transcription elongation (left), or alters the chromatin structure to increase its accessibility to the transcription apparatus. For RNF20-suppressed genes, which reside preferentially in closed chromatin and are associated with higher levels of paused Pol II, H2Bub inhibits the binding of TFIIIS to hPAF1 (right), thus interfering with transcriptional elongation along those genes.

support the conclusion that this transcriptional regulation is indeed mediated via H2B ubiquitylation.

The diverse regulatory effects of RNF20 depletion on different subgroups of genes implied that the role of RNF20 in transcription regulation might be more complex than initially suspected, and might depend on a crosstalk with other transcriptional regulators or chromatin modifiers. In an attempt to resolve this quandary and further characterize the mechanisms underlying the opposite responses of the two groups of genes to RNF20 depletion, the distribution patterns and levels of H2Bub on these genes were determined using H2Bub ChIP followed by high-throughput sequencing. While RNF20-dependent genes showed a pattern of H2Bub similar to that of the rest of the genome, the RNF20-suppressed genes were found to possess significantly higher levels of H2Bub associated with their transcribed regions [61]. This was particularly intriguing because these genes tend to be less expressed than RNF20-independent genes, contradicting the overall positive correlation between H2Bub and transcript levels. Moreover, further analysis revealed that the RNF20-suppressed genes, despite being expressed at relatively low levels, are associated with higher levels of RNA polymerase II (Pol II) throughout their transcribed region compared to RNF20-dependent and RNF20-independent genes.

The RNF20-suppressed genes were also found to have constitutively elevated levels of two well-studied histone modifications – trimethylation of lysine 4 on histone H3 (H3K4me3) and acetylation of histone H3 on lysines 9 and 14 (H3K9/14Ac) – which are widely believed to play a positive role in transcription regulation. On the other hand, genome-wide low resolution data of higher order chromatin structure [64] indicated that the RNF20-suppressed genes reside preferentially within “closed” chromatin [61]. This association with closed, less readily accessible chromatin may explain the observation that the RNF20-suppressed genes are lowly transcribed despite bearing positive histone marks and high constitutive levels of RNA polymerase II. The unfavorable chromatin structure may hinder transcriptional elongation, resulting in the “trapping” of many RNA Pol II molecules that are stalled, paused or slowed down along those genes. In that chromatin context, H2Bub may contribute as an inhibitory signal to transcriptional elongation. Indeed, subsequent work established that TFIIIS, a highly conserved transcription elongation factor [65], is involved in the mechanism by which RNF20/H2Bub suppresses transcription [62].

TFIIIS is an elongation factor that helps RNA Pol II pass through transcriptional blocks on DNA; it does so by reactivating arrested

RNA Pol II, through stimulating the endonucleolytic cleavage of nascent RNA by RNA Pol II [65–67]. hPAF1 is an RNA Pol II-associated factor that facilitates transcription elongation [68]. hPAF1 was shown to be required for H2B ubiquitylation, as the RNF20/RNF40 complex associates with Pol II through binding to hPAF1 [69]. It was recently shown that TFIIS binds directly to both RNA Pol II and hPAF1, and the direct interaction between hPAF1 and TFIIS results in their cooperative binding to RNA Pol II, exerting a strong synergistic effect on transcription elongation [68].

In line with these observations, the catalytic activity of the RNF20–RNF40–hRAD6A complex was found to interfere with TFIIS binding to the hPAF1 complex, presumably through H2B ubiquitylation (see model in Fig. 1). This interference resulted in selective impairment of the transcription elongation of RNF20-suppressed genes, but not of other genes. Upon depletion of RNF20, the amount of hPAF1-associated TFIIS was increased, augmenting transcription elongation. Furthermore, over-expression of TFIIS alone led to selective up-regulation of the RNF20-suppressed genes, suggesting that the inhibitory effect of H2Bub can be overcome by an excess of TFIIS [62].

While the inhibitory effects of H2Bub on the recruitment of TFIIS to chromatin and its association with hPAF1 are general, only the subset of RNF20-suppressed genes seems to benefit from the relief of that interference. It is conceivable that this subset of genes is excessively dependent on TFIIS for the transcription elongation process, owing to their residing in an unfavorable chromatin context that causes frequent RNA polymerase II pausing.

As mentioned above, microarray analysis identified a second, highly RNF20-dependent gene cluster (Fig. 1) whose transcription was significantly reduced upon RNF20 depletion. The mechanism by which RNF20/H2Bub stimulates the transcription of those genes remains obscure. It can be speculated that for those genes, RNF20/H2Bub is involved in recruiting some positive elongation factor(s) that is rate-limiting for those but not all other genes (Fig. 1). It is also plausible that H2Bub alters chromatin structure in the specific context of those genes and makes it more accessible to the transcription machinery [70]. Alternatively, RNF20 may affect the activity of some transcriptional regulators, especially since it was shown to directly bind and influence the transcriptional activity of the transcription factor and tumor suppressor protein p53 [55]. However, a significant enrichment for specific transcription factor binding motifs in RNF20-dependent genes could not be confirmed (data not shown).

While the various mechanisms by which RNF20 and H2B ubiquitylation regulate transcription are still not fully understood, the identity of the genes regulated by RNF20 provides strong clues to its importance for cellular homeostasis. The list of genes suppressed by RNF20 comprises numerous proto-oncogenes and proliferation-related genes, including many that are known to be induced by epidermal growth factor (EGF). Conversely, RNF20 is required for optimal expression and activity of p53, and several studies indicate that the p53 response to DNA damage is significantly attenuated in cells lacking RNF20 [55,61,63]. Consistent with the transcriptomic effect of RNF20 depletion, RNF20 exhibits a variety of *in vitro* and *in vivo* biological effects that suggest it may act as a tumor suppressor [61]. For example, cells depleted of RNF20 manifest increased migration, as well as an augmented response to EGF. Stable knockdown of RNF20 in mouse NIH3T3 cells leads to increased formation of colonies in soft agar, a strong indicator of neoplastic transformation. Moreover, such RNF20-depleted cells are more tumorigenic in mice. Of note, the *RNF20* promoter harbors a CpG island that is hypermethylated in a series of human breast cancer tumors, lending further credibility to the notion that attenuation of RNF20 activity and subsequent down-modulation of H2Bub promote a clinically relevant neoplastic process [61].

In contrast to RNF20, and in agreement with its inhibition by RNF20, TFIIS may contribute positively to cancer development. Depletion of TFIIS in cancer cell lines was shown to inhibit cellular proliferation [71], and TFIIS was found to directly regulate the transcriptional elongation of the anti-apoptotic gene *BCL2L1* encoding the Bcl-XL protein, an important contributor to the survival of many types of cancer cells [72,73]. In addition, the expression of the *TCEA1* gene, which encodes the major species of TFIIS, is elevated in a variety of human tumors [62].

In conclusion, H2Bub may restrain the expression of a subset of cancer-promoting genes, whereas TFIIS may positively regulate the same subset of genes. In normal cells, H2Bub probably gets the upper hand, keeping those genes in tight check. But, when this homeostatic interaction is perturbed by an increase in TFIIS expression or a decrease in H2Bub, a battery of pro-cancer genes may be unleashed, facilitating cancer development.

### 3. RNF20–RNF40 is called to emergency action upon DNA damage induction

The complex, multi-level organization of chromatin [74] makes it necessary to reorganize its configuration to allow DNA transactions. DNA repair is no exception, and dynamic changes in chromatin condensation and accompanying histone marks have been recognized as inevitable DDR pathways [15,75–80]. Here, too, the DDR relies on recruitment of existing players in the chromatin organization arena [32–34,75–79,81–85]. H2B monoubiquitylation was previously implicated in the DDR in the budding yeast [86–88]. Three recent studies – by Nakamura et al. [89], Moyal et al. [90] and Chernikova et al. [91] – demonstrated the importance of this process in the DSB response in human cells and provided further insight into its mechanistic aspects: H2Bub was found to be induced by DSBs, most likely at DSB sites, and required for timely repair of this lesion. The results of these studies will be summarized here collectively, with a few differences between them noted.

As is typical for a pathway involved in DSB repair, abrogation of histone H2B monoubiquitylation, either via RNF20–RNF40 depletion or over-expression of non-ubiquitylatable histone H2B, led to cellular hypersensitivity to DSB-inducing agents. Further examination revealed the expected reduction in the efficiency of DSB repair caused by compromising this pathway. The retardation in DSB repair was observed by indirect readouts such as the disappearance of the nuclear foci of phosphorylated histone H2AX ( $\gamma$ H2AX) or the 53BP1 protein, or by direct measurement of DSBs using the I-SceI assay [92,93] or the comet assay [94]. Indeed, H2Bub was found to be induced following DSB induction (the damage-induced H2B monoubiquitylation was seen more clearly when background H2Bub associated with active transcription was reduced using transcription inhibitors [90]). RNF20 was found to be recruited to the damaged sites, suggesting that this process occurred at these sites.

Importantly, RNF20 and RNF40 interacted physically with ATM [90] and NBS1 [89] (a member of the MRN complex [17]) and were found to be ATM targets. Not surprisingly, therefore, damage-induced H2B monoubiquitylation was ATM- and RNF20–RNF40-dependent. Furthermore, this reaction required the presence of ATM's phosphorylation sites in RNF20; replacement of endogenous RNF20 by ectopic, non-phosphorylatable protein abolished the process [90]. On the other hand, end resection and damage-induced release of H2B from chromatin were NBS1-dependent [89].

A natural corollary of these observations was examination of the two main DSB repair pathways, NHEJ and HRR, in cells in which damage-induced H2Bub was abrogated. Collectively, the three studies [89–91] showed that both pathways (including the critical preliminary step of DNA end resection) were retarded under these conditions, suggesting that the H2Bub-driven process occurred

upstream of both repair processes. Indeed, abrogation of damage-induced H2B monoubiquitylation led to reduced recruitment of players in both pathways to the damage sites [89,90]. Notably, H2Bub formation was not required for the initial recruitment of players in the “sensor” layer of the DDR [89,90] but was specifically needed for recruiting the repair proteins, and was thus independent of  $\gamma$ H2AX formation [89].

The temporary anchoring of RNF20–RNF40 at damage sites may be due to interactions with other damage response proteins that accumulate at these sites, including the basal interactions with ATM and NBS1 [89,90]. This temporary residence of RNF20–RNF40 at the damage sites is expected to induce H2Bub locally at these sites. It cannot be ruled out that, in parallel, the balance between the actions of this E3 ubiquitin ligase and opposing deubiquitylating proteases [95–98] may be shifted towards prolonged ubiquitylation of H2B at these sites. Importantly, the temporary service of the H2B monoubiquitylation module in the DDR was uncoupled from its role in transcription in undamaged cells [89,90]. Thus, while carrying out its regular enzymatic activity in the DDR context, it was presumably physically and functionally detached from its regular context. In the transcription context, H2B monoubiquitylation in yeast and mammals is dependent on the early steps in transcription initiation and elongation, and the corresponding E3 ligase closely interacts with many proteins that take part in this process, some of which associate directly with RNA Pol II [41,49–54,99,100]. It is not entirely clear how many of the proteins surrounding RNF20–RNF40 in the transcription context accompany this heterodimer to the DNA damage sites. hRAD6, the corresponding E2 ubiquitin conjugating enzyme, probably does. Some of the proteins that surround RNF20–RNF40 at transcription sites are involved in di- and trimethylation of Lys4 and Lys79 of histone H3 (reviewed in [41,101]), and H2Bub was shown to stimulate DOT1L – the Lys79 methylase [102]. Methylated Lys79 of histone H3 was previously suggested to be an anchor of the budding yeast damage response protein Rad9 [103] and its human ortholog 53BP1 [104]. Conflicting data argued later for binding of 53BP1 and its fission yeast ortholog to methylated Lys20 of histone H4 [105–107].

It was of interest, therefore, to examine whether the H2Bub-dependent methylations accompany H2B monoubiquitylation also at DSB sites. Here, some differences are noted between two of the studies. Moyal et al. [90] observed that, while the density of H3K4Me2 along laser-induced damage tracks was not altered, that of H3K79Me3 was, but this increase was not affected by abrogation of H2Bub induction. This observation was in agreement with previous suggestions that histone modifications implicated as 53BP1 anchors were not induced de novo at damage sites, but became exposed due to alterations in chromatin organization at these sites [104,106,108]. On the other hand, using chromatin immunoprecipitation analysis, Nakamura et al. [89] observed an RNF20-dependent increase in H3K4 methylation in the vicinity of localized DSBs induced by the restriction endonuclease I-SceI [92,93]. The different results of the two studies can be ascribed to the use of very different methods of DSB induction. The I-SceI-induced DSB may simulate better DSBs evoked during normal DNA transactions, compared to the extensive and heterogeneous laser-induced damage. Interestingly, Nakamura et al. [89] also noticed that the chromatin remodeling factor SNF2h, known to be recruited to transcribed sites via its interaction with methylated H3K4, was recruited to the I-SceI-induced DSB site, and SNF2h depletion abrogated the HRR pathway and the recruitment of HRR players. Their conclusion was that, at least in the HRR pathway, RNF20-dependent methylation of H3K4 may be part of the process, similar to the transcription context.

The physical role of H2Bub in facilitating the recruitment of repair proteins could be attributed to the effect of this histone PTM

on chromatin organization. Fierz et al. [70] recently showed that this modification interferes with compaction of the 30 nm chromatin fiber and leads to an open, biochemically accessible fiber conformation. This conformation may facilitate nucleosome dis- and reassembly during transcription and repair, enhancing the accessibility of DNA embedded in chromatin to the corresponding enzymes in these various DNA transactions.

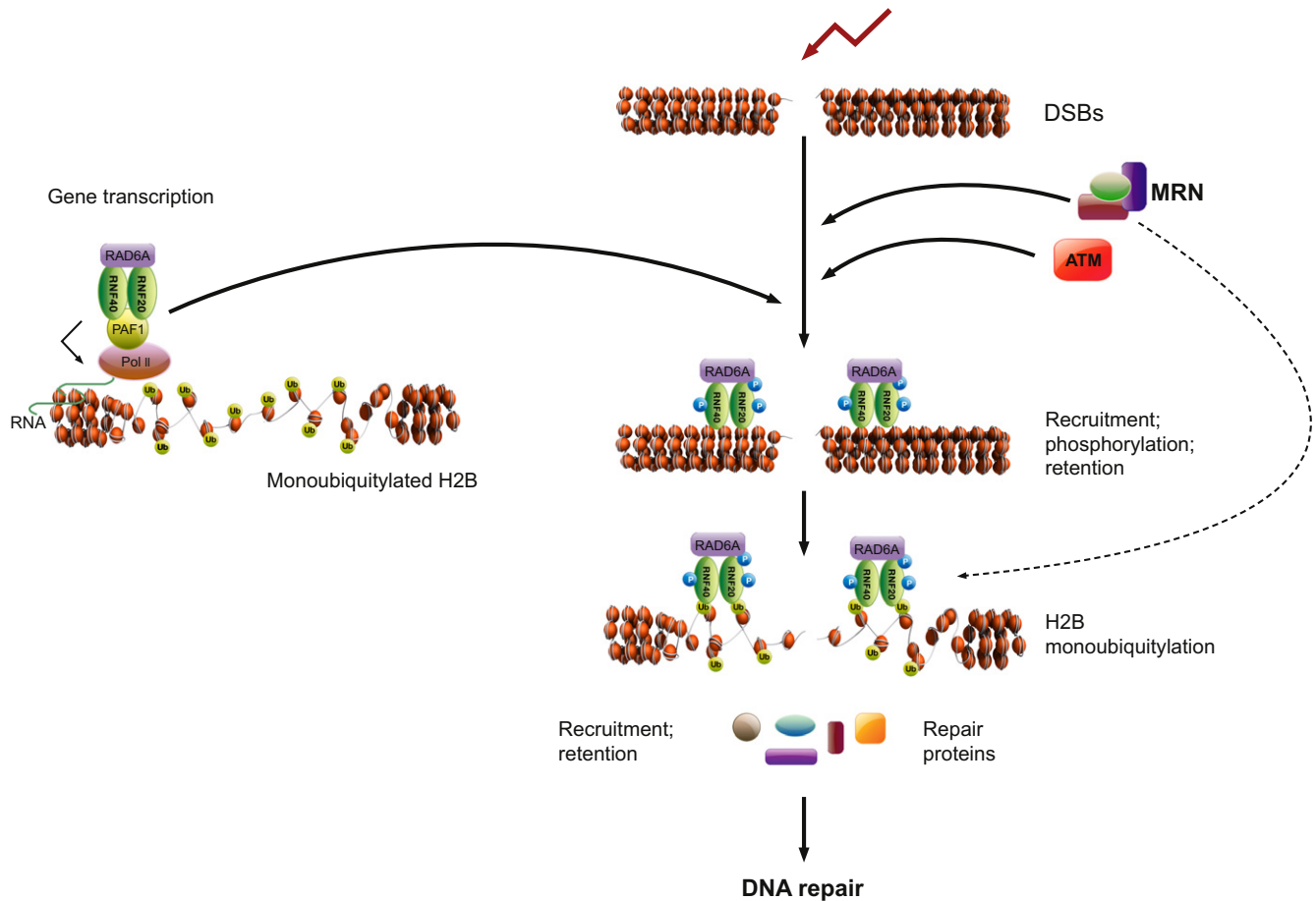
An important example of the involvement in the DDR of another histone mark that is normally associated with gene regulation is histone H2A monoubiquitylation, which was linked to transcription silencing (reviewed in [41,109]). This process was recently implicated in facilitating DSB repair and was associated with two DDR players, the RING finger proteins RNF8 and RNF168 [15,110–115]. Interestingly, unlike with H2Bub, the H2A ubiquitin ligases that function in the gene regulation context [116] were not implicated in DNA damage-induced H2A monoubiquitylation. There are additional differences in the appearance of the two apparently analogous histone marks at DNA damage sites, such as the dependence of the RNF8-mediated pathway on MDC1, and its requirement for 53BP1 recruitment – contrary to what is seen in damage-induced H2Bub. Importantly, H2Aub was recently shown to be involved in ATM-mediated transcriptional silencing and prevention of RNA Pol II elongation-dependent chromatin decondensation at regions distal to DSBs [117]. Thus, the two histone monoubiquitylations may play opposing roles in the dynamics of chromatin organization: H2Bub in the immediate vicinity of DSBs, and H2Aub farther away from these lesions. Indeed, despite the apparent analogy between the two modifications, H2Aub, at the opposing side of the nucleosomal surface, does not lead to chromatin fiber decompaction [70].

Information about the interface between the DDR and chromatin organization is rapidly accumulating [75,76,78,108,118–120]. An early, extremely rapid process at damage sites is the CK2-mediated phosphorylation and eviction of the heterochromatin protein 1 (HP1) [121]. Nucleosome destabilization at DSB sites was recently shown to depend on the ATPase activity of the p400 SWI/SNF ATPase and histone acetylation by the Tip60 acetyltransferase [122]. Damage-induced monoubiquitylation of histone H4 by the BBAP E3 ligase was reported [123], histone acetylation by Trapp-Tip60 was implicated in modulating the loading of repair proteins at DSBs [35], and the chromatin remodeling factor CHD4 was recently shown to be recruited to action at DSB sites [82,83,85]. Loss of histone H2B at DSB sites was previously reported in the budding yeast [124], and shown to be ATM-dependent and required for XRCC4 recruitment in human cells [125]. Such displacement may follow the decompaction of the chromatin fiber caused by H2Bub [70].

A. Nussenzweig's laboratory previously showed an initial, very rapid chromatin relaxation at DSB sites that was ATP-dependent and ATM-independent, and was required for the recruitment of the damage sensors [126,127]. The recent data suggested that the ATM–RNF20–RNF40–H2Bub axis functions independently and later, and is required for the actual DSB repair process. Collectively, the data call for a model of chromatin relaxation at DSB sites as a two-stage process consisting of a rapid, ATM-independent chromatin decondensation that facilitates the recruitment of sensors, and an ATM-dependent stage at the level of the 30-nm chromatin fiber, which is mediated by ATMs substrate, RNF20–RNF40 [90].

#### 4. Conclusions

Protein ubiquitylation is rapidly rising as a post-translational modification with a prominent role in signal transduction. The wealth of previous and recent studies on the functional significance of histone H2B monoubiquitylation attests to the importance of this histone mark in the dynamic shaping of chromatin in



**Fig. 2.** The ATM-mediated DDR borrows the H2B monoubiquitylation module for temporary emergency service at the sites of DNA damage. RNF20 and RNF40, presumably with the E2 ubiquitin conjugating enzyme RAD6A, are recruited, and RNF20 and RNF40 are phosphorylated by ATM. The MRN complex is required for ATM activation and also interacts with RNF20–RNF40 via its member, NBS1. H2Bub is induced at the damage sites and remains there for a while, to allow chromatin fiber decompaction and timely recruitment of repair proteins.

conjunction with several cardinal DNA transactions. In the transcription context, H2Bub is now emerging as a highly selective, task-oriented histone mark that up- or down-regulates the expression of specific groups of genes controlling the delicate balance between normal cellular growth and neoplasia. Histone H2B monoubiquitylation is also a prototypic example of the temporary but swift and decisive manner in which the DDR calls to action existing functional modules in the face of a DNA damage emergency (Fig. 2). It is expected that this mode of action of the DDR will be exemplified with many more branches of cellular metabolism.

### Acknowledgements

We thank K. Komatsu for critical comments on the manuscript. Work in the authors' laboratories is supported in part by grants from the Dr. Miriam and Sheldon Adelson Medical Research Foundation (to Y.S. and M.O.), The A-T Medical Research Foundation, the Israel Cancer Research Fund and the A-T Ease Foundation (to Y.S.), The Lower Saxony-Israel Cooperation Program, and the National Cancer Institute (R37 CA40099) (to M.O.). M.O. is incumbent of the Andre Lwoff chair in Molecular Biology. Y.S. is a Research Professor of the Israel Cancer Research Fund.

### References

- [1] Halazonetis, T.D., Gorgoulis, V.G. and Bartek, J. (2008) An oncogene-induced DNA damage model for cancer development. *Science* 319, 1352–1355.
- [2] Jackson, S.P. and Bartek, J. (2009) The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078.
- [3] Ciccia, A. and Elledge, S.J. (2010) The DNA damage response: making it safe to play with knives. *Mol. Cell* 40, 179–204.
- [4] Giglia-Mari, G., Zotter, A. and Vermeulen, W. (2010) DNA Damage Response. *Cold Spring Harb. Perspect. Biol.* 3, A000745.
- [5] Hartlerode, A.J. and Scully, R. (2009) Mechanisms of double-strand break repair in somatic mammalian cells. *Biochem. J.* 423, 157–168.
- [6] Hiom, K. (2010) Coping with DNA double strand breaks. *DNA Rep. (Amst.)* 9, 1256–1263.
- [7] Lieber, M.R. (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* 79, 181–211.
- [8] Holthausen, J.T., Wyman, C. and Kanaar, R. (2010) Regulation of DNA strand exchange in homologous recombination. *DNA Rep. (Amst.)* 9, 1264–1272.
- [9] Wyman, C. and Kanaar, R. (2006) DNA double-strand break repair: all's well that ends well. *Annu. Rev. Genet.* 40, 363–383.
- [10] Warmerdam, D.O. and Kanaar, R. (2010) Dealing with DNA damage: relationships between checkpoint and repair pathways. *Mutat. Res.* 704, 2–11.
- [11] Bekker-Jensen, S. and Mailand, N. (2010) Assembly and function of DNA double-strand break repair foci in mammalian cells. *DNA Rep. (Amst.)* 9, 1219–1228.
- [12] Polo, S.E. and Jackson, S.P. (2011) Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes Dev.* 25, 409–433.
- [13] Bensimon, A., Schmidt, A., Ziv, Y., Elkon, R., Wang, S.Y., Chen, D.J., Aebersold, R. and Shiloh, Y. (2010) ATM-dependent and -independent dynamics of the nuclear phosphoproteome after DNA damage. *Sci Signal* 3, rs3.
- [14] Bensimon, A., Aebersold, R. and Shiloh, Y. (2011) Beyond ATM: The protein kinase landscape of the DNA damage response. *FEBS Lett.* 585, 1625–1639.
- [15] Bekker-Jensen, S. and Mailand, N. (2010) The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. *FEBS Lett.* 585, 2914–2919.
- [16] Xu, Y. and Price, B.D. (2011) Chromatin dynamics and the repair of DNA double strand breaks. *Cell Cycle* 10, 261–267.

- [17] Stracker, T.H. and Petrini, J.H. (2011) The MRE11 complex: starting from the ends. *Nat. Rev. Mol. Cell Biol.* 12, 90–103.
- [18] Lamarche, B.J., Orazio, N.I. and Weitzman, M.D. (2010) The MRN complex in double-strand break repair and telomere maintenance. *FEBS Lett.* 584, 3682–3695.
- [19] Derheimer, F.A. and Kastan, M.B. (2010) Multiple roles of ATM in monitoring and maintaining DNA integrity. *FEBS Lett.* 584, 3675–3681.
- [20] Matsuoka, S. et al. (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166.
- [21] Lempiainen, H. and Halazonetis, T.D. (2009) Emerging common themes in regulation of PIKKs and PI3Ks. *EMBO J.* 28, 3067–3073.
- [22] Meek, K., Dang, V. and Lees-Miller, S.P. (2008) DNA-PK: the means to justify the ends? *Adv. Immunol.* 99, 33–58.
- [23] Flynn, R.L. and Zou, L. (2010) ATR: a master conductor of cellular responses to DNA replication stress. *Trends Biochem. Sci.* 36, 133–140.
- [24] Lopez-Contreras, A.J. and Fernandez-Capetillo, O. (2010) The ATR barrier to replication-born DNA damage. *DNA Repair (Amst.)* 9, 1249–1255.
- [25] Lovejoy, C.A. and Cortez, D. (2009) Common mechanisms of PIKK regulation. *DNA Rep. (Amst.)* 8, 1004–1008.
- [26] Eyfjord, J.E. and Bodvarsdottir, S.K. (2005) Genomic instability and cancer: networks involved in response to DNA damage. *Mutat. Res.* 592, 18–28.
- [27] Lavin, M.F. (2008) Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat. Rev. Mol. Cell Biol.* 9, 759–769.
- [28] Chun, H.H. and Gatti, R.A. (2004) Ataxia-telangiectasia, an evolving phenotype. *DNA Rep. (Amst.)* 3, 1187–1196.
- [29] Li, S. et al. (2010) Radiation Sensitivity and Tumor Susceptibility in ATM Phospho-Mutant ATF2 Mice. *Genes Cancer* 1, 316–330.
- [30] Bhoumik, A., Singha, N., O'Connell, M.J. and Ronai, Z.A. (2008) Regulation of TIP60 by ATF2 modulates ATM activation. *J. Biol. Chem.* 283, 17605–17614.
- [31] Bhoumik, A., Takahashi, S., Breitweiser, W., Shiloh, Y., Jones, N. and Ronai, Z. (2005) ATM-dependent phosphorylation of ATF2 is required for the DNA damage response. *Mol. Cell* 18, 577–587.
- [32] Ziv, Y. et al. (2006) Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat. Cell Biol.* 8, 870–876.
- [33] Goodarzi, A.A., Noon, A.T., Deckbar, D., Ziv, Y., Shiloh, Y., Lohrich, M. and Jeggo, P.A. (2008) ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol. Cell* 31, 167–177.
- [34] Chou, D.M. et al. (2010) A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. USA* 107, 18475–18480.
- [35] Murr, R., Loizou, J.I., Yang, Y.G., Cuenin, C., Li, H., Wang, Z.Q. and Herceg, Z. (2006) Histone acetylation by Trapp-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. *Nat. Cell Biol.* 8, 91–99.
- [36] Salton, M., Lereenthal, Y., Wang, S.Y., Chen, D.J. and Shiloh, Y. (2010) Involvement of matrin 3 and SFPQ/NONO in the DNA damage response. *Cell Cycle* 9, 1568–1576.
- [37] West, M.H. and Bonner, W.M. (1980) Histone 2B can be modified by the attachment of ubiquitin. *Nucleic Acids Res.* 8, 4671–4680.
- [38] Wood, A. et al. (2003) Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell* 11, 267–274.
- [39] Hwang, W.W., Venkatasubrahmanyam, S., Ianculescu, A.G., Tong, A., Boone, C. and Madhani, H.D. (2003) A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. *Mol. Cell* 11, 261–266.
- [40] Kao, C.F., Hillyer, C., Tsukuda, T., Henry, K., Berger, S. and Osley, M.A. (2004) Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. *Genes Dev.* 18, 184–195.
- [41] Weake, V.M. and Workman, J.L. (2008) Histone ubiquitination: triggering gene activity. *Mol. Cell* 29, 653–663.
- [42] Ng, H.H., Xu, R.M., Zhang, Y. and Struhl, K. (2002) Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J. Biol. Chem.* 277, 34655–34657.
- [43] Dover, J., Schneider, J., Tawiah-Boateng, M.A., Wood, A., Dean, K., Johnston, M. and Shilatifard, A. (2002) Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *J. Biol. Chem.* 277, 28368–28371.
- [44] Sun, Z.W. and Allis, C.D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* 418, 104–108.
- [45] Schneider, J. et al. (2005) Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. *Mol. Cell* 19, 849–856.
- [46] Xiao, T., Kao, C.F., Krogan, N.J., Sun, Z.W., Greenblatt, J.F., Osley, M.A. and Strahl, B.D. (2005) Histone H2B ubiquitylation is associated with elongating RNA polymerase II. *Mol. Cell Biol.* 25, 637–651.
- [47] Wood, A., Schneider, J., Dover, J., Johnston, M. and Shilatifard, A. (2005) The Bur1/Bur2 complex is required for histone H2B monoubiquitination by Rad6/Bre1 and histone methylation by COMPASS. *Mol. Cell* 20, 589–599.
- [48] Larabee, R.N., Krogan, N.J., Xiao, T., Shibata, Y., Hughes, T.R., Greenblatt, J.F. and Strahl, B.D. (2005) BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. *Curr. Biol.* 15, 1487–1493.
- [49] Lee, J.S., Shukla, A., Schneider, J., Swanson, S.K., Washburn, M.P., Florens, L., Bhaumik, S.R. and Shilatifard, A. (2007) Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. *Cell* 131, 1084–1096.
- [50] Li, B., Carey, M. and Workman, J.L. (2007) The role of chromatin during transcription. *Cell* 128, 707–719.
- [51] Schulze, J.M. et al. (2009) Linking cell cycle to histone modifications: SBF and H2B monoubiquitination machinery and cell-cycle regulation of H3K79 dimethylation. *Mol. Cell* 35, 626–641.
- [52] Kim, J. et al. (2009) RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. *Cell* 137, 459–471.
- [53] Fleming, A.B., Kao, C.F., Hillyer, C., Pikaart, M. and Osley, M.A. (2008) H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. *Mol. Cell* 31, 57–66.
- [54] Ezhkova, E. and Tansey, W.P. (2004) Proteasomal ATPases link ubiquitylation of histone H2B to methylation of histone H3. *Mol. Cell* 13, 435–442.
- [55] Kim, J., Hake, S.B. and Roeder, R.G. (2005) The human homolog of yeast BRE1 functions as a transcriptional coactivator through direct activator interactions. *Mol. Cell* 20, 759–770.
- [56] Zhu, B., Zheng, Y., Pham, A.D., Mandal, S.S., Erdjument-Bromage, H., Tempst, P. and Reinberg, D. (2005) Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol. Cell* 20, 601–611.
- [57] Minsky, N., Shema, E., Field, Y., Schuster, M., Segal, E. and Oren, M. (2008) Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. *Nat. Cell Biol.* 10, 483–488.
- [58] Henry, K.W. et al. (2003) Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes Dev.* 17, 2648–2663.
- [59] Wyce, A., Henry, K.W. and Berger, S.L. (2004) H2B ubiquitylation and deubiquitylation in gene activation. *Novartis Found Symp.* 259, 63–73.
- [60] Marzluff, W.F., Gongidi, P., Woods, K.R., Jin, J. and Maltais, L.J. (2002) The human and mouse replication-dependent histone genes. *Genomics* 80, 487–498.
- [61] Shema, E. et al. (2008) The histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. *Genes Dev.* 22, 2664–2676.
- [62] Shema, E., Kim, J., Roeder, R.G. and Oren, M. (2011) RNF20 Inhibits TFIIIS-Facilitated Transcriptional Elongation to Suppress Pro-oncogenic Gene Expression. *Mol. Cell* 42, 477–488.
- [63] Zhang, F. and Yu, X. (2011) WAC, a functional partner of RNF20/40, regulates histone H2B ubiquitination and gene transcription. *Mol. Cell* 41, 384–397.
- [64] Gilbert, N., Boyle, S., Fiegler, H., Woodfine, K., Carter, N.P. and Bickmore, W.A. (2004) Chromatin architecture of the human genome: gene-rich domains are enriched in open chromatin fibers. *Cell* 118, 555–566.
- [65] Fish, R.N. and Kane, C.M. (2002) Promoting elongation with transcript cleavage stimulatory factors. *Biochim. Biophys. Acta* 1577, 287–307.
- [66] Labhart, P. and Morgan, G.T. (1998) Identification of novel genes encoding transcription elongation factor TFIIIS (TCEA) in vertebrates: conservation of three distinct TFIIIS isoforms in frog, mouse, and human. *Genomics* 52, 278–288.
- [67] Reinberg, D. and Roeder, R.G. (1987) Factors involved in specific transcription by mammalian RNA polymerase II. Transcription factor IIS stimulates elongation of RNA chains. *J. Biol. Chem.* 262, 3331–3337.
- [68] Kim, J., Guermah, M. and Roeder, R.G. (2010) The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIIS. *Cell* 140, 491–503.
- [69] Ng, H.H., Dole, S. and Struhl, K. (2003) The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J. Biol. Chem.* 278, 33625–33628.
- [70] Fierz, B., Chatterjee, C., McGinty, R.K., Bar-Dagan, M., Raleigh, D.P. and Muir, T.W. (2011) Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. *Nat. Chem. Biol.* 7, 113–119.
- [71] Hubbard, K., Catalano, J., Puri, R.K. and Gnat, A. (2008) Knockdown of TFIIIS by RNA silencing inhibits cancer cell proliferation and induces apoptosis. *BMC Cancer* 8, 133.
- [72] Ito, T. et al. (2006) Transcription elongation factor S-II is required for definitive hematopoiesis. *Mol. Cell Biol.* 26, 3194–3203.
- [73] Nagata, M., Ito, T., Arimitsu, N., Koyama, H. and Sekimizu, K. (2009) Transcription arrest relief by S-II/TFIIIS during gene expression in erythroblast differentiation. *Genes Cells* 14, 371–380.
- [74] van Steensel, B. (2011) Chromatin: constructing the big picture. *EMBO J.* 30, 1885–1895.
- [75] van Attikum, H. and Gasser, S.M. (2009) Crosstalk between histone modifications during the DNA damage response. *Trends Cell Biol.* 19, 207–217.
- [76] Corpet, A. and Almuzni, G. (2009) A histone code for the DNA damage response in mammalian cells? *EMBO J.* 28, 1828–1830.
- [77] Falk, M., Lukasova, E. and Kozubek, S. (2010) Higher-order chromatin structure in DSB induction, repair and misrepair. *Mutat. Res.* 704, 88–100.
- [78] Groth, A., Rocha, W., Verreault, A. and Almuzni, G. (2007) Chromatin challenges during DNA replication and repair. *Cell* 128, 721–733.
- [79] Palomera-Sanchez, Z. and Zurita, M. (2011) Open, repair and close again: chromatin dynamics and the response to UV-induced DNA damage. *DNA Rep. (Amst.)* 10, 119–125.
- [80] Pan, M.R., Peng, G., Hung, W.C. and Lin, S.Y. (2011). Monoubiquitination of H2AX regulates DNA damage response signaling. *J Biol Chem.*, in press.
- [81] Noon, A.T., Shibata, A., Rief, N., Lohrich, M., Stewart, G.S., Jeggo, P.A. and Goodarzi, A.A. (2010) 53BP1-dependent robust localized KAP-1 phosphorylation is essential for heterochromatic DNA double-strand break repair. *Nat. Cell Biol.* 12, 177–184.

- [82] Larsen, D.H. et al. (2010) The chromatin-remodeling factor CHD4 coordinates signaling and repair after DNA damage. *J. Cell Biol.* 190, 731–740.
- [83] Polo, S.E., Kaidi, A., Baskcomb, L., Galanty, Y. and Jackson, S.P. (2010) Regulation of DNA-damage responses and cell-cycle progression by the chromatin remodelling factor CHD4. *EMBO J.* 29, 3130–3139.
- [84] Scimone, M.L., Meisel, J. and Reddini, P.W. (2010) The Mi-2-like Smed-CHD4 gene is required for stem cell differentiation in the planarian *Schmidtea mediterranea*. *Development* 137, 1231–1241.
- [85] Smeenk, G., Wiegant, W.W., Vrolijk, H., Solari, A.P., Pastink, A. and van Attikum, H. (2010) The NuRD chromatin-remodeling complex regulates signaling and repair of DNA damage. *J. Cell Biol.* 190, 741–749.
- [86] Game, J.C. and Chernikova, S.B. (2009) The role of RAD6 in recombinational repair, checkpoints and meiosis via histone modification. *DNA Rep. (Amst.)* 8, 470–482.
- [87] Giannattasio, M., Lazzaro, F., Plevani, P. and Muzi-Falconi, M. (2005) The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6-Bre1 and H3 methylation by Dot1. *J. Biol. Chem.* 280, 9879–9886.
- [88] Game, J.C., Williamson, M.S., Spicakova, T. and Brown, J.M. (2006) The RAD6/BRE1 histone modification pathway in *Saccharomyces* confers radiation resistance through a RAD51-dependent process that is independent of RAD18. *Genetics* 173, 1951–1968.
- [89] Nakamura, K. et al. (2011) Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. *Mol. Cell* 41, 515–528.
- [90] Moyal, L. et al. (2011) Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. *Mol. Cell* 41, 529–542.
- [91] Chernikova, S.B., Dorth, J.A., Razorenova, O.V., Game, J.C. and Brown, J.M. (2010) Deficiency in Bre1 impairs homologous recombination repair and cell cycle checkpoint response to radiation damage in mammalian cells. *Radiat. Res.* 174, 558–565.
- [92] Pierce, A.J., Johnson, R.D., Thompson, L.H. and Jasin, M. (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev.* 13, 2633–2638.
- [93] Moynahan, M.E., Chiu, J.W., Koller, B.H. and Jasin, M. (1999) Brca1 controls homology-directed DNA repair. *Mol. Cell* 4, 511–518.
- [94] Liao, W., McNutt, M.A. and Zhu, W.G. (2009) The comet assay: a sensitive method for detecting DNA damage in individual cells. *Methods* 48, 46–53.
- [95] Samara, N.L., Datta, A.B., Berndsen, C.E., Zhang, X., Yao, T., Cohen, R.E. and Wolberger, C. (2010) Structural Insights into the Assembly and Function of the SAGA Deubiquitinating Module. *Science* 328, 1025–1029.
- [96] Nicassio, F. et al. (2007) Human USP3 is a chromatin modifier required for S phase progression and genome stability. *Curr. Biol.* 17, 1972–1977.
- [97] van der Knaap, J.A., Kumar, B.R., Moshkin, Y.M., Langenberg, K., Krijgsveld, J., Heck, A.J., Karch, F. and Verrijzer, C.P. (2005) GMP synthetase stimulates histone H2B deubiquitylation by the epigenetic silencer USP7. *Mol. Cell* 17, 695–707.
- [98] Hossain, M.A., Claggett, J.M., Nguyen, T. and Johnson, T.L. (2009) The cap binding complex influences H2B ubiquitination by facilitating splicing of the *SUS1* pre-mRNA. *RNA* 15, 1515–1527.
- [99] Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A. and Reinberg, D. (2006) Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell* 125, 703–717.
- [100] Wood, A. et al. (2007) Ctk complex-mediated regulation of histone methylation by COMPASS. *Mol. Cell Biol.* 27, 709–720.
- [101] Smith, E. and Shilatifard, A. (2010) The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. *Mol. Cell* 40, 689–701.
- [102] Chatterjee, C., McGinty, R.K., Fierz, B. and Muir, T.W. (2010) Disulfide-directed histone ubiquitylation reveals plasticity in hDot1L activation. *Nat. Chem. Biol.* 6, 267–269.
- [103] Grenon, M., Costelloe, T., Jimeno, S., O'Shaughnessy, A., Fitzgerald, J., Zgheib, O., Degerth, L. and Lowndes, N.F. (2007) Docking onto chromatin via the *Saccharomyces cerevisiae* Rad9 Tudor domain. *Yeast* 24, 105–119.
- [104] Huyen, Y. et al. (2004) Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 432, 406–411.
- [105] Greeson, N.T., Sengupta, R., Arida, A.R., Jenuwein, T. and Sanders, S.L. (2008) Di-methyl H4 lysine 20 targets the checkpoint protein Crb2 to sites of DNA damage. *J. Biol. Chem.* 283, 33168–33174.
- [106] Sanders, S.L., Portoso, M., Mata, J., Bahler, J., Allshire, R.C. and Kouzarides, T. (2004) Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. *Cell* 119, 603–614.
- [107] Botuyan, M.V., Lee, J., Ward, I.M., Kim, J.E., Thompson, J.R., Chen, J. and Mer, G. (2006) Structural basis for the methylation state-specific recognition of histone H4–K20 by 53BP1 and Crb2 in DNA repair. *Cell* 127, 1361–1373.
- [108] Rossetto, D., Truman, A.W., Kron, S.J. and Cote, J. (2010) Epigenetic modifications in double-strand break DNA damage signaling and repair. *Clin. Cancer Res.* 16, 4543–4552.
- [109] Higashi, M., Inoue, S. and Ito, T. (2010) Core histone H2A ubiquitylation and transcriptional regulation. *Exp. Cell Res.* 316, 2707–2712.
- [110] Wang, B. and Elledge, S.J. (2007) Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brc1/Brc36 complex in response to DNA damage. *Proc. Natl. Acad. Sci. USA* 104, 20759–20763.
- [111] Kolas, N.K. et al. (2007) Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 318, 1637–1640.
- [112] Huen, M.S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M.B. and Chen, J. (2007) RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 131, 901–914.
- [113] Mailand, N., Bekker-Jensen, S., Fastrup, H., Melander, F., Bartek, J., Lukas, C. and Lukas, J. (2007) RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 131, 887–900.
- [114] Doil, C. et al. (2009) RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 136, 435–446.
- [115] Stewart, G.S. et al. (2009) The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* 136, 420–434.
- [116] Vidal, M. (2009) Role of polycomb proteins Ring1A and Ring1B in the epigenetic regulation of gene expression. *Int. J. Dev. Biol.* 53, 355–370.
- [117] Shanbhag, N.M., Rafalska-Metcalf, I.U., Balane-Bolivar, C., Janicki, S.M. and Greenberg, R.A. (2010) ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141, 970–981.
- [118] Misri, S., Pandita, S., Kumar, R. and Pandita, T.K. (2008) Telomeres, histone code, and DNA damage response. *Cytogenet. Genome Res.* 122, 297–307.
- [119] Escargueil, A.E., Soares, D.G., Salvador, M., Larsen, A.K. and Henriques, J.A. (2008) What histone code for DNA repair? *Mutat. Res.* 658, 259–270.
- [120] Karagiannis, T.C. and El-Osta, A. (2007) Chromatin modifications and DNA double-strand breaks: the current state of play. *Leukemia* 21, 195–200.
- [121] Ayoub, N., Jeyasekharan, A.D., Bernal, J.A. and Venkiteswaran, A.R. (2008) HPI-beta mobilization promotes chromatin changes that initiate the DNA damage response. *Nature* 453, 682–686.
- [122] Xu, Y., Sun, Y., Jiang, X., Ayrappetov, M.K., Moskwa, P., Yang, S., Weinstock, D.M. and Price, B.D. (2010) The p400 ATPase regulates nucleosome stability and chromatin ubiquitination during DNA repair. *J. Cell Biol.* 191, 31–43.
- [123] Yan, Q., Dutt, S., Xu, R., Graves, K., Juszczynski, P., Manis, J.P. and Shipp, M.A. (2009) BBAP monoubiquitylates histone H4 at lysine 91 and selectively modulates the DNA damage response. *Mol. Cell* 36, 110–120.
- [124] Tsukuda, T., Fleming, A.B., Nickoloff, J.A. and Osley, M.A. (2005) Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. *Nature* 438, 379–383.
- [125] Berkovich, E., Monnat Jr., R.J. and Kastan, M.B. (2007) Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nat. Cell Biol.* 9, 683–690.
- [126] Kruhlik, M.J., Celeste, A., Dellaire, G., Fernandez-Capetillo, O., Muller, W.G., McNally, J.G., Bazett-Jones, D.P. and Nussenzweig, A. (2006) Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol.* 172, 823–834.
- [127] Kruhlik, M.J., Celeste, A. and Nussenzweig, A. (2006) Spatio-temporal dynamics of chromatin containing DNA breaks. *Cell Cycle* 5, 1910–1912.