

SURVEY AND SUMMARY

The multi-functionality of UHRF1: epigenome maintenance and preservation of genome integrity

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ABSTRACT

During S phase, the cooperation between the macromolecular complexes regulating DNA synthesis, epigenetic information maintenance and DNA repair is advantageous for cells, as they can rapidly detect DNA damage and initiate the DNA damage response (DDR). UHRF1 is a fundamental epigenetic regulator; its ability to coordinate DNA methylation and histone code is unique across proteomes of different species. Recently, UHRF1's role in DNA damage repair has been explored and recognized to be as important as its role in maintaining the epigenome. UHRF1 is a sensor for interstrand crosslinks and a determinant for the switch towards homologous recombination in the repair of double-strand breaks; its loss results in enhanced sensitivity to DNA damage. These functions are finely regulated by specific post-translational modifications and are mediated by the SRA domain, which binds to damaged DNA, and the RING domain. Here, we review recent studies on the role of UHRF1 in DDR focusing on how it recognizes DNA damage and cooperates with other proteins in its repair. We then discuss how UHRF1's epigenetic abilities in reading and writing histone modifications, or its interactions with ncRNAs, could interlace with its role in DDR.

INTRODUCTION

Several studies in the past 15 years have revealed that disruption of Ubiquitin-like with PHD and RING Finger domain 1 (UHRF1) results in hypersensitivity to DNA damage (1–3), suggesting a critical role for this factor in the maintenance of genome stability. UHRF1 disruption sensi-

tize murine embryonic stem cells to DNA-damaging agents (4), while human UHRF1 transcript and protein levels are negatively regulated in response to cellular damage (5). Although a complete picture remains elusive, the relevance of UHRF1 in DNA damage repair has been emphasized in recent years, as scientists are gaining a deeper understanding of the fine regulatory mechanisms underlying UHRF1's multi-functional role.

During S phase, cells need to accomplish two major tasks: (i) faithfully propagate the genetic code and (ii) correctly maintain the epigenetic pattern in a timely and precise manner. These important tasks are carried out by macromolecular complexes containing DNA replication and epigenetic machineries that work together to accurately copy the information in the daughter strands; the same complexes are responsible for the rapid detection of DNA damage and the recruitment of DNA repair factors (6–8). Recent evidence indicate that UHRF1 is one of the key regulators for both of these processes (9–11). The ability of UHRF1 to orchestrate the genetic (recognition of DNA damage), molecular (recruitment of DNA repair factors) and epigenetic (DNA methylation maintenance) (9–14) levels during S phase is of paramount importance for the correct cell cycle progression. In fact, this allows to rapidly restore both the DNA integrity and the correct epigenetic information during DNA replication. Many reviews have widely discussed the epigenetic functions of UHRF1 and its role in cancer (15–18). Here, we recall the multiple functions of UHRF1, while enlightening for the first time the important role of this factor in coupling the maintenance of the epigenetic information with the DNA damage repair processes. We focus on the most recent findings about the different roles of UHRF1 in the DNA damage repair, integrating and discussing the contribution of UHRF1 to this cellular process.

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UHRF1: EPIGENETIC FUNCTIONS AND REGULATION

In physiological conditions (19–21), UHRF1 is present only in actively proliferating tissues (e.g. the bone marrow and lower part of the intestinal crypt) and its expression is not detectable in terminally differentiated tissues (e.g. the central nervous system, liver, upper part of the intestinal crypt) (22,23). The protein levels are tightly regulated during cell cycle (24,25): UHRF1 expression increases in late G1/beginning of the S phase, it reaches its peak during mid S-phase, when heterochromatic regions are replicated, and it is down-regulated at the end of the M phase (26–28). This precise regulation is lost in almost every type of cancer such as prostate (29), colorectal (30), liver (31) and gastric (32) cancer among others, where UHRF1 is overexpressed regardless of the cell cycle phase, and produces a great impact on tumorigenesis and cancer progression (16,33,34). Indeed, its overexpression is sufficient to rewire gene expression, inducing terminally differentiated cells to re-enter cell cycle (28) and mediating tumour formation in zebrafish livers (35). For these reasons, it is considered a potential universal tumour biomarker and an even more a promising target for therapy in cancers (16,36).

UHRF1 functions are performed through its 5 domains: ubiquitin-like domain (UBL), tandem tudor domain (TTD), plant homeodomain (PHD), SET and RING-associated domain (SRA) and really interesting new gene domain (RING), connected to each other by linker regions (Figure 1A) (37). Its uniqueness lies in the SRA domain, required for the recognition of methylated DNA and shared in primates only with UHRF2, that possess high homology in structure and in sequence with UHRF1 but lacks functional redundancy in terms of DNA methylation maintenance (38,39). The coordination of UHRF1 domains is involved in recognizing and interacting with a plethora of epigenetic factors, among which DNA methyltransferases (in particular DNMT1) (40), proliferating cell nuclear antigen (PCNA) (41), histone deacetylase 1 (HDAC1) (13), ubiquitin specific protease 7 (USP7/HAUSP) (42), euchromatic histone-lysine N methyltransferase 2 (G9a/EHMT2) (43), histone methyltransferase SUV39H1 (29), Poly(ADP-ribose) Polymerase 1 (PARP1) (44), DNA ligase 1 (LIG1) (45,46) and Tat Interacting Protein 60 (TIP60) (47).

The most studied epigenetic function of UHRF1 is the DNA methylation maintenance, that engages its central SRA and C-terminal RING domains. UHRF1 recognizes and binds hemi-methylated DNA through its SRA domain (13,48,49) and recruits DNMT1 during the S phase to propagate the methylation pattern of cytosines on newly synthesized DNA (40,50,51). The ubiquitin E3 ligase activity of the RING domain promotes DNMT1 localization onto replication foci by ubiquitinating Lys23 and Lys18 of histone H3 (H3K18Ub, H3K23Ub) (52,53). The binding of the UIM domain of DNMT1 to the ubiquitinated H3 mediates DNMT1 chromatin binding, allowing the methyltransferase to initiate conformational changes that culminate in its enzymatic activity toward hemi-methylated CpGs site (54). The pivotal role of the SRA domain in these steps is to determine the base-flipping of the methylated cytosine (49,55,56), facilitating the exposure of the un-

methylated cytosine of the CpG to DNMT1 activity (57); at the same time, a conformational opening in UHRF1 enables histone recognition (58), facilitating the correct propagation of the DNA methylation pattern. In addition, the N-terminal UBL domain functionally cooperates with the RING domain in recruiting the E2 factor, Ube2D, to UHRF1 (59,60).

UHRF1 is involved in heterochromatin formation and in sustaining a repressive chromatin landscape. Via the SRA domain, UHRF1 recruits HDAC1, a histone lysine deacetylase involved in chromatin compaction (13), and it interacts with PARP1, resulting in the accumulation of H4K20me3, a heterochromatin repressive mark (44). By TTD and PHD domains, UHRF1 binds to the di- and trimethylated lysine 9 (H3K9me2/3), the main heterochromatin mark associated with DNA methylation, and by TTD it can recognize the unmodified lysine 4 (H3K4me0) (61–65). Despite the well-known interaction between UHRF1 and heterochromatin regions, the PHD domain also binds unmodified H3R2, a histone mark mainly associated with euchromatin, and this opens important perspectives on the role of UHRF1 in euchromatin recognition (66). The combination of these histone marks is required for the correct ubiquitination of H3K18 and H3K23 (52–54), and for the correct maintenance of DNA methylation during replication (63,67) and in the newly identified mechanism of replication-uncoupled maintenance (68) (Figure 1B). We and others have shown that, in cancer cells, deregulated UHRF1 can bind gene promoters (29,30,32,69,70), triggering or maintaining an altered epigenetic landscape to mediate their silencing. Dimethylation of H3R2 results in significant reduction of UHRF1 binding to H3, regardless of H3K4 and H3K9 methylation status, showing the importance of this mechanism for targeting UHRF1 to chromatin (66).

Little is known about the transcriptional regulation of UHRF1, and the transcription factors regulating its expression are yet to be elucidated. The activation of p53/p21^{Cip1/WAF1}-dependent G1 checkpoint inhibits UHRF1 expression (71) and, in human cancer cells, the binding of E2F-1 on intron 1 of UHRF1 enhances its transcription (13,33). The functions, stability, and localization of UHRF1 are mainly controlled through a defined pattern of post-translational modifications (PTMs) in the domains and the linker regions (37) mediated by a network of CDK/cyclin complexes and de-ubiquitinases (Figure 1C). Phosphorylation or methylation of specific residues can affect UHRF1 functions, while its degradation is mostly regulated by ubiquitination. In specific conditions, the same residue can be differentially modified to obtain diverse outcomes, e.g. methylation of K385 by SET7 confers the ability to promote DNA repair by homologous recombination during S phase (72,73), while methylation of the same residue by SET8 during G2 mediates UHRF1 destabilization and degradation (74). UHRF1 can perform auto-ubiquitination via the intrinsic ubiquitin E3 ligase activity of its RING domain (5,75). During DNA replication, when co-localized with DNMT1, USP7 de-ubiquitinates UHRF1 enhancing UHRF1 stability (42). This association is destabilized by the CDK1/cyclin B, a specific complex of M phase, that phosphorylates UHRF1 on serine S652 (in isoform 2, corresponding to S639 in isoform 1).

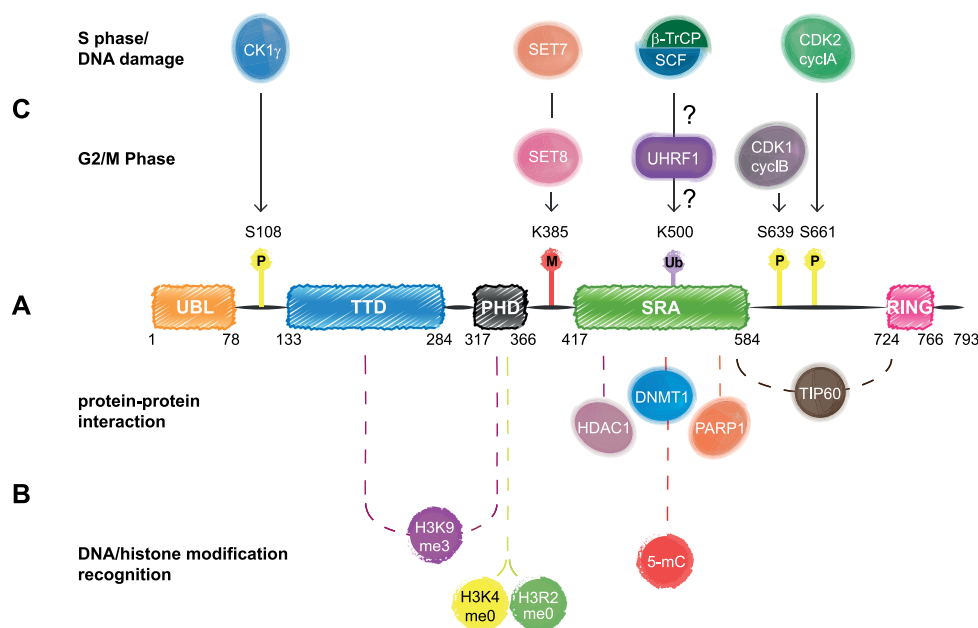


Figure 1. Domains, interactors and post-translational modifications of UHRF1. (A) UHRF1 is a large multidomain protein consisting of five domains: ubiquitin-like domain (UBL), tandem tudor domain (TTD), plant homeodomain (PHD), SET and RING-associated domain (SRA) and really interesting new gene domain (RING), connected by linker regions that undergo post-translational modifications, conferring different conformational states to UHRF1 that regulate its stability and functions. Numbers below the structure show the amino acid position in human UHRF1 Isoform 1. (B) Through these domains UHRF1 can interact with different factors and recognise a pattern of histone modifications. Via the SRA domain, UHRF1 binds DNMT1, HDAC1 and PARP1, and also hemi-methylated DNA, while via the SRA and RING domains it binds to TIP60. Via PHD it recognizes unmodified H3R2 and H3K4 on chromatin, while via PHD and TTD it recognizes trimethylated H3K9. Dashed lines represent domain-specific interactions (C) UHRF1 is subject to different PTMs; in particular, it is phosphorylated, methylated and ubiquitinated in specific sites. These modifications are involved in its stabilization (S108, S639, K385, K500) and/or in the functionality (S661, K385) of the protein. S108 is phosphorylated by CK1 δ , mainly following DNA damage; S639 is phosphorylated by the CDK1-cyclin B complex in M phase; K385 is methylated by SET8, required in the G2-M transition. All these modifications determine UHRF1 proteasome-dependent degradation via ubiquitination of K500 or of other unknown residues by UHRF1 or SCF $^{\beta\text{-TrCP}}$. K385 is methylated also by SET7 in S phase, in response to DSB; S661 is phosphorylated by CDK2/cyclin A during S phase, again in response to DSB. Both are required for UHRF1 recruitment at the site of DNA damage. Above are reported the specific factors responsible for the highlighted PTMs in the different cellular context; the connections are shown as arrows. The question marks on the arrow between UHRF1/SCF $^{\beta\text{-TrCP}}$ and K500ub are determined by the absence of direct evidence. The SCF $^{\beta\text{-TrCP}}$ complex ubiquitinates UHRF1 following phosphorylation of S108 by CK1 δ , but the exact residue was not determined. Following methylation of K385 by SET8, ubiquitination of UHRF1 on K500 was observed, but the responsible E3 enzyme was not identified. Since UHRF1 can auto-ubiquitinate via RING domain, the exact E3 ligase responsible for K500ub remains to be elucidated.

The phosphorylation of S652, harboured within the USP7-interacting domain of UHRF1, determines its ubiquitination and degradation by disrupting the UHRF1–USP7 interaction (76). Besides the regulation of stability and degradation of UHRF1, its PTMs introduce an additional layer of regulation that allows UHRF1 to directly contribute to DNA damage response.

UHRF1 AND SENSITIVITY TO DNA DAMAGE

UHRF1 is tightly connected to DNA damage sensitivity and repair. Depletion of UHRF1 in cells increases occurrence of chromosomal aberration, especially fragmentation (3), as well as of spontaneous sister chromatid exchange (4), indicating that UHRF1 is essential for genome stability. In the absence of exogenous DNA damage induction, UHRF1 depletion triggers cell cycle arrest in G2/M, caspase 8-dependent apoptosis (2) and activation of DDR, with high levels of serine 139 phosphorylation of histone H2AX (p^{Ser139}H2AX, known as γ H2AX (77)) (2). This modification, performed by ATM, extends for megabases around the site of damage constituting a recruiting hub—the γ H2AX foci—for DNA repair complexes

(78). In zebrafish, zUHRF1 knock-out causes DNA replication defects, increased DNA damage and apoptosis (79). Cells with reduced levels of UHRF1 also displays higher levels of basal γ H2AX (80). The lack, or reduced amounts, of UHRF1 impairs the cells' ability to repair the physiological DSBs encountered during S phase, leading to persistent DNA damage signalling (γ H2AX) and elongation of the cell cycle, with accumulation of cells in G2/M (3,80). In the absence of proper DNA repair, bypassing this checkpoint could generate chromosomal aberration and aneuploidy, resulting in genomic instability. Conversely, loss of UHRF1 hypersensitize the cells to DNA-damaging agents. Disruption of mUHRF1 in murine embryonic stem cells increases the sensitivity to X-rays, UV light, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and hydroxyurea (4). Functional ablation of UHRF1 followed by γ -radiation results in a decreased tendency to cell cycle arrest, with only minor cell accumulation in the G2/M phase and no noticeable decline in the S phase fraction, suggesting an impairment in the activation of the G2 checkpoint. Moreover, when UHRF1-silenced cells are challenged with DNA damaging agents, γ H2AX fails to accumulate at foci, significantly reducing percentage of γ H2AX-positive cells (3).

Similarly, UHRF1 hypomorphic cells show reduced accumulation of γ H2AX when exposed to DNA damage, suggesting a defective DDR in absence of physiological levels of UHRF1 that could explain the increased sensitivity to DNA damage (80).

On the other hand, UHRF1 levels decrease in response to cellular damage, with the maximum effect following exposure to DNA damaging agents (cisplatin, etoposide and bleomycin) (5). Although the impact of UHRF1 stability and its PTMs on the sensitivity to specific agents is not fully elucidated, it has been reported that the ubiquitin E3 ligase activity of the RING domain of hUHRF1 is key for cell survival following exposure to various cytotoxic and genotoxic agents (5). A possible mechanism for the regulation of UHRF1 stability is mediated by the SCF ^{β -TrCP} E3 ligase and CK1 γ . Upon DNA damage ATM phosphorylates CK1 γ , mediating its translocation to the nucleus where it phosphorylates UHRF1 on S108, in the linker region between UBL and TTD domains. After UHRF1 phosphorylation, β -TrCP binds UHRF1 and recruits the SCF complex that targets it for ubiquitination (24). Indeed, loss of β -TrCP or CK1 δ in cancer cell lines resulted in higher levels of UHRF1.

The majority of the studies suggests that UHRF1 has a role in the response to agents that induce double-strand breaks, pyrimidine dimers, and interstrand crosslinks (2,3,5,11,80), but interestingly, the cellular outcomes of UHRF1 loss are correlated to the type of DNA damaging agent and insult. UHRF1 is able to recognize more efficiently interstrand crosslinks formed by compounds that cause minor distortions of the DNA helix such as psoralen or mitomycin C, and less proficiently compounds causing major distortion like cisplatin (11). At the molecular level, the DNA damage recognition is mediated mainly by the SRA domain, that is extremely flexible and can recognize diverse damages (48,55,56): in particular, the SRA domain is shown to be fundamental for the recognition of pyrimidine dimers and for ICLs (11,80). This domain is frequently present in plants and in bacteria (81,82). In *Deinococcus radiodurans*, a bacterial strains capable of surviving the exposure of very high doses of DNA damaging agents, such as, the SRA-containing protein is involved in the resistance against extreme DNA damage, even if its role has not been completely elucidated (83). Although these microorganisms lack cytosine methylation, SHP (SRA-HNH protein) binds mismatched cytosines and cooperates in DNA damage repair; indeed, its loss leads to higher survival following γ -irradiation but also to higher mutation rate, suggesting its involvement in the maintenance of genome integrity. In humans, the SRA domain, with the RING domain, was demonstrated to be involved in the response to double-strand breaks: coordination between these two domains is essential for the correct activation of the response, as well as in the choice of the mechanism involved in the repair (10,72).

Altogether, these findings enlighten the crucial role of UHRF1 not only in response to DNA damage induction but also in the maintenance of chromosome integrity in normal conditions, significantly contributing to genomic stability; for this reason, UHRF1 can be defined a 'genome caretaker'.

UHRF1 AT THE CROSSROAD BETWEEN EPIGENOME MAINTENANCE AND GENOME INTEGRITY PRESERVATION

Cells are constantly exposed to numerous DNA damaging agents and deleterious events that profoundly impact the DNA integrity, resulting in the formation of various types of DNA damage. It is currently understood that the pathway chosen to recognize and repair DNA damage is strictly dependent on the specific type of damage, the cellular environment and the phase of the cell cycle in which the damage occurs. In particular, it was shown that genes involved in mismatch repair (MMR), Fanconi anaemia (FA) and homologous recombination (HR) have their highest expression in S phase, whereas base excision repair (BER), nucleotide excision repair (NER), and non-homologous end joining (NHEJ) genes expression is independent of cell cycle phase (84). As a factor absent in quiescent and terminally differentiated cells, and predominantly operating during DNA replication, UHRF1 exerts its function in DNA repair mechanisms particularly active in S phase, such as ICL and DSB repair, interacting with multiple epigenetic factors (10,11).

Expression and activity of DNA repair genes are regulated at several levels (85,86): given the cell cycle-phase specificity, it is not surprising that multiple enzymes required for efficient recognition, resection and repair are controlled by CDK-cyclin phosphorylation (87,88). PTMs have long been known to play a major role in the recruitment and activation of the repair factors, i.e. protein kinase ataxia telangiectasia mutated (ATM) phosphorylates key players in DDR network (78). Answering to how ATM is activated in order to initiate DSB repair, or how the correct site of damage is 'flagged' to be specifically recognized by the different sensor proteins allowed the discovery of the involvement of the epigenetic machinery in DDR, paving the way for the definition of UHRF1 role in DNA damage.

The first chromatin marker identified to be involved in DNA damage repair, in particular DSBs, was γ H2AX (77). γ H2AX interacts with several factors involved in checkpoint signalling and DSB repair such as NBS1, one of the factors of the MRN complex that is involved in the activation of ATM (89). Soon other canonical histone marks were found to possess functions strictly correlated to the DNA damage recognition and repair processes. Kim, Lee and Miller in 2019 published a comprehensive analysis on the importance of the pattern of histone modifications driving the cellular response towards different repair mechanisms (90), while a very detailed analysis of the different histone modifications involved in DSBs was performed by Clouaire *et al.* in 2018 (91). Acetylation and ubiquitination appear to be the most frequent histone PTMs involved in the binding and interaction with DNA repair factors. Histone acetylations and de-acetylation are involved in a chromatin remodelling mechanism, the 'access-repair-restore', that facilitates the accessibility of DNA repair factors to damaged DNA (92,93). Ubiquitination determines specific recruitment of DNA repair factors and selective exclusion of others via alteration of the steric hindrance (94). For example, acetylation of H4K16 by TIP60 (95), and ubiquitination of H2BK120 by RNF20/RNF40, followed by acetyla-

tion of the same residue by PCAF (96,97) drive DSB repair towards HR. Conversely, H2AK15 can be ubiquitinated by RNF8/RNF168, leading towards NHEJ (98,99), or acetylated by TIP60, directing the repair towards HR (100). Acetylation of H3K18 (H3K18ac), methylation of H3K36 (H3K36me1/2/3), of H3K9, and of H4K20 (H4K20me1/2) are all examples of histone marks contributing to the DDR pathway choice (90). Multiple epigenetic and replication factors are now recognized as fundamental in the repair processes, especially for the maintenance of genome stability. For example, PARP1 acts both in single-strand and in double-strand break repair and it can facilitate nucleosome disassembly by PARylating histones and recruiting multiple chromatin remodellers (101). PCNA also has long been associated with DNA repair, mainly MMR and NER, together with heterochromatin protein 1 (HP1) (102,103). It is also associated with DSB repair, where it recruits DNMT1 to the lesion; this DNA methyltransferase was shown to be involved as an early responder to DSBs (104). Various PTMs of PCNA regulate its role in DNA repair (105); in particular, ubiquitination was demonstrated to favour different processes depending on the number of ubiquitin groups added to the protein. Mono-ubiquitination of PCNA is induced by a high number of DNA damaging agents (106). Accumulation at stalled forks of RAD18-dependent mono-ubiquitinated PCNA was demonstrated to direct the repair towards the process of ICL resolution (107). All the aforementioned factors are very well-known interactors of UHRF1; many have been directly associated with UHRF1 in DNA damage (72,73), while others are still being evaluated. Among the latter, the interaction between the MYST domain of TIP60 and the SRA and RING domains of UHRF1 (47) is peculiar because UHRF1 is commonly associated to DNA methylation and heterochromatin formation, two processes that are not expected to be associated with histone acetylation, generally linked to chromatin relaxation and activation of transcription. Nevertheless, TIP60 has been identified as a binding partner in the epigenetic complex formed by UHRF1, DNMT1 and HDAC1; the presence of TIP60 determines the acetylation of H2AK5, which is linked to genome stability (108). UHRF1 binding to TIP60 also impairs its interplay with p53, suppressing the ability of TIP60 to acetylate p53 and therefore blocking p53-mediated apoptosis (108,109). The inhibition of the apoptotic process following UHRF1 binding to TIP60 could promote HR repair via acetylation of H4K16, that prevents the localization of 53BP1 in response to both ICLs and DSBs (110).

Finally, it has been reported that DNA repair via HR can also alter the DNA methylation pattern at the site of the corrected damage (111,112). In a model of targeted DNA damage, DNMT1 and DNMT3A are recruited at the site of repair together with UHRF1 and GADD45A and mediate the selective methylation on the promoter distal region of the repaired DNA, determining changes in its expression (111). Also alterations in chromatin structure have been observed, such as transient gain of H3K9me2/3 and loss of H3K4me2/3, as well as formation of chromatin loops connecting the 5' and 3' ends of the repaired gene (112). Therefore, in this context, the epigenetic remodelling post-repair

could represent a further checkpoint in the assessment of genome integrity, providing a DNA damage recognition code, in which UHRF1 is an active participant.

UHRF1 and interstrand crosslinks repair

DNA interstrand crosslinks (ICLs) are one of the major obstacles encountered by the replisome machinery (113–115). In ICLs, the two complementary DNA strands are covalently bound to each other (116). Consequently, DNA replication and transcription are hampered due to inhibition of DNA strand separation. Such barriers determine the stalling of the replication fork and the consequent replication stress that, if not properly resolved, produces the collapse of the fork with the dissociation of the replication machinery from the DNA strand (117). The Fanconi's anaemia repair complex (118), which is composed by a family of 22 proteins (FANCA to FANCT) as well as many associated proteins, plays a major role in the sensitivity to ICLs (113,114). The evidence that link UHRF1 to the Fanconi pathway are numerous, but the exact mechanism of its involvement is not fully elucidated yet. As the SRA domain of UHRF1 recognizes DNA distortions, it is not surprising that UHRF1 can recognize ICLs and has an active role in their repair (11,80). During S phase, the presence of UHRF1 in the replication machinery facilitates the recognition of the DNA damage and it can initiate a very rapid response in case of ICLs. This temporal and spatial localization could be favourable also for the FA proteins that are involved in specific steps of the DNA replication, such as common fragile sites (CFS) (119).

Recognition of ICLs initiates DNA damage response (DDR) and, in case of incorrect or incomplete repair, activates cell death mechanisms to avoid DNA breakage and chromosomal rearrangements (118). Since ICLs affect both strands of the DNA, the repair of these lesions is complex, and it requires involvement of numerous other DNA repair mechanisms such as NER and HR (120,121). Briefly, FANCM, after being phosphorylated by ataxia telangiectasia and RAD3-related (ATR), functions as a landing platform for the FA core complex (122,123) (Figure 2A, step 1). This complex acts as a ubiquitin ligase for two other FA proteins, FANCD2 and FANCI, that are recruited as a heterodimer, phosphorylated, and ubiquitinated (124,125) (Figure 2A, steps 2 and 3); they perform the 'unhooking', the nucleolytic incision at replication forks necessary to release the ICL from one of the two parental strands. FANCD2 and FANCI bind the nuclease FANCP (SLX4) via FAN1 (FANCD2 and FANCI-associated nuclease 1); FANCP recruits and activates specific endonucleases, such as FANQ(ERCC4/XPF)–ERCC1, MUS81–EME1 and SLX1 (126) (Figure 2A, steps 4 and 5). In the complementary strand, the lesion is bypassed while the corrected strand is ligated. This intact duplex will be the template for the repair of the DNA double-strand break (DSB) created in the other strand, with the FA complex coordinating the activity of HR and MMR proteins (127,128).

Two groups investigated simultaneously the role of UHRF1 in ICL repair; the authors of both these studies

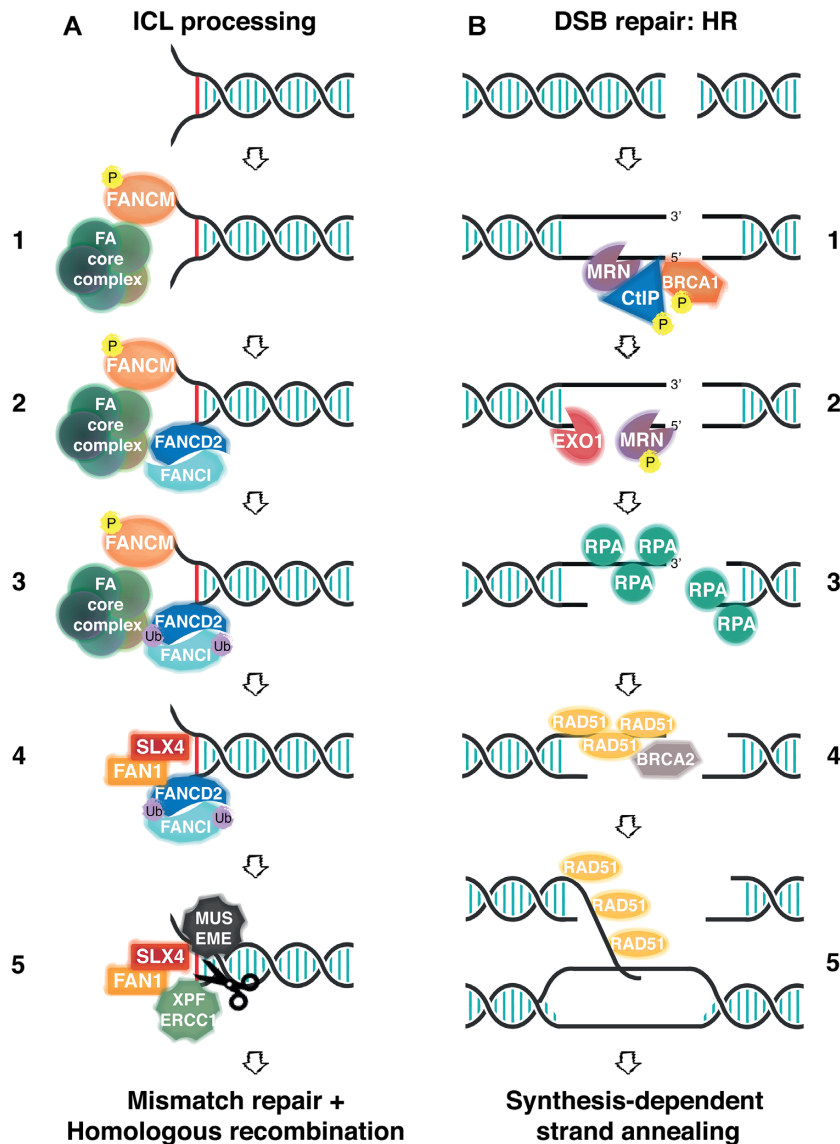


Figure 2. Mechanisms of DNA repair during S phase. The pathway chosen to recognize and repair DNA damage is strictly dependent on the specific type of damage, the cellular environment and the phase of the cell cycle. During S phase, factors of Fanconi anaemia pathway and homologous recombination have their highest expression. (A) In DNA ICLs the two complementary DNA strands are covalently bound to each other and cannot be separated. FANCM is phosphorylated by ATR and recruits the FA core complex on ICL site, that in turn ubiquitinates the FANCD2/FANCI heterodimer; once ubiquitinated they perform the nucleolytic incision at replication forks necessary to release the ICL from one of the two parental strands by binding SLX4. SLX4 recruits and activates endonucleases XPF–ERCC1, MUS81–EME1 and SLX1. In the complementary strand the lesion is bypassed while the corrected strand is ligated via MMR mechanism. This intact duplex will be the template for the repair of the DSB created in the other strand via homologous recombination. (B) DSB repair pathway choice is determined by the processing of DNA ends; 5′-to-3′ nucleolytic resection, leaving long 3′ DNA tails addresses the repair to HR. BRCA1 promotes the removal of 53BP1 from the damaged DNA, allowing resection by recruiting phosphorylated CtIP and the nucleolytic MRN complex. Phosphorylation of MRN activates it, starting the resection of 5′ ends together with EXO1 (and DNA2/BLM). The RPA complex coats the 3′ tails generated from resection, protecting them from further processing. RAD51, with the assistance of BRCA2, replaces RPA and catalyses homologous pairing and DNA strand exchange. Following strand invasion, the DNA synthesis proceeds, and the damage is repaired on the basis of the homologous sequence, mainly via synthesis-dependent strand annealing.

identified UHRF1 as part of the sensing machinery, but their conclusions substantially differ in the mechanism, as one study reported the direct interaction between the FA pathway and UHRF1, while the other suggested an independent and parallel role of UHRF1 from the FA factors (11,80). Both studies showed that UHRF1 recruitment is an early event in ICL response, and the SRA domain is most critical for the ability of UHRF1 to recognize DNA

crosslinks through the direct binding of SRA to the lesions, as deletion of the SRA domain completely abolished UHRF1 interaction with the ICL. Liang *et al.* demonstrated that UHRF1 binding precedes and is required for the recruitment of FANCD2; UHRF1 recognizes and binds to ICL *in vivo* within seconds after their appearance in the genome, and knock-down of UHRF1 completely abolished FANCD2 recruitment to ICL sites (11). They also reported

that UHRF1 has a stronger affinity for DNA containing ICLs compared to undamaged DNA; structural investigations of the ICL/UHRF1/FANCD2 complex, to better elucidate the nature of this DNA–protein interaction, were not performed, as the authors did not determine whether UHRF1 directly interacts with FANCD2. Conversely, Tian *et al.* concluded that UHRF1 function is independent from the FA pathway, since FANCD2 mono-ubiquitination and activation were not affected by UHRF1 partial depletion (80). In this model, UHRF1 has a convergent but different mechanism since loss of UHRF1 and SLX4 or FANCL caused an enhancement of the sensitivity to ICLs compared to the loss of one factor alone. However, a residual amount of UHRF1 was present in this hypomorphic model, and this condition can be crucial to discriminate UHRF1 role, since a reduced amount could still be sufficient to serve as ICLs sensor.

It is therefore plausible that UHRF1 is responsible for the recruitment of FANCD2 on ICL sites, but not for its ubiquitination (Figure 3A, step 1). Indeed, mono-ubiquitination of FANCD2 by the FA core complex has been demonstrated to be subsequent to the recruitment of the FANCD2/FANCI complex to stalled forks, and could determine acquisition of higher affinity for ICLs (129). This evidence is supported by a recent work from Motnenko *et al.*, which showed the involvement of UHRF2 as an ICL sensor protein. They demonstrated a direct protein–protein interaction between UHRF1, UHRF2 and FANCD2 (Figure 3A, step 2); this interaction is fundamental for the subsequent mono-ubiquitination of FANCD2 by the FA complex through retention of FANCD2 on chromatin (130) (Figure 3A, step 3). The role of UHRF1 in ICL repair is not only limited to the recognition of the damage but encompasses the recruitment of the ICL-processing factors, as it directly binds both MUS81/EME1 (associated with FANCP) and ERCC1/XPF complexes upon DNA damage (Figure 3A, step 4). Truncation of the C-terminal portion containing SRA and RING domains disrupted the association with both ERCC1 and MUS81 (80), but the co-localization with the MUS81/EME1 complex was not achieved via RING-dependent ubiquitination of EME1 (131); thus, it is plausible to hypothesize that the RING domain is responsible for protein–protein interactions, and it works in cooperation with the SRA domain to bind to specific lesions and recruit designated factors. Therefore, the dual activity of UHRF1—damaged DNA binding and nuclease recruitment—may allow it to sense ICLs and initiate lesion-processing pathways to promote DNA damage removal (Figure 3A).

UHRF1 and DNA double-strand breaks repair

Double-strand breaks (DSBs) are highly toxic lesions that pose an important threat to the stability of the genome as they may cause various mutations, deletions and rearrangements with loss of genetic material and disruption of gene structure and function. (132). They may be caused directly by ionizing radiation or indirectly by endogenous/exogenous challenges; one of the major sources of DSBs is the formation of ICLs. UHRF1 has been shown to play an important

role for resolving DSBs via homologous recombination (HR) (10,133).

Two pathways lead the repair of DSBs in mammalian cells: non-homologous end joining (NHEJ) in all cell cycle phases and homologous recombination during S and G2 phase, when sister chromatids are available (134–136). DSB repair pathway choice is determined at the molecular level by the type of processing of DNA ends. If the damaged ends undergo 5′-to-3′ nucleolytic resection, and bear long 3′ DNA tails, they will be repaired via HR. instead, unprocessed ends will be recognized by NHEJ factors (137). Three distinct nucleases perform the ends resection process (Figure 2B): the 5′-to-3′ exonuclease EXO1, the endonuclease/helicase DNA2-BLM and the MRN complex (MRE11-RAD50-NBS1) (138). Regulators of the process are 53BP1, an ATM kinase target, and the tumour suppressor protein BRCA1 (139). 53BP1 negatively regulates the resection in G1 (140), while BRCA1 promotes the removal of 53BP1 in S phase, thus allowing for the processing (141). CtIP, an interactor of the MRN complex, is phosphorylated at the G1-S transition by CDKs, promoting complex formation between CtIP, MRN, and BRCA1 (phosphorylated on Ser327) (142) and activating the resection by MRN (phosphorylated on Thr847) (143) (Figure 2B, steps 1 and 2). The stability of the complex is regulated by ubiquitination and SUMOylation of the lesion-processing factors (144). The replication protein A (RPA) complex coats the 3′ tails of single-stranded DNA (ssDNA) generated during resection, protecting them from unwanted processing (145) (Figure 2B, step 3). These tails are substrates for the recombinase RAD51 that catalyse the homologous pairing and DNA strand exchange, together with BRCA2 that assists RAD51 loading onto RPA-coated ssDNA (146) (Figure 2B, step 4). Following strand invasion, the DNA synthesis proceeds and the damage is repaired based on homology (136) (Figure 2B, step 5).

In 2016, Zhang *et al.* revealed that the direct interaction between UHRF1 and BRCA1 is important in the choice of the correct repair pathway following DSBs, inducing a switch towards homologous recombination (10). During S phase, S661-phosphorylated UHRF1 (S674 in isoform 2) is recruited at DSBs by BRCA1 through the BRCT domain (Figure 3B, step 1–2). This phosphorylation is fundamental for the recruitment of UHRF1 at DSB sites and for regulating DSB repair choice; intriguingly, the phosphorylation of the corresponding residue in zebrafish (S648) by CDK2/cycA2 is required for correct embryogenesis (147). UHRF1 promotes the switch towards HR by ubiquitinating the telomere-associated protein RIF1. In fact, RIF1 suppresses 5′ end resection, limiting BRCA1 accumulation and promoting NHEJ (133). In human cell lines, upon DSBs formation, poly-ubiquitination of RIF1 on K63 by UHRF1 determines the removal of RIF1 from the site of the damage, possibly through the disruption of the RIF1–53BP1 interaction, allowing BRCA1 to activate the downstream events of HR (10) (Figure 3B, step 3). This role of UHRF1 in HR seems separated and independent from its function in DNA methylation and histone ubiquitination. In human cells, when S674A phosphorylation is impaired, UHRF1 is unable to interact with BRCA1 and ubiquitinate RIF1, while maintaining its epigenetic role in DNA methylation

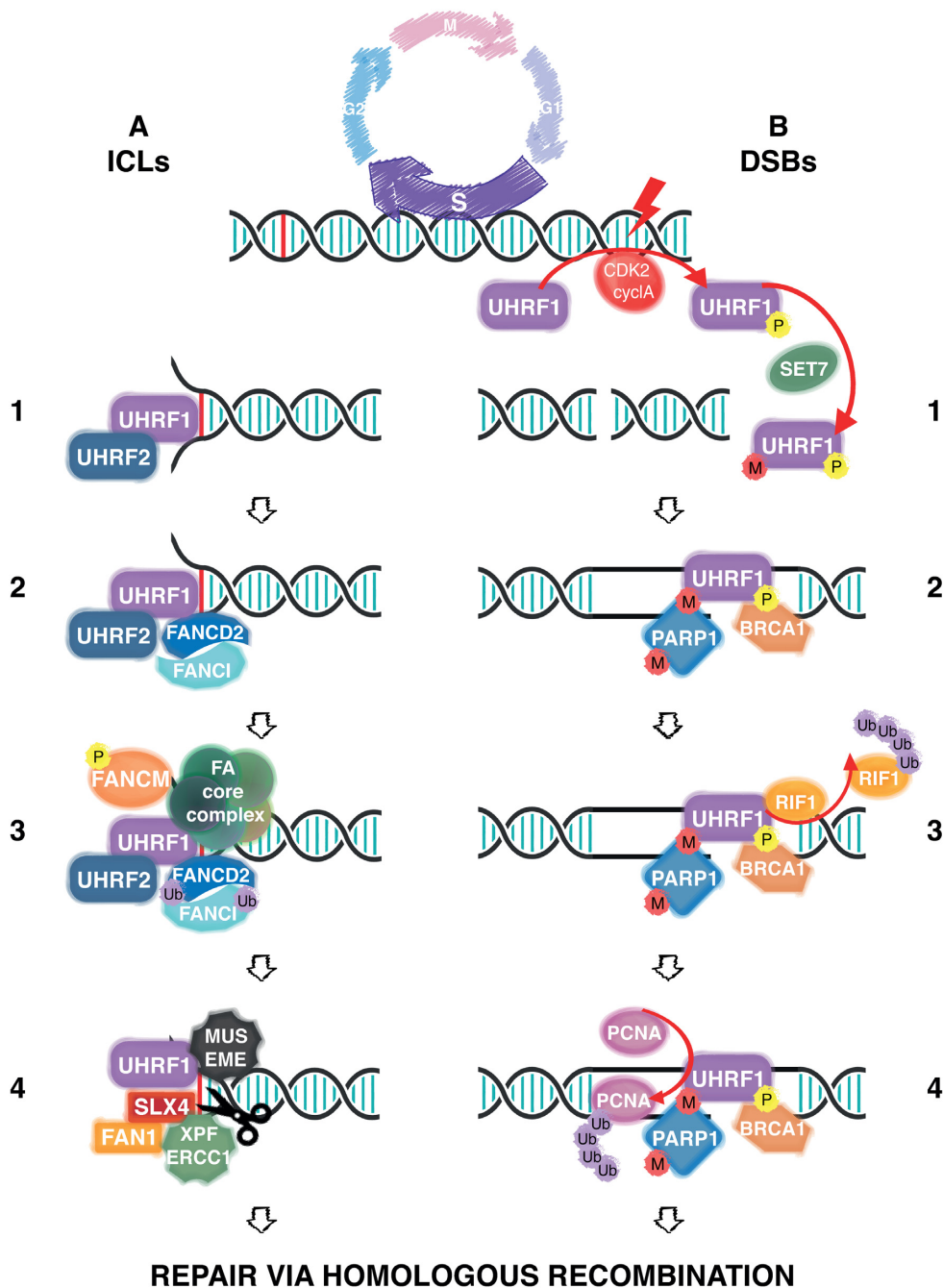


Figure 3. UHRF1 roles in DNA damage response pathways. (A) During S phase UHRF1 can act as a sensor for interstrand crosslinks. It binds the damaged region through its SRA domain, together with UHRF2 (step 1), and recruits FANCD2 through RING domain (step 2). Once ubiquitinated by the FA core complex, FANCD2/FANCI activate the FA pathway (step 3). UHRF1 could also cooperate in the final step of ICR resolution by recruiting nucleases such as MUS81/EME1, via RING domain (step 4). The double-strand lesion produced by such nucleases is repaired in S phase via homologous recombination. (B) UHRF1 is involved in the recognition of double-strand breaks during S phase. Following DNA damage, UHRF1 is phosphorylated by CDK2/cyclin A on S661 and is subsequently methylated by SET7 on K385 (step 1). These two modifications are necessary for the recruitment of UHRF1 on damaged sites; phosphorylation of S661 is essential for UHRF1 interaction with BARD domain of BRCA1, methylation of K385 for the interaction with PARP1, which is also methylated by SET7 (step 2). Phosphorylated UHRF1 poly-ubiquitinates RIF1, dissociating it from 53BP1 and removing it from the damage; the removal of 53BP1 activates the 5'-to-3' processing of DNA ends leading to the formation of 3' single-strand tails recognized by the RPA complex and directing the repair towards HR (step 3). Methylated UHRF1 is also responsible for poly-ubiquitination of PCNA at K164. While mono-ubiquitination is commonly linked to processes of DNA damage tolerance pathways (DDT), poly-ubiquitination could be determined by persistence of PCNA on damaged end, representing a signal of the switch towards HR pathway (step 4).

and mono-ubiquitination of H3 (10). These results suggest that the two functions of UHRF1 are integrated but possess unique features; the two abilities might cooperate in the replication process, as genome integrity must be preserved before the correct pattern of DNA methylation is propagated.

UHRF1 function in DSB repair was further revealed by Hahm *et al.* in 2019. They demonstrated that phosphorylation of UHRF1 at S661 during S phase is essential for the subsequent methylation *in vivo* of UHRF1 at K385 by SET7; SET7 action is counteracted by the activity of LSD1 that removes the methyl group, tightly controlling the levels of methyl-UHRF1 during DNA damage response (72). This methylation is a determinant for the interaction with PARP1 and for the recruitment of UHRF1 on damaged sites (73) (Figure 3B, step 1–2). Inhibition of UHRF1–PARP1 interaction in presence of DNA damage resulted in cell cycle arrest in G2/M phase and increased apoptosis, denoting reduced efficiency in the repairing processes. It is interesting to notice that PARP1 is similarly methylated by SET7 at K508 to enhance its recruitment to damaged site in response to oxidative stress (148). Therefore, the activity of SET7 appears to be fundamental for the recruitment of necessary factors in the process of DNA damage recognition. In addition, methylation of K385 of UHRF1 promotes poly-ubiquitination of PCNA on K164, facilitating the switch towards HR repair (72) (Figure 3B, step 4). It is hypothesized that persisting PCNA at stalled primer ends could be a signal for its poly-ubiquitination, possibly determined by UHRF1, that is responsible for the switch towards HR. This modification is less abundant compared to mono-ubiquitination, conceivably due to the relative overall efficiency of the ubiquitinating/deubiquitinating enzymes or to the very quick temporal presence of the PTM (106). In this intricate network, UHRF1 can be considered a key mediator of the switch towards HR in S phase, thanks to its ability to bind to different factors fundamental for the activation of the homologous recombination pathway such as BRCA1, PARP1 and TIP60 (Figure 3B). UHRF1 post-translational modifications appear to be fundamental for the correct functioning of this factor and the recruitment of specific interactors in DNA damage sensing and repairing.

UHRF1 IN DNA DAMAGE RESPONSE AS A POTENTIAL THERAPEUTIC TARGET

UHRF1 has been recently explored for its potential in targeted therapy against cancer. Since UHRF1 is overexpressed in a wide variety of solid tumours and it is not required in non-replicating cells, its downregulation could be used to overcome the resistance to DNA damaging agents by impairing DNA repair mechanisms. While several approaches have been tested to inhibit the different domains of UHRF1 (149,150), a recent work focused specifically on targeting UHRF1 functions in DNA damage repair (151). Here it is shown that in prostate cancer cells, the combined treatment with HDAC and PARP inhibitors (SAHA and veliparib) disrupts the interaction between UHRF1 and BRCA1 and decreases UHRF1 and BRCA1 levels, resulting in inhibition of the HR pathway and cell death (151).

While UHRF1 depletion resulted in reduced BRCA1 protein levels, BRCA1 depletion did not alter UHRF1 levels, suggesting that the treatment with the two inhibitors could act directly on UHRF1 stability. A possible explanation for the HDACi-dependent degradation of UHRF1 could be the interference with the activity of USP7 (152), that regulates UHRF1 levels *in vivo* by counteracting the auto-ubiquitination activity of the RING domain of UHRF1 and removing ubiquitin adducts (42). HDAC1, recruited by UHRF1 on the replication fork during DNA synthesis (13), and all the HDAC family proteins play a critical role in the DDR, since they are involved in the switch between NHEJ and HR (153). In particular, HDAC1 de-acetylates H4K16, leading to ubiquitination of H2A by RNF8/RNF168 at the site of the damage and recruitment of 53BP1 (153), stimulating the NHEJ pathway. This action is counteracted by TIP60-dependent acetylation of H4K16, that by contrary stimulates HR during S phase by preventing the localization of 53BP1 in response to both ICLs and DSBs (110). As previously mentioned, both HDAC1 and TIP60 are interactors of UHRF1 during S phase (108); the two proteins equally bind to the SRA domain (together with the RING domain in case of TIP60) (13,47). Thereby, it is possible to hypothesize that TIP60 and HDAC1 could bind to UHRF1 in distinct conditions, depending on the functions exerted by UHRF1 in response to the precise cell state (DNA repair versus DNA methylation). Further studies are necessary to determine if known or unknown PTMs of UHRF1 is involved in this hypothetical differential activation, determining the correct affinity for the specific interactor. Considering the role of HDAC1 in promoting NHEJ, its inhibition should promote HR by avoiding de-acetylation of H4K16. However, as shown in different studies, a pan-HDACi such as SAHA downregulates also RAD50 and MRE11 in cancer cells, together with UHRF1 and BRCA1 (151,154), while a specific HDAC1/2/3i (MS-275) transcriptionally downregulates FANCD2 (155), therefore prejudicing the functionality of the DDR at different levels. Molecular insights of this general impairment, as well as the precise mechanism behind UHRF1 downregulation, are still missing. However, the potential for targeted regulation of UHRF1—the ability to reduce or even overcome chemoresistance by compromising the ability of the cells to repair the DNA damage—once again depicts this factor as a really promising target for therapy in cancers.

FUTURE PERSPECTIVES: UNEXPLORED CONNECTIONS

Although enormous strides have been made in the past few years, there is still a huge amount of knowledge that awaits to be discovered by researcher. In DDR, the ability of UHRF1 to read and write specific histone modifications has not been explored; indeed, recognition of specific patterns of chromatin modification by distinct domains within ‘reader’ proteins, such as UHRF1, plays a critical role in the maintenance of genomic stability. Definitely, as for its epigenetic role, we can hypothesise that UHRF1 recognizes and binds to damaged chromatin on histones via PHD and TTD domains, similarly to what experimentally demonstrated for the binding to damaged DNA via SRA domain (11).

The binding to trimethylated H3K9, normally associated with heterochromatin formation (9), was demonstrated to be necessary to stimulate phosphorylated TIP60 activity to acetylate ATM and different histone residues (156,157). It is therefore plausible to hypothesize that UHRF1 binding to H3K9me3 could be involved in its role in HR promotion via the mutual interaction UHRF1/TIP60/H3K9me3, leading to the correct positioning of the two factors on damaged chromatin (Figure 4A). H3R2me0, essential for UHRF1 binding to histones (66), has also been recently identified as critical for DNA damage repair in ESCs; the combination of H3R2me0 with H3K4me0 is recognized by TRIM66 via its PHD domain (158). Chromatin-bound TRIM66 recruits Sirt6 to deacetylate H3K56ac, negatively regulating its levels (158); biphasic decrease-increase of this epigenetic mark has been shown in response to UV-induced DNA damage during the early steps of NER (159). Conceivably, UHRF1 binding to damaged chromatin could exploit the same pattern, as its PHD recognizes the same precise combination between H3R2me0 and H3K4me0. Understanding the role of H3R2 methylation/demethylation balance (160) for the recruitment of UHRF1 and other proteins involved in DNA repair will allow the deepening of our knowledge on the role this histone modification has in the DNA repair process, mainly due to the fact that presence of mono and di-methylation marks (both symmetrical and asymmetrical) strongly inhibits UHRF1 binding to chromatin (66). The involvement of H4K20me3, another histone modification associated with UHRF1 (44), deserves to be investigated in DSB repair. It is actually known that mono- and dimethylated H4K20 provide docking sites for the DDR factor 53BP1, favouring NHEJ (161). ADP ribosylation of UHRF1 is required for the correct trimethylation of H4K20 in heterochromatin (44); the early binding of PARP1 to the DNA damaged sites induces PARylation of several proteins involved in DNA repair and the recruitment of chromatin modifying complexes at the site of damage (162). Since heterochromatic histone marks play a role in the activation of DNA damage checkpoint (163), UHRF1 and PARP1 could work in concert with KDM3B to maintain the correct balance between methylation and demethylation of this residue during DDR (164). Better investigation of the role of UHRF1 and PARP1 on H4K20me3 during the DDR is warranted, mainly in light of the evidence showing that differential methylation of the same histone residue can regulate the choice of DSB pathways: for example, H3K36me3 is important for HR repair, while H3K36me2 is involved in NHEJ (165,166). Methylation readers could recognize these different marks to promote specific branches of DSB repair; whereas H4K20me1/2 leads toward NHEJ via binding of 53BP1, H4K20me3 accumulation could be linked to HR. Likewise, the ability of UHRF1 to write histone modification could be of impact in DDR. H3K18 and H3K23, ubiquitinated by UHRF1 to facilitate DNMT1 binding and activity (52–54), were found to be acetylated in damaged chromatin (167). These acetylations, catalysed by GCN5, are involved in the recruitment of the SWI/SNF chromatin remodelling complex to damaged chromatin to promote DSB repair by NHEJ. Therefore, presence of a ubiquitin group deposited by UHRF1 could be of hindrance to the NHEJ pathway, possibly favouring HR (Figure 4B).

Finally, another aspect not yet evaluated in UHRF1-DDR relationship is the potential interaction between non-coding RNAs and UHRF1 in DNA damage. Recently, UHRF1 has been identified as an interactor/target of lncRNAs and miRNAs (69,168,169) that regulate its expression and stability, as well as the expression of UHRF1 targets. While the lncRNA paRCDH1-AS directly binds to UHRF1 with a decoy function to avoid CDH1 silencing (69), the lncRNA UPAT interacts with UHRF1 in the linker region between SRA and RING domains and stabilizes it by interfering with β -TrCP-mediated ubiquitination of UHRF1 (168); the presence of UPAT could counteract the downstream effects of UHRF1 phosphorylation on S108 by CK1 γ upon DNA damage, avoiding β -TrCP binding and UHRF1 degradation. Whereas the role of proteins and modifications in DSB repair have been fairly well defined, the study of the direct functions of small and long non-coding RNAs (miRNAs, lncRNAs, diRNAs) in the DNA damage response is just at its beginning. Their emerging importance is perceived by the intricate roles they are shown to play in DDR, although their precise mechanism of action is still under evaluation: lncRNAs can act as guides, scaffolds or decoys (170). Most interestingly, they are important for spatial regulation of protein complexes in DNA repair foci, and for the commitment to a repair pathway (171,172). Damage-induced long non-coding RNAs (dilncRNAs) are generated at the DSBs via RNA polymerase II and act both as precursor of small DNA-damage RNAs (DDRNs) or by recruiting DDRNs through RNA-RNA pairing, allowing for site-specific localization of the DDR machinery. In particular, the long known interaction of 53BP1 with RNA through its Tudor domain (173) has been confirmed with DDRNs and dilncRNAs, and it leads to the formation of DDR foci containing 53BP1, inhibiting DNA-end resection and directing the repair towards NHEJ (171). Conversely, during S/G2 phase dilncRNAs can pair to resected DNA-ends forming DNA:RNA hybrids that recruit BRCA1, BRCA2 and RAD51, guiding DNA repair via HR (172). Considering that during S phase phosphorylated UHRF1 interacts with BRCA1, we could hypothesize that this binding, and the correct localization on damaged ends, may be reinforced by the interaction of UHRF1 with DNA:RNA hybrids, possibly via its Tudor domain in a manner similar to 53BP1 (Figure 4C). The hypothesis that UHRF1 could interact with damage-induced non-coding RNAs is further strengthened by evidence showing the recruitment of TIP60 via small RNAs upon DSB formation (174), as well as by the observation that BRCA2 and FA factors localize to damaged sites via DNA:RNA hybrids (175). Therefore, a further perspective in unveiling the complex framework of DNA repair should evaluate the link between UHRF1 and ncRNAs, concerning not only UHRF1 stabilization but also their functional interaction in the DDR.

CONCLUSIONS

For many years, UHRF1 has mainly been studied for its properties as a fundamental epigenetic regulator in the correct maintenance of DNA methylation during replication. Indeed, its ability to coordinate the information stored in DNA methylation and histone code is unique among all

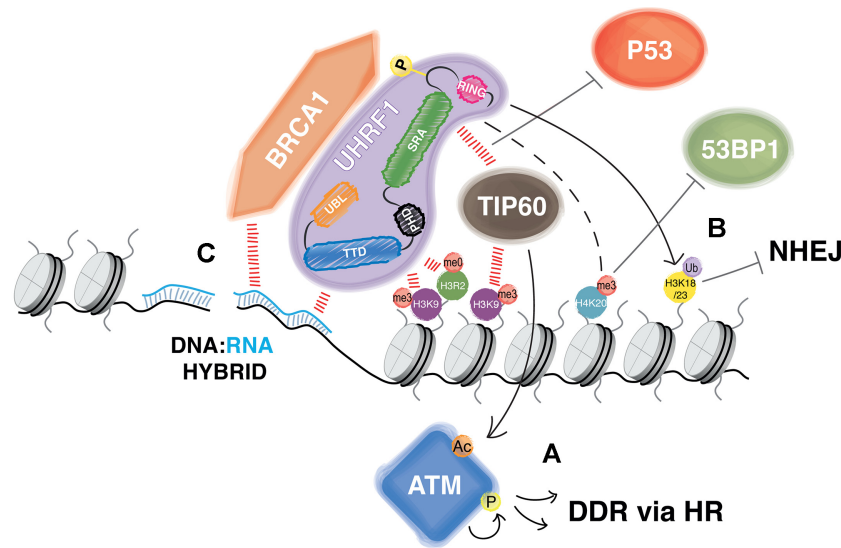


Figure 4. Hypothetical model representing the complexity of UHRF1 known or potential roles/interactions in DNA damage repair. Numerous aspects remain to be investigated about UHRF1 interactions and roles during the DNA damage response, as some of its known epigenetic abilities could be also involved in the detection of DNA damage and choice of repair pathway. The model attempts to integrate these different functions to show the complexity that could hide behind every player in the DDR, specifically UHRF1. (A) Recognition and binding of H3K9me3 and H3R2me0 by the TTD and PHD domains could be critical to UHRF1 positioning on damaged DNA; the presence of H3K9me3 could reinforce UHRF1 binding to TIP60, blocking TIP60 interaction with P53 and avoiding the activation of the apoptosis process. TIP60 would be free to acetylate ATM, starting the DDR. (B) The RING domain-dependent ubiquitination of H3K18 and H3K23 could facilitate HR repair by blocking their acetylation, detected in DSB repair via NHEJ, or functioning as docking sites for HR proteins. Trimethylation of H4K20, performed in the presence of UHRF1 and PARP1, could prevent 53BP1 recognition of H4K20me1/2, further impairing the NHEJ repair pathway. (C) UHRF1 is a known RNA-binding protein via SRA domain. Following DNA damage, it could also bind the RNA:DNA hybrids formed in S/G2 phase together with BRCA1, possibly via TTD, facilitating HR; the hypothesised mechanism is similar to the binding of RNA:RNA duplex by the Tudor domain of 53BP1 that directs the repair towards NHEJ.

mammal proteins. In this review we summarized a new function of UHRF1 that is the cooperation in the DNA damage repair and the pivotal role in the switch between NHEJ and HR during DSBs repair. The role of UHRF1 in DDR is finely regulated by the SRA and RING domains, responsible for the binding to the damaged DNA and for the recruitment of DNA repair interactors, and by specific PTMs of the protein. Coupling the maintenance of the epigenetic code with the correct propagation of the genetic information is key for cells during S phase: UHRF1 cooperates to both ‘sides of the coin’, enabling the DNA repair machinery to act quickly in checking, repairing and replacing the correct epigenetic information onto the newly synthesized DNA.

It is important to highlight that UHRF1 presence is essential for mammals, as demonstrated by the embryonic lethality resulting from UHRF1 knockout. However, the outcome of UHRF1 loss can be tracked in models such as zebrafish, where UHRF1 is maternally provided during the initial steps of embryogenesis, or *in vitro* cells. Here, UHRF1 depletion has long been known to lead to DNA hypomethylation, DNA damage and apoptosis, as well as to the re-expression of transposable elements both *in vivo* and *in vitro*. Loss of DNA methylation and activation of transposons are both known to contribute to DNA damage and genomic instability. These observations open an important dilemma regarding the role of UHRF1 in DDR mechanisms: does the lack of UHRF1 result in detectable DNA damage due to impaired DDR, or is the loss of DNA methylation, induced by UHRF1 depletion, that contributes the

DNA damage? A clear answer is not available with our current understanding of UHRF1’s functions. Although here we described the evidence of the contribution of UHRF1 in DDR, whether and how UHRF1 loss leads to direct or indirect mechanisms of DNA damage formation is still not known. Answering this question remains an open challenge that would need to be addressed in order to fully understand the role of UHRF1 during development and in complex scenarios such as cancer formation and progression.

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