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Review Article

Implications of ubiquitination and the maintenance of replication fork stability in cancer therapy

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DNA replication forks are subject to intricate surveillance and strict regulation by sophisticated cellular machinery. Such close regulation is necessary to ensure the accurate duplication of genetic information and to tackle the diverse endogenous and exogenous stresses that impede this process. Stalled replication forks are vulnerable to collapse, which is a major cause of genomic instability and carcinogenesis. Replication stress responses, which are organized via a series of coordinated molecular events, stabilize stalled replication forks and carry out fork reversal and restoration. DNA damage tolerance and repair pathways such as homologous recombination and Fanconi anemia also contribute to replication fork stabilization. The signaling network that mediates the transduction and interplay of these pathways is regulated by a series of post-translational modifications, including ubiquitination, which affects the activity, stability, and interactome of substrates. In particular, the ubiquitination of replication protein A and proliferating cell nuclear antigen at stalled replication forks promotes the recruitment of downstream regulators. In this review, we describe the ubiquitination-mediated signaling cascades that regulate replication fork progression and stabilization. In addition, we discuss the targeting of replication fork stability and ubiquitination system components as a potential therapeutic approach for the treatment of cancer.

Introduction

DNA replication machinery is controlled by sophisticated regulatory mechanisms to maintain the faithful duplication and transmission of genetic information. However, the accuracy of DNA replication is often challenged by exogenous and endogenous stresses at active replication forks. These include DNA–protein cross-links (DPCs), intra- or inter-strand cross-links, secondary DNA structures, DNA lesions, and imbalanced dNTP pools [1]. Ultimately, replication stress causes chromosome instability, which can in turn lead to carcinogenesis [2]. As a result, the proper regulation and maintenance of DNA replication forks is of critical importance.

When replication stress arises, replication forks may stall in response. Stalling leads to the uncoupling of Cdc45-MCM-GINS (CMG) helicase and DNA polymerase and the generation of single-stranded DNA (ssDNA), which is rapidly bound and protected by the heterotrimeric replication protein A (RPA) complex (RPA1, RPA2, and RPA3). The ensuing recruitment of regulators to RPA-coated ssDNA results in the initiation of replication fork stabilization, replication fork reversal and restart [3], or DNA damage tolerance (DDT) [4]. This also triggers global responses: for example, the activation of cell cycle checkpoints, repression of origin firing, and balancing of the dNTP pool. This is mediated by the ataxia telangiectasia and RAD3-related kinase (ATR)-checkpoint kinase 1 (CHK1) signaling cascade [5].

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Table 1 E3 ligases that regulate replication fork stability

E3 ligase	Substrate	Function	REF.
OBI1	ORC	Promotes origin firing and recruitment of DNA replication factors	[16]
SCF-Skp2 DDB1-Cul4	CDT1	Maintains low levels of CDT1 in S and G2 phase	[17]
SPOP	Geminin	Blocks the binding of CDT1 to MCM and prevents the overfiring of replication origins	[18]
SCF^{Dia2}	MCM7	Recruits CDC48 to disassemble CMG helicase, mediating termination in budding yeast	[19,20]
$CUL2^{LRR1}$	MCM7	Recruits CDC48 to disassemble CMG helicase, mediating termination in metazoans	[21,22]
SCF^{Pof3}	Pol ε/δ MCM-2,-4,-6	Delays replication fork progression via the ubiquitin-proteasome system in Swi1-deficient cells	[23]
PRP19	RPA	Promotes ATR-ATRIP activation	[12]
RFWD3	RPA	Activates ATR-ATRIP and promotes PCNA ubiquitination	[24,25]
HectH9	TopBP1	Reduces TopBP1 recruitment to chromatin and attenuates ATR-dependent signaling	[26]
RAD18	PCNA	Promotes monoubiquitination of PCNA, facilitating binding to TLS polymerase, and helps to bypass problematic lesions	[27]
$CRL4^{Cdt2}$	PCNA	Facilitates the TLS pathway in undamaged cells	[28].
Rad5 HLTF/SHPRH	PCNA	Promotes the TS pathway and recruits the DNA translocase ZRANB3 to stalled replication forks	[13–15]
SCF^{FBH1}	RAD51	Prevents reassociation of RAD51 with DNA	[29]
RNF168	H2A	Prevents MRE11-dependent fork degradation	[30]
Bre1	H2B	Facilitates fork stalling and recovery during replication stress	[31,32]
MDM2	PARP1	Increases the activity of RECQ1 and PRIMPOL, promoting restart and repriming of DNA synthesis	[33]
SIAH2	CtIP	Promotes the loading of SIAH2 at stalled replication forks, facilitating efficient restart	[34]
TRAIP	DPCs	Promotes degradation of DPCs via the ubiquitin-proteasome pathway	[35]
TRAIP	CMG	Promotes CDC48/p97-dependent disassembly of CMG at ICLs and NEIL3 pathway	[36,37]
FANCL PHF9	FANCD2/FANCI	Promotes ICL repair via nuclease-dependent DNA incision carried out by XPF (FANCQ)-ERCC1 and SLX4 (FANCP) and diminishes R-loop accumulation	[38,39]

Abbreviations: DPC, DNA–protein cross-link; ICL, DNA interstrand cross-link; TLS, translesion synthesis; TS, template-switching.

The replication stress response signaling network is modulated via a series of post-translational modifications, with phosphorylation and ubiquitination being the most important of these. Ubiquitination is a reversible post-translational modification which involves the covalent attachment of ubiquitin (Ub), a compact 76-amino acid protein, on to the lysine residues of various substrates. This modification is catalyzed by three different classes of enzymes: Ub-activating enzymes (E1), Ub-conjugating enzymes (E2), and Ub ligases (E3). During ubiquitination, an E1 enzyme activates the C-terminus of Ub and transfers it to the active cysteine site of an E2 enzyme. Subsequently, an E3 ligase, which specifically recognizes the substrates, acts as an intermediate recruiter of Ub-loaded E2 and catalyzes the ubiquitination of target proteins [6,7]. Polyubiquitination involves the formation of polyubiquitin chains by linking additional Ub molecules together via their lysine residues (K6, K11, K27, K29, K33, K48, or K63) or N-terminal methionine (M1). Meanwhile, attached Ub or Ub chains are removed by the Ub-cleavage activity of deubiquitinases (DUBs) [8].

The location and type of ubiquitination confers different fates to substrates. For example, polyubiquitination via linkage at K48 or K11 marks substrates for degradation via the proteasome system [9]. K63-linked polyubiquitination is non-proteolytic in most scenarios, but has been reported to regulate substrate degradation under restricted circumstances [10]. Furthermore, mono-, multi-mono-, and polyubiquitination at M1, K6, K11, K27, K29, K33, and K63 residues is known to regulate the localization, interactome, and activity of various substrates [9,11]. In particular, the ubiquitination of critical regulators allows for a cascade of molecular interactions and events to counteract replication stress (Tables 1 and 2). For example, RPA ubiquitination promotes the recruitment of the ATR-ATR interacting protein (ATRIP) complex, ensuing activation of the ATR-CHK1 signaling cascade [12], while proliferating cell nuclear antigen (PCNA) ubiquitination is a prerequisite for DDT and the reversal of stalled replication forks [13–15]. Balanced ubiquitination is crucial for the efficient switching of substrate activity and signal transduction during the progression of replication and the resolution of replication stress. Furthermore, if the ubiquitination of regulatory molecules is dysregulated, the stability of replication forks is threatened. When this occurs, chromosome instability may follow, and cancer with it.

The activation of oncogenes is also known to induce severe replication stress. As a result, this hallmark of cancer may provide various promising targets for the development of therapeutic drugs. In this review, we summarize

Table 2 Deubiquitinases that regulate replication fork stability

Deubiquitinase	Substrate	Function	REF.
USP36	PRIMPOL	Enhances PRIMPOL stability at stressed forks	[40]
USP1	PCNA	Removes the monoubiquitin moiety from PCNA to switch off the TLS pathway	[41]
USP1/UAF1	FANCD2	Removes the monoubiquitin moiety from FANCD2 to enable efficient completion of DNA crosslink repair	[42–45]
USP10	PCNA	Cooperates with ISGylated PCNA to suppress the TLS pathway	[46]
USP7	SPRTN	Antagonizes the auto-cleavage and subsequent inactivation of SPRTN	[47]
USP11	SPRTN	Prevents auto-cleavage of monoubiquitinated SPRTN	[48]
VCPIP1/VCIP135	SPRTN	Promotes the relocalization of SPRTN onto chromatin	[49]

Abbreviations: TLS, translesion synthesis; TS, template-switching.

the ubiquitination modifications that regulate DNA replication and stalled replication fork stabilization, including replication fork reversal and restart, DNA damage tolerance, and the involvement of DNA repair. We also discuss the potential targets yielded by this system for the development of small molecules as anticancer therapies.

The involvement of ubiquitination in the regulation of replication fork progression

DNA replication comprises several steps, including origin licensing, origin firing, replication fork elongation and termination, and is monitored and regulated by precise, sophisticated cellular machinery. Preparation for DNA replication begins in G1 phase with the recognition of replication origins by the origin recognition complex (ORC), a multi-subunit AAA+ ATPase (ORC1-6 and LRWD1/ORCA in vertebrates) [50]. ORC, together with cell division cycle 6 protein (CDC6), recruits CDC10-dependent transcript 1 (CDT1), which binds to and assembles the minichromosome maintenance 2-7 (MCM2-7) complex. In turn, this complex licenses replication origins. Only a small fraction of origins licensed by pre-replicative complexes (pre-RCs) are fired in S phase. Cyclin-dependent kinase (CDK) and DBF4-dependent kinase (DDK) are responsible for activating the MCM2-7 helicase, which facilitates the formation of CMG helicase and the subsequent unwinding of DNA to form active replication forks [51,52]. The E3 ligase ORC-ubiquitin-ligase-1 (OBI1, also known as C13ORF7/RNF219) catalyzes the multi-monoubiquitination of chromatin-bound ORC3 and ORC5. OBI1 deficiency inhibits the firing of replication origins and recruitment of DNA replication factors such as DNA polymerase α and PCNA; however, pre-RC assembly and origin licensing are unaffected by deficient OBI1-mediated ORC ubiquitination [16].

DNA replication takes place at multiple origins to guarantee efficiency, with replication of genome information occurring only once per cell cycle. Balanced origin firing is essential for the accurate duplication of genomic information: replication overfiring and re-replication increase the likelihood of stalled forks, which can lead to fork collapse if improperly repaired. Thus, origin licensing is carefully regulated and restricted in G1 phase to avoid origin licensing/reactivation after the G1/S transition, and potentially catastrophic outcomes such as amplified replication and chromosome breakage. For example, the expression of CDT1, a pivotal regulator of pre-RC assembly, is subject to strict cell cycle-dependent control. Low levels of CDT1 during S and G2 phases are achieved via the Ub-proteasome system, which is under the regulatory control of the E3 ligases SKP1-cullin1-F-box protein (SCF)-Skp2 and DNA damage-binding protein 1(DDB1)-Cul4 [17]. In S phase, binding of CDT1 to the MCM complex is blocked by geminin protein, preventing the overfiring of replication origins. This process requires the K27-linked polyubiquitination of geminin at K100 and K127 by the E3 ligase SPOP [18]. In prostate cancer, mutant SPOP fails to ubiquitinate geminin, leading to origin overfiring, re-replication, and genomic instability [18,53]. However, geminin can be stabilized by the deubiquitinases DUB3 and ubiquitin carboxyl-terminal hydrolase 7 (USP7), and DUB3 deficiency is known to increase re-replication events [54].

The replisome initiates DNA replication at fired origins by assembling the DNA polymerase α-primase complex via TIMELESS/ TIMELESS-interacting protein (TIPIN)/AND-1 proteins [55]. After the assembly of the CTF18-replication factor complex (RFC) and PCNA, efficient elongation of the leading and lagging strands of replication forks is carried out by DNA polymerase ε and DNA polymerase δ, respectively [56]. Following elongation, the replisome is disassembled during final step of chromosomal DNA replication: termination. This step is accomplished by the degradation of the core component, CMG helicase, which functions to bind and unwind the parental DNA during replication. Once DNA synthesis is terminated through the convergence of opposing replication forks, the CMG helicase is ubiquitinated and disassembled [57,58]. Ubiquitination-mediated destruction of the CMG helicase

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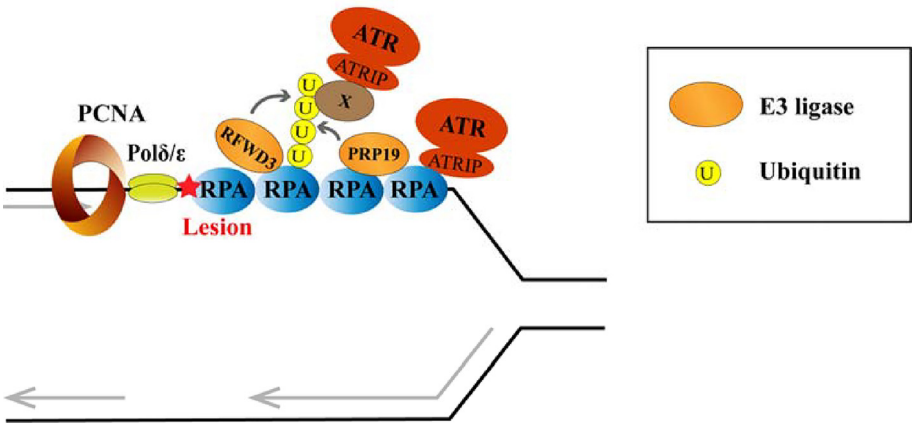

Figure 1. Ubiquitination at the RPA-coated ssDNA platform under replication stress

ATR activation promotes PRP19 recruitment to RPA-coated ssDNA, PRP19 and RFWD3 catalyze RPA poly-ubiquitination at the stalled replication fork. ATR-ATRIP is recruited to stalled replication forks by directly binding to RPA. Recruitment of ATR-ATRIP is also promoted by RPA ubiquitination, and that might be mediated by an uncharacterized protein (protein X) as the lack of ubiquitin binding domain on ATR-ATRIP.

is catalyzed by the E3 ligase complex SCF^{Dia2} in budding yeast and $CUL2^{LRR1}$ in metazoans [21,19]. The leucine-rich repeat domains of Dia2 and LRR1 bind to the zinc-finger domains of MCM3 and MCM5 within the CMG complex, but this interaction is occluded by the excluded DNA strand at replication forks before termination [59]. In budding yeast, ubiquitination of CMG helicase on MCM7 K29 residues occurs at the end of DNA replication. This ubiquitination signal is recognized by the UFD1-NPL4 complex, which recruits the segregase cell division control protein 48 (CDC48, also known as p97) to drive the disassembly of CMG helicase [20]. $CUL2^{LRR1}$-mediated ubiquitination of MCM7 also drives the disassembly of CMG helicase by UFD1-NPL4-CDC48 in S phase. Moreover, a backup pathway involving CDC48 and its cofactor UBXN-3 is activated during prophase, where it removes the accumulated CMG helicase on chromatin under conditions of $CUL2^{LRR1}$ deficiency [22].

In addition to the replisome, the replication fork protection complex (FPC), exemplified by the Swi1-Swi3 complex in fission yeast, moves with the replication forks and acts to stabilize them [60]. Importantly, Swi1 deficiency triggers the destruction of DNA polymerases (Polε and Polδ) and DNA helicases (MCM-2,-4,-6) via the ubiquitin-proteasome system, which is regulated by the E3 ligase SCF^{Pof3}. This degradation delays replication fork progression, avoiding aberrant DNA replication and mitotic catastrophe [23]. In general, ubiquitination and deubiquitination targeting the core components of the replisome or related factors, which regulate the assembly or disassembly of the replication machinery in cell cycle-dependent manner, contribute to the precise progression of replication forks.

Ubiquitination coordinates a concerted response at stalled replication forks

Replicating DNA is predisposed to collapse if it is insufficiently stabilized or unable to efficiently counteract threats that impede its progression. Stalling DNA polymerases are uncoupled from CMG helicase, leading to the generation of ssDNA that is rapidly bound and protected by the RPA complex. RPA-coated ssDNA serves a platform for the loading of downstream regulators capable of responding to replication stress. Among these factors, ATR kinase is a key regulator that stabilizes stalled replication forks and orchestrates a global response to trigger cell cycle checkpoints, repress origin firing, and sustain a balanced dNTP pool [61]. ATR knockout leads to chromosome breakage and cell death, while ATR haploinsufficiency and mutation cause tumorigenesis [62,63]. ATR, together with ATR-interacting protein (ATRIP), is recruited to stalled replication forks by RPA [64]. Although ATR-ATRIP can directly bind to RPA-ssDNA, it is proposed that optimal loading of the ATR-ATRIP complex on to stalled replication forks also requires K63-linked polyubiquitination of RPA, which is mediated by the E3 ligase pre-mRNA-processing factor 19 (PRP19) (Figure 1) [12]. However, whether ATR-ATRIP could directly recognize this K63-linked polyubiquitination on RPA still need further elucidation. Moreover, the lack of ubiquitin binding domains on the reported ATR-ATRIP cryo-EM structure raises the possibility of the existence of a potential protein X that bridges the connection between ATR-ATRIP and the ubiquitin chains [12]. The replication stress/DNA damage-induced interaction between PRP19

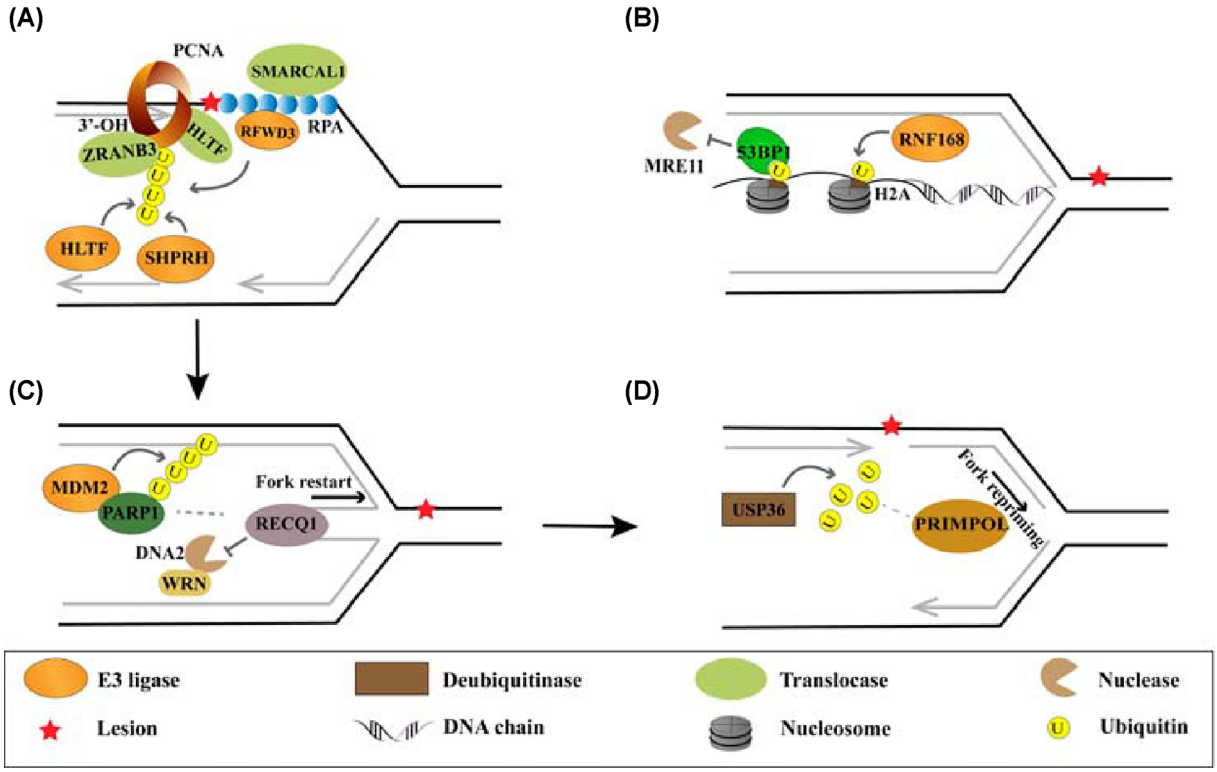

Figure 2. Regulation of fork reversal and restart by ubiquitination

(**A**) Fork reversal is initiated by DNA translocases such as SMARCAL1, ZRANB3, and HLTF. SMARCAL1 binds to RPA-coated ssDNA, while ZRANB3 is recruited at stalling forks via polyubiquitinated PCNA mediated by RFWD3, HLTF, or SHPRH, and HLTF has an affinity for available 3′-OH groups located near the fork junction. These three translocases may act on different types of stalled forks or perform distinct functions at the same fork. (**B**) The regressed arm of a reversed fork requires protection against nucleolytic attacks. RNF168-mediated monoubiquitination of H2A on K13/15 at stalled forks is recognized by 53BP1, protecting the regressed arms from degradation by MRE11: this modification also regulates nucleosome deposition, facilitating efficient fork restart. (**C**) Replication fork restart or repriming requires specific factors such as RECQ1 and PRIMPOL. RECQ1 activity is increased following the ubiquitination and inactivation of PARP1 by the oncogenic E3 ligase MDM2, which accelerates DNA replication progression. (**D**) Deubiquitination of PRIMPOL by USP36 enhances its stability at stressed forks, contributing to replication repriming.

and RPA is dependent on PI3K kinase (ATR, ATM, and DNA-PK)-mediated phosphorylation of RPA, forming a feed-forward loop that accelerates ATR activation [12,65]. In addition, the E3 ligase RFWD3 interacts constitutively with RPA, promoting the optimized ubiquitination of RPA and the activation of ATR [24]. Notably, recruitment of ATR-ATRIP is essential for the activation of ATR kinase, but is not in itself sufficient: ATR kinase activation also requires the allosteric activators topoisomerase II binding protein 1 (TopBP1) and Ewing tumor-associated antigen 1 (ETAA1). TopBP1 and ETAA1 are recruited to stalled replication forks via RFC-RAD17/RAD9-RAD1-HUS1 complexes and RPA, respectively [66,67]. Following its recruitment to chromatin by MIZ1, TopBP1 is protected from proteasomal degradation mediated by the ubiquitin ligase HectH9 (Mule, ARF-BP1, and HUWE1); however, the oncoprotein MYC disrupts the association between TopBP1 and MIZ1. This results in reduced TopBP1 levels and attenuated ATR-dependent signaling [26].

Under normal conditions, RFWD3, which promotes the ubiquitination of RPA, is reported to localize at replication forks and is essential for efficient DNA replication, and it is suggested that RFWD3 loads onto replication forks via interaction with PCNA [68]. Under conditions of fork collapse, the RPA-mediated localization of RFWD3 to ssDNA is critical for gap-filling repairs [25, 69, 70]. The interaction between RFWD3 and RPA can be disrupted by the mutation of RFWD3 in the WD40 repeat region (I639K) or by single point mutations in the RPA32 subunit of RPA (F248, E252, or H254), which inhibits the recruitment of RFWD3 to DNA damage sites [70]. RFWD3 also promotes the ubiquitination of PCNA and the ensuing recruitment of downstream effectors (Figure 2A) [25]. Similar to RPA-coated ssDNA, polyubiquitinated PCNA serves as a platform for concerted molecular events to stabilize stalled replication

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forks. PCNA is monoubiquitinated at lysine 164 by the E3 ligase RAD18, which is recruited to stalled replication forks by RPA [27]. Further K63-linked polyubiquitination of PCNA at K164, which is catalyzed by Rad5 in yeast or by its homologs helicase-like transcription factor (HLTF) and SNF2 histone-linker PHD-finger RING-finger helicase (SHPRH) in mammalian cells [71,72], leads to the recruitment of the DNA translocase zinc finger RANBP2-type containing 3 (ZRANB3) to stalled replication forks [13,14]. HLTF has a strong preference for available 3′-OH groups near fork junctions, and in addition to its E3 ligase activity, exhibits translocase activity that contributes to replication fork remodeling [73]. Meanwhile, another translocase, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A like 1 (SMARCAL1), is loaded via interaction with RPA [74]. The DNA translocases SMARCAL1, ZRANB3, and HLTF initiate the reversal of stalled replication forks, forming a protective four-way junction (also termed the 'chicken foot') to overcome replication stress (Figure 2A). An *in vitro* study showed that SMARCAL1, ZRANB3, and HLTF recognize different fork structures, indicating a need for different remodelers to cope with the diverse fork structures generated by challenges to replication.

The regressed arms at fork reversal sites resemble free DNA double-strand break (DSB) ends and, as such, are potentially vulnerable to attack by nucleases such as DNA2, exonuclease 1 (EXO1), C-terminal binding protein interacting protein (CtIP), and meiotic recombination 11 homolog 1 (MRE11). The recruitment of specific DSB repair regulators to reversed forks is, therefore, necessary to protect the DNA from nucleolytic threats [30]. For example, the formation of BRCA2-dependent RAD51 filaments at stalled replication forks protects the nascent DNA from MRE11-, MUS81-, CtIP-, and EXO1-mediated degradation; this protective effect partially underlies the chemosensitivity of BRCA2-deficient tumors [75,76]. Ubiquitination of RAD51 by the E3 ligase SCF^{FBH1} complex at K58 and K64 also serves to prevent any unregulated activity [29]. The E3 ligase RNF168, a key regulator in DSB repair, is also localized at replication forks, and its deficiency leads to elevated fork reversal. RNF168-mediated monoubiquitination of H2A on K13/15 at stalled forks leads to recognition by the DSB repair protein 53BP1, which promotes non-homologous end joining-mediated repair and counteracts the homologous recombination (HR)-mediated repair of DSB. This protects the regressed arms from degradation by MRE11, and regulates nucleosome deposition for efficient fork restart (Figure 2B) [30]. In addition, PCNA ubiquitination at K164 results in the recruitment of the translocase ZRANB3, which promotes fork reversal and protects stalled forks from nucleolysis (Figure 2A). Mutations in K164R cause enhanced retention of PCNA on chromatin, leading to aberrant nucleosome packaging and susceptibility to degradation by DNA2 at stalled replication forks [77].

The prompt restart of reversed forks following the resolution of replication threats is necessary for the effective completion of DNA synthesis. The restarting of reversed forks is promoted by the human helicase RECQ1 in a DNA2- and WRN-dependent manner, with limited degradation of nascent DNA [78]. As a key contributor to the DNA damage response, PARP1 also participates in the regulation of stalled replication fork reversal and restart. It is proposed that PARP1 binds to stalled replication forks and recruits the translocases SMARCAL1, ZRANB3, and HLTF to initiate fork reversal [79]. The PARylation activity of PARP1 further prevents the RECQ1-dependent premature restart of reversed forks [80]. PARP1 also recruits MRE11 to stalled replication forks, promoting end resection and efficient fork restart [81]. The oncoprotein mouse double minute 2 homolog (MDM2), a well-known E3 ligase of tumor suppressor P53, is known to ubiquitinate PARP1, targeting it for proteasomal degradation. Correspondingly, elevated levels of MDM2 destabilize PARP1, reduce stalled replication fork reversal, and induce nascent DNA elongation as mediated by the RECQ1 and primase/polymerase PRIMPOL. These effects ultimately lead to replication fork instability and micronuclei accumulation (Figure 2C) [33]. As a mechanism of alleviating replication stress, the deubiquitination of PRIMPOL at K29-linked polyubiquitination sites is carried out by USP36, leading to PRIMPOL stabilization. Replication stress enhances the interaction between USP36 and PRIMPOL, but this is dependent on the deubiquitination of USP36 at lysine 329 and lysine 338 (Figure 2D) [40]. Moreover, the E3 ligase SIAH2 interacts with and ubiquitinates CtIP at K62, K78, K115, K132, and K133, which promotes the loading of CtIP at stalled replication forks and facilitates efficient fork restart. [34].

The particular structure of stalled replication forks determines the finely tuned responses orchestrated by multiple molecular events involved in different repair pathways. The regressed arms should be protected from nucleases as its resemblance with free DSB ends, moreover, pathway choice with DNA damage tolerance is also noteworthy as it also depends on PCNA ubiquitination at K164.

DNA damage tolerance (DDT) pathway

To support the progression of DNA replication forks, RPA-coated ssDNA localized at stalled forks triggers the DDT pathway. This pathway includes error-prone translesion synthesis (TLS) and error-free template switching (TS), which are driven by PCNA ubiquitination [82,83]. Under replication stress, RPA-coated ssDNA recruits E3 ligase RAD18

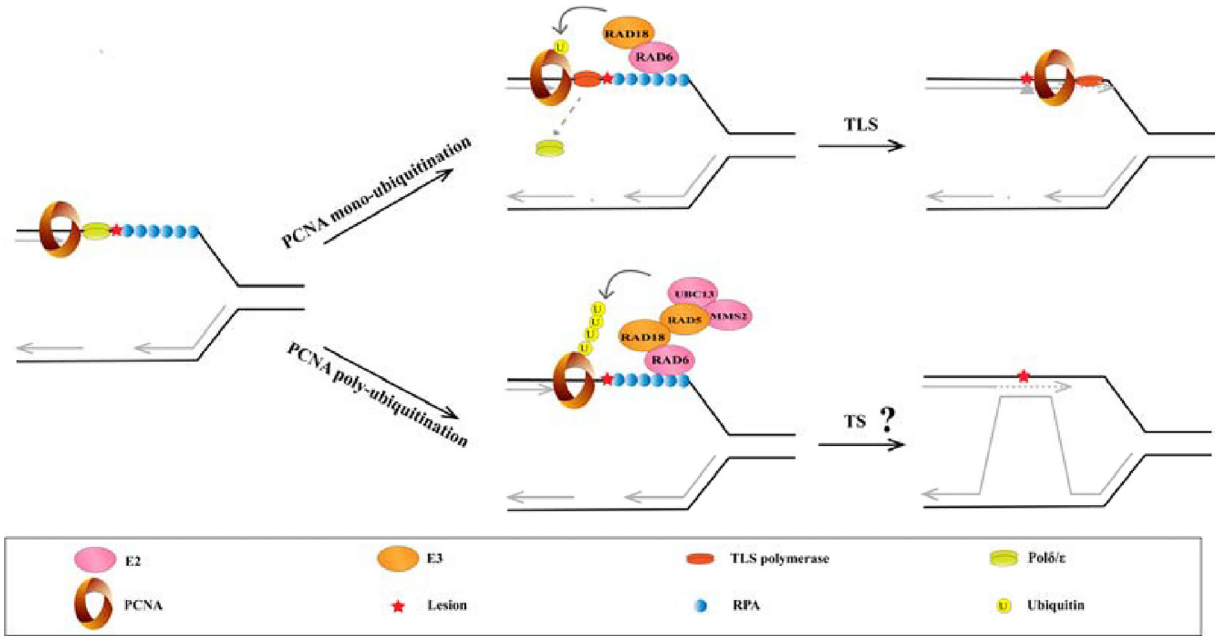

Figure 3. PCNA ubiquitination in the translesion synthesis and template switching pathways

PCNA monoubiquitination promotes polymerase switching to activate the translesion synthesis pathway (TLS) and enable the bypassing of problematic lesions. Under replication stress, RPA-coated ssDNA recruits a complex of RAD18 and RAD6, leading to PCNA monoubiquitination at K164. This modification disrupts the affinity of PCNA for B-family DNA polymerases (Polδ and Polε) and increases its affinity for specialized Y-family polymerases (including Polη, Polι, Polκ, and Rev1); these events lead to error-prone TLS. In template switching (TS), following the initial RAD6/RAD18-dependent monoubiquitination of PCNA at K164, UBC13-MMS2, and RAD5 are required to generate K63-linked polyubiquitin chains. However, the exact details of TS following PCNA polyubiquitination remain unknown.

and E2 enzyme RAD6, leading to PCNA monoubiquitination at K164 (Figure 3) [27,84–87]. This modification alters PCNA conformation, disrupting its affinity for B-family DNA polymerases (Polδ and Polε) and increasing its affinity for the specialized Y-family polymerases (including Polη, Polι, Polκ, and Rev1), which carry out error-prone TLS [83,88]. Although the Y-family polymerases are characterized by both low processivity and low fidelity, Polη can accurately replicate thymidine-thymidine dimers and bypass UV-induced cyclobutane pyrimidine dimers [89]. Polη deficiency causes the genetic disorder xeroderma pigmentosum variant, which causes predisposition to sunlight-induced skin cancer [90]. In addition to the putative RAD18/RAD6-catalyzed monoubiquitination of PCNA described above, PCNA is monoubiquitinated at K164 by the E3 ligase complex $CRL4^{Cdt2}$ in unperturbed cells [28]. However, to ensure replication accuracy, monoubiquitination by $CRL4^{Cdt2}$ is constitutively antagonized by ubiquitin-specific protease 1 (USP1)-mediated deubiquitination [28]. Conversely, UV-induced auto-cleavage of USP1 enhances the accumulation of monoubiquitinated PCNA, promoting TLS [41]. At the termination of TLS, monoubiquitinated PCNA binds to EFP, an E3 ligase for the ubiquitin-like protein interferon-stimulated gene 15 (ISG15). EFP-catalyzed ISGylation of PCNA leads to recognition by the deubiquitinase USP10, which removes the monoubiquitin moiety from PCNA and allows the release of the Y-family polymerases. Subsequently, deISGylation by USP43 enables PCNA to rebind to replicative DNA polymerases in order to resume normal DNA replication [46]. In addition, the E3 ligases HERC2 and RNF8 promote TLS across abasic sites at (or close to) stalled replication forks in a monoubiquitinated PCNA-independent manner [91]. However, the exact targets of HERC2 and RNF8 remain to be determined.

As well as mediating the transition from replicative to TLS polymerases, ubiquitinated PCNA also recruits other regulators, including the AAA+ class ATPase Werner helicase interacting protein 1 (WRNIP1), which has multiple functions. WRNIP1 (Mgs1 in yeast) contains a ubiquitin-binding zinc finger (UBZ) domain, which directs the binding of WRNIP1 to ubiquitinated PCNA and disrupts the association between PCNA and polymerase δ at stalled replication forks [92,93]. Simultaneously, WRNIP1 promotes stabilization of RAD51, preventing uncontrolled nucleolytic attack by MRE11 [94,95]. During replication stress, WRNIP1 also bridges ATM signaling by interacting with ATM interactor protein (ATMIN), thereby preventing aberrant mitotic segregation [96].

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In contrast to TLS, TS uses the undamaged nascent sister chromatid as a template to carry out limited DNA replication [97]. This process occurs after fork reversal, enabling the bypassing of DNA lesions, and is dependent on PCNA polyubiquitination. K63-linked polyubiquitination of PCNA mediated by UBC13-MMS2/RAD5 (an E2, E2 variant, and E3 ubiquitin ligase, respectively) in yeast (Figure 3) follows its initial RAD6/RAD18-dependent monoubiquitination at K164 [98,99]. In mammalian cells, two human RAD5 homologs, HLTF and SHPRH, are responsible for the K63-linked polyubiquitination of PCNA at K164 [71,72]. While helicase-coupled repriming occurs downstream of the DNA lesions for continued replication fork progression, the DNA lesions and gaps left behind replication forks due to repriming are reported to be resolved accurately by template switch [100]. In *Saccharomyces cerevisiae*, Polα/Primase/Ctf4 deficiency disturbs the scheduled repriming, leading to longer ssDNA, increased fork reversal, decreased error-free DTT, and increased error-prone DTT. Although long stretch of ssDNA seems to repress error-free DTT, it is required for the loading of recombinase RAD51 and PCNA monoubiquitination E3 ligase RAD18. More precise mechanisms keep the delicate balance that guide the response at stalled replication forks to fork repriming, fork reversal, and error-free or error-prone DTT worth further elucidation. The entire procedure of template switching is not restricted in S phase. TS intermediates pseudo-double Holliday-Junctions may persist till G2/M phase and is processed by Mus81-Mms4 and Slx4 nucleases in cell cycle-dependent manner [101–104].

TS-dependent K63-linked polyubiquitination is elongated at the basis of PCNA monoubiquitination at K164 residue which mediates TLS. Inhibition of this type of PCNA polyubiquitination is known to skew DNA damage tolerance toward TLS and to increase UV-induced mutagenesis [105]. Ready access to the recombination template of TS due to sister chromatid cohesion established during DNA replication and the recruitment of recombinase RAD51 provide the preference of TS over TLS during S phase [101,106]. Besides, chromatin status and chromosome architecture are both proposed to regulate the DDT pathway choice [97,107–109].

PCNA is also modified by the attachment of the small ubiquitin-like modifier (SUMO) at K164 site. SUMOylated PCNA is recognized by the helicase Srs2, which represses HR by disrupting RAD51 filaments in yeast [110,111]. RAD51-mediated HR represents an alternative mechanism for bypassing DNA lesions. PCNA SUMOylation represses unscheduled recombination and helps maintain the balance of DDT and HR at stalled replication forks [112]. In a DDT-deficient scenario, DNA DSBs generated at collapsed replication forks are repaired via HR to initiate DNA replication. Monoubiquitination of histone H2B (H2Bub) at K123, catalyzed by Bre1, is critical for both the DDT and HR pathways during replication stress. In the absence of H2Bub, cells are more susceptible to hydroxyurea-induced replication stress, exhibiting increased RPA foci and decreasing Rad51 recruitment in S phase [31,32]. Another anti-recombination helicase, FBH1, which also functions as an F-box E3 ligase that targets RAD51 for degradation, acts as a master regulator of HR by limiting RAD51-mediated recombination at blocked replication forks [113]. FBH1 is recruited by PCNA via PCNA-interacting peptide (PIP), leading to repressed HR at replication forks. Subsequently, the PIP box is recognized by the E3 ligase complex $CRL4^{Cdt2}$, triggering the ubiquitin-proteasome degradation of FBH1 and ensuing recruitment of TLS polymerases [114]. As a highly conserved site, K164 residue of PCNA is subjected to three post-translational modifications, namely SUMOylation, monoubiquitination, and polyubiquitination. The recombinase RAD51 also plays pivotal role in both DDT and HR pathways at stalled replication forks. The multifunction of these key players reflects the delicate balances of pathway choice according to specific scenarios.

Engagement of DNA repair pathways

In addition to becoming uncoupled from DNA polymerase at stalled replication forks, CMG helicase can also be blocked when encountering specific DNA lesions. Such lesions include DPCs and DNA interstrand cross-links (ICLs). The proteasome-dependent removal of DPCs relies partially on E3 ligase TRAIP-mediated DPC polyubiquitination during DNA replication (Figure 4A) [35]. DPCs can also be degraded by the metalloprotease SPARTAN (SPRTN), independent from the ubiquitin-proteasome system (Figure 4A). Germline SPRTN mutations cause Ruijs-Aalfs syndrome, which is characterized by premature aging and hepatocellular carcinoma [115,116]. Monoubiquitination by an unknown E3 ligase triggers the autocleavage and proteasomal degradation of SPRTN; however, under DPC-induced replication stress conditions, the DUBs USP7 and USP11 are reported to deubiquitinate SPRTN following its localization to chromatin [47,48]. The DUB VCPIP1/VCIP135 also deubiquitinates SPRTN after being phosphorylated and activated by ATM/ATR. The deubiquitination of SPRTN promotes its acetylation and ability to localize to chromatin [49]. As SPRTN acts on loosely folded DPCs, ubiquitin-modified DPCs must first be unfolded by p97 in association with the ubiquitin adapter complex Ufd1-Npl4 to facilitate SPRTN-mediated proteolysis [117]. Moreover, SPRTN also recognizes ubiquitinated PCNA, which promotes the recruitment of RAD18 and removal of USP1 from chromatin. This further accelerates PCNA monoubiquitination [118]. However, further investigation into the mechanisms underlying the resolution of DPCs is still required. For example, although the TRAIP-proteasome system and

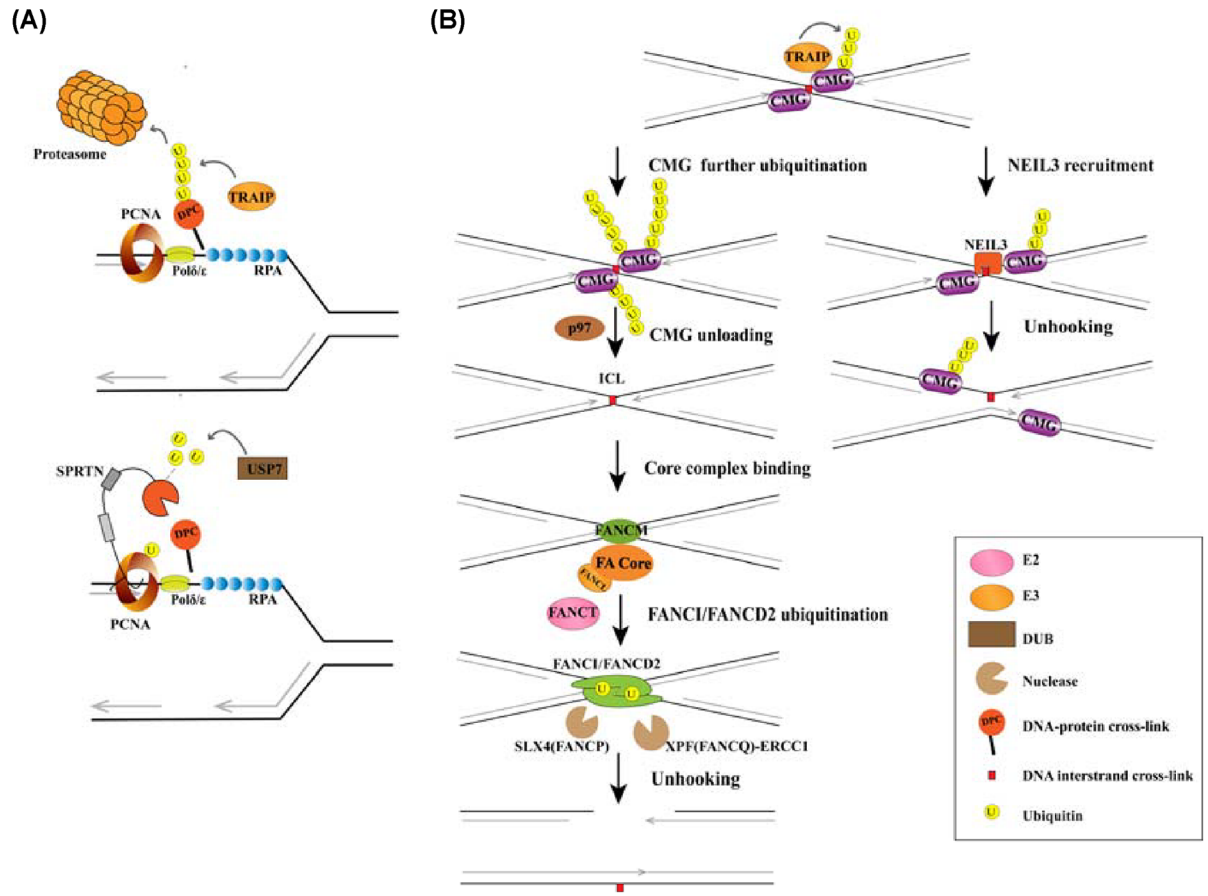

Figure 4. The role of ubiquitination in the removal of DNA-protein cross-links and interstrand cross-links

(**A**) The removal of DNA–protein cross-links (DPCs) relies mainly on TRAIP/proteasomes (top), which degrade polyubiquitinated DPCs, or SPRTN (bottom), which is recruited by ubiquitinated PCNA and deubiquinated by USP7. (**B**) Interstrand cross-link (ICL) removal is mainly mediated by the FA pathway. When two replication forks converge on an ICL, TRAIP-generated Ub chains recruit the glycosylase NEIL3 to directly cleave cross-links, while longer Ub chains promote CDC48/p97-dependent CMG disassembly. Next, a heterotetrameric FANCM complex recognizes the stressed replication forks and recruits the FA core complex. Within the core complex, FANCL (E3 ubiquitin ligase) and FANCT (E2) monoubiquitinate FANCD2 at K561 and FANCI at K523. This step promotes the nuclease-dependent DNA incision by XPF (FANCQ)-ERCC1 and SLX4 (FANCP), leading to the unhooking of ICLs and generation of DSB intermediates.

SPRTN-dependent pathway are known to be involved in DPC removal, the functions of other DPC proteases in response to different types of DPCs should also be characterized. In addition, while ubiquitination and deubiquitination are known to function as molecular switches of SPRTN activity, the corresponding E3 ligase is yet to be identified.

ICLs halt replication fork progression and transcription by covalently connecting the two strands of DNA [119]. Defective ICL repair causes Fanconi anemia (FA), an autosomal recessive disorder characterized by developmental abnormalities, bone marrow failure, and predisposition to cancers such as leukemias and carcinomas [120–122]. The DNA replication-dependent FA pathway, which involves ubiquitination of the core component FANCD2/FANCI, is well known for its critical role in ICL repair (Figure 4B). Unloading of the CMG helicase from chromatin is a prominent event in the initiation of the FA pathway, in a similar manner to replication termination. Replication termination involves the polyubiquitination of MCM7 and CDC48/p97-dependent disassembly of CMG, while polyubiquitination of CMG at ICLs requires TRAIP but not $CUL2^{LRR11}$ [36,37]. Short TRAIP-generated Ub chains recruit the glycosylase NEIL3 to directly cleave cross-links, while longer Ub chains promote p97-dependent CMG unloading [36]. Subsequently, the anchoring complex, which consists of the ATPase FANCM, FAAP24, MHF1, and MHF2, recognizes the stalled replication fork and mediates recruitment of the FA core complex [119,123,124]. The E3 ubiquitin ligase FANCL (PHF9) then cooperates with FANCT (UBE2T), an E2 enzyme, to monoubiquitinate FANCD2

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Table 3 Small molecules designed to target proteasomes for cancer therapy

Target	Inhibitor	Cancer types	Phase	REF.
Proteasome	Bortezomib carfilzomib ixazomib	Multiple myeloma, Mantle cell lymphoma	FDA-approved	[137,138,141]
Proteasome	marizomib oprozomib delanzomib	Haematological malignancies, solid tumors	Early-phase clinical trials	[142]

and FANCI on K561 and K523, respectively [125–127]. Intriguingly, FANCM complex-deficient cells exhibit only partially reduced levels of mono-ubiquitinated FANCD2/FANCI [124], suggesting the existence of an alternative FA core complex recruitment pathway. Consistent with this notion, UHRF1 has been found to bind to ICLs and promote the recruitment of FANCD2 [128]. Ubiquitination of the FANCI/FANCD2 complex promotes nuclease-dependent DNA incision by XPF (FANCQ)-ERCC1 and SLX4 (FANCP) [38, 129, 130], leading to the unhooking of ICLs. The resulting intermediate DSBs are then repaired via HR, classical nonhomologous end joining, alternative nonhomologous end joining and single-strand annealing [120]. To bypass DNA lesions, the unhooking step also includes the recruitment of the TLS polymerases Rev1 and Polι [131]; however, the adducts remaining in the parental strand cannot be removed completely [132]. FANCD2 is also recruited to R-loops, which are DNA:RNA hybrid structures with displaced ssDNA, via binding the displaced ssDNA strand and ssRNA tail. Monoubiquitinated FANCD2/FANCI is also known to promote the resolution of R-loops, but the precise mechanism remains elusive [39].

The TLS and FA pathways are closely interconnected. For example, RAD18/RAD6 is required for efficient FANCD2/FANCI monoubiquitination and chromatin localization [133,134]. Meanwhile, FAAP20, an FA complex subunit responsible for core complex stability and FANCD2 monoubiquitination, binds to monoubiquitinated Rev1 via its Ub-binding zinc finger 4 domain and stabilizes Rev1 nuclear foci [135,136]. In addition, monoubiquitinated FANCD2/FANCI proteins are deubiquitinated by the USP1/UAF1 complex after DNA removal, completing the ICL repair procedure [42–45].

Targeting ubiquitination and replication fork stability in cancer therapy

Drug resistance is one of the major challenges faced when developing treatment strategies for various types of cancer. The ubiquitin–proteasome system (UPS) regulates nearly all cellular activities as it controls protein turnover, and aberrant expression levels of oncoproteins or tumor suppressors increase the likelihood of carcinogenesis. As a result, the UPS represents an emerging target for the development of novel anticancer drugs and chemosensitizers, with the aim of overcoming drug resistance. This approach has already borne fruit: bortezomib, the first therapeutic proteasome inhibitor to be approved by the Food and Drug Administration (FDA) for the treatment of multiple myeloma (MM) [137] and mantle cell lymphoma (MCL) [138], exhibits favorable selectivity towards tumor cells over normal cells. Bortezomib functions by inhibiting chymotrypsin-like activity, primarily through its interactions with the proteasome β5 core particle [139]. Bortezomib retreatment in patients with myeloma has no cumulative toxicity, and a combination of bortezomib with a rituximab, cyclophosphamide, doxorubicin, and prednisone treatment regimen (VcR-CAP) has shown improved outcomes compared with the R-CHOP regimen [138]. This latter regimen involves a combination of bendamustine plus rituximab with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. However, bortezomib has no obvious effect in on solid tumors such as prostate cancer, breast cancer, and non-small cell lung cancer [140]. The potency of bortezomib and apparent lack of treatment resistance has driven the development of next generation proteasome inhibitors, such as carfilzomib and ixazomib, for the treatment of blood cancers. More inhibitors are in the preclinical investigation phase, or are currently undergoing clinical trials (Table 3).

Alternative strategies for the development of chemotherapeutic drugs involve blocking the catalytic activity of enzymes such as E2, E3, and DUBs, or disrupting the interaction between these enzymes and specific substrates. E3 ligases and DUBs are of critical importance to the maintenance of replication fork stability (Tables 1 and 2) and are aberrantly expressed in several types of cancer (Table 4). Several small-molecule inhibitors targeting these enzymes have now been designed to inhibit cancer cell proliferation (Table 5). Inhibition of RAD18/RAD6 activity seems to represent yet another promising approach: RAD18/RAD6 activity regulates the ubiquitination of PCNA and FANCI/FANCD and is therefore required for both the DDT and FA pathways. Moreover, aberrant overexpression of RAD18 is common across a broad spectrum of cancers, including esophageal cancer [143] and gastric cancer

Table 4 Aberrant expression of E3 ligases and DUBs regulating replication fork stability in cancers

Gene	Cancer type	Observed alterations	REF.
MDM2	Gastric cancer	Gene amplification	[149]
	Prostate cancer	Overexpression	[150]
	Non-small cell lung cancer	Gene polymorphisms	[151]
	Leukemias and lymphomas	Gene amplification	[152]
RAD18	Esophageal cancer	Overexpression	[143]
	Ovarian cancer	Overexpression	[153]
	Glioma	Overexpression	[154]
	Cervical cancer	Overexpression	[155]
	Melanoma	Overexpression	[156]
	Triple-negative breast cancer	Overexpression	[157]
SPOP	Prostate cancer	Gene mutation and reduced expression	[158]
	Non-small cell lung cancer	Reduced expression	[159]
	Colorectal cancer	Reduced expression	[160]
	Endometrial cancer	Gene mutation	[161]
	Kidney cancer	Overexpression	[162]
RFWD3	Bladder cancer	Overexpression	[163]
	Gastric cancer	Overexpression	[164]
	Colorectal cancer	Overexpression	[165]
	Non-small cell lung cancer	Overexpression	[166]
	Hepatocellular carcinoma	Overexpression	[167]
TRAIP	Liver cancer	Overexpression	[168]
	Osteosarcoma	Overexpression	[169]
	Lung cancer	Overexpression	[170]
	Triple-negative breast cancer	Overexpression	[171]
$CRL4^{Cdt2}$	Hepatocellular carcinomas	Overexpression	[172]
	Breast cancer	Overexpression	[173]
	Gastric cancers	Overexpression	[174]
USP1	Breast cancer	Overexpression	[175]
	Lung cancer	Gene mutation	[176]
	Non-small cell lung cancer	Overexpression	[177]
	Cervical cancer	Overexpression	[176]
	Gastric cancer	Overexpression	[176]
	Hepatocellular carcinoma	Overexpression	[178]
	Osteosarcoma	Overexpression	[179]
	Multiple myeloma	Overexpression	[180]
	Glioblastoma	Overexpression	[181]
USP7	Prostate cancer	Overexpression	[182]
	Hepatocellular carcinoma	Overexpression	[161]
	Ovarian cancer	Overexpression	[183]
USP36	Ovarian cancer	Overexpression	[184]

Table 5 Potential inhibitors targeting replication fork stability for chemotherapy

Target	Inhibitor	Cancer types	Phase	REF.
RAD6	SMI#9, SMI#9-GNP	Triple-negative breast cancer	Preclinical studies	[145,146]
	TZ9	Ovarian cancer		[185]
$CRL4^{Cdt2}$	MLN4924	Ovarian cancer	Early-phase clinical trials	[147]
MDM2	RG7112	Neuroblastoma	Early-phase clinical trials	[186]
	AMG-232, Idasanutlin	Multiple myeloma, glioblastoma		[148]
SPOP	6b	Kidney cancer	Preclinical studies	[187]
UBE2T/FANCL	CU2	Bladder cancer, lung cancer	Preclinical studies	[188]
USP1/UAF1	SJB3-019A	Multiple myeloma	Preclinical studies	[189]
	Pimozide, GW7647	Non-small cell lung cancer		[190]
	ML323	Non-small cell lung cancer, osteosarcoma		[191]
USP7	P5091, HBX41108	Colorectal cancer	Preclinical studies	[159,192]
	GNE-6776	Triple-negative breast cancer		[193]

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(GC) [144] (Table 4). In GC, RAD18 depletion suppresses cell proliferation and invasiveness and increases cisplatin sensitivity [144]. In addition, SMI#9, a RAD6-selective inhibitor, attenuates chemotherapy agent-induced PCNA monoubiquitination and enhances the chemosensitivity of triple-negative breast cancer (TNBC) [145]. Notably, the development of a nanotherapy-mediated RAD6 inhibitor (SMI#9-GNP) has overcome the solubility limitations of SMI#9 [146]. Meanwhile, as the role of $CRL4^{Cdt2}$ in origin relicensing and rereplication becomes increasingly apparent, MLN4924, a NEDD8-activating enzyme inhibitor, has been used to inhibit $CRL4^{Cdt2}$ activity in ovarian cancer cells. The resulting persistence of the DNA replication licensing factor CDT1 at replication origins suggests that this E3 ligase may constitute a novel target in cancer therapy [147]. Other E3 inhibitors, such as MDM2 inhibitors, are currently under investigation in clinical studies (Table 5) [148].

USP1 is a well-characterized DUB that regulates the FA and TLS pathways in partnership with UAF1. It is aberrantly expressed in various types of cancer, such as non-small cell lung cancer (NSCLC), MM, osteosarcoma, and glioblastoma, where it influences cancer development and progression (Table 4) [177, 179, 181, 189]. Treatment of MM cells with USP1 inhibitor SJB3-019A leads to increased levels of Ub-FANCD2, Ub-FANCI and Ub-PCNA, and decreased RAD51 foci formation; blocking the FA pathway and inhibiting HR repair. Moreover, SJB3-019A triggers synergistic anti-MM activity when combined with other anti-MM agents, such as bortezomib, ACY1215 (an HDAC6 inhibitor), lenalidomide, or pomalidomide [189]. Additionally, the USP1/UAF1 inhibitors pimozide and GW7647 exert synergetic activity with cisplatin in cisplatin-resistant NSCLC cells (H596, NCI), but not in cisplatin-sensitive NSCLC cells (H460, NCI) [190], which may be a viable means of overcoming drug resistance. Another USP1/UAF1 inhibitor, ML323, has also been developed to enhance the cytotoxicity of cisplatin in NSCLC and osteosarcoma cells [191]. Several additional small-molecule inhibitors of E3 ligases and DUBs are also being investigated in preclinical studies [163, 166, 184].

However, intervention to the proteasome basically disrupt the turnover of the entire cellular proteome, and targeting the activities of E3 ligases or DUBs also faces specificity problems due to their multiple substrates. Development of the proteolysis-targeting chimeras (PROTACs) and deubiquitinase-targeting chimeras (DUBTACs) systems seems promising and more specific. PROTACs consists of an E3 ligand, a protein of interest, and a linker to form a ternary complex, followed by the proteasome-mediated degradation of ubiquitinated protein [194]. This technique has potential advantages in overcoming drug resistance and targeting nonenzymatic proteins compared with small molecular inhibitors. With advent of the first PROTAC bifunctional molecule for the degradation of methionine aminopeptidase 2 (MetAP-2) in 2001 [195], different PROTACs have been designed and synthesized for degradation of targeted protein, such as CDK, transcription factors, and BET protein family [196]. Moreover, oral PROTACs (ARV-110 and ARV-471) have entered clinical trial of prostate and breast cancer treatment [197,198]. Given that deubiquitination and stability of tumor suppressors or oncoproteins play important roles in carcinogenesis, DUBTACs also potentially to be a potent therapeutic intervention. For example, A DUBTAC consisting of EN523 OTUB1 recruiter and lumacaftor stabilizes ΔF508-CFTR, which improves cystic fibrosis [199]. Although still in the initial stage, the prospective PROTACs and DUBTACs molecules are attracting more attention and promising in cancer treatment. Considering the intimate relationship between replication fork stability and carcinogenesis, specific targeting of the key regulator's stability may be promising in cancer treatment. If we can further improve our understanding of the molecular machinery that stabilizes replication forks, further novel targets for treating cancer and alleviating drug resistance are likely to emerge.

Conclusions

DNA replication forks are extremely vulnerable to a range of endogenous and exogenous stressors. To cope with such frequent insults, which force replication forks to halt, organisms have evolved sophisticated and highly conserved mechanisms to sustain replication fork stability while the threats are resolved. Post-translational modifications, including ubiquitination, play critical roles in organizing the replication stress response machinery. Global responses triggered by ubiquitinated RPA-mediated ATR activation and ubiquitinated PCNA-mediated fork reversal/lesion bypass constitute a well-coordinated signaling network that maintains replication fork stability [12,24]. PCNA undergoes polyubiquitination following monoubiquitination by RAD18/RAD6, and PCNA mono- and polyubiquitination are essential for the coordination of fork reversal, TLS, and TS at stalled replication forks [27,84–87]. RAD18/RAD6 activity is also required for the efficient monoubiquitination of the FA core components FANCD2 and FANCI, indicating their potential involvement in resolving ICLs [133,134]. Intricate cross-talk between different signaling pathways maintains the balance of this system, and ensures that the appropriate responses are mounted to counteract replication stress under various circumstances.

Ubiquitination is a common modification that is involved in almost all cellular activities. Harnessing the ubiquitin system as a means to treat cancer is currently the focus of intensive study, and three proteasome inhibitors (bortezomib, carfilzomib, and ixazomib) have been approved by the FDA for the treatment of MM and MLC [142]. More inhibitors are currently undergoing early-phase clinical trials and preclinical studies. However, a lack of target specificity could lead to indiscriminate toxicity in both malignant and normal tissues. More specific PROTACs and DUBTACs techniques are promising in the chemotherapeutic development. Oncogenic activation itself is also known to result in the dysregulation of DNA replication, making replication stress a hallmark of cancer. The administration of chemotherapeutic drugs, such as PARP inhibitors and platinum drugs, also induces severe replication stress, providing an additional target for drug synergism. For example, kinase inhibitors targeting ATR-CHK1-WEE1 have shown promising results in combination with other chemotherapy drugs, and can overcome PARP inhibitor resistance [200].

The involvement of the ubiquitination system in the replication stress response also offers potential targets for chemotherapeutic drugs. In particular, the multiple roles of RFWD3 and RAD18 in stabilizing stalled replication forks suggests that these proteins represent candidate targets for chemotherapeutic drug development. Indeed, both RFWD3 and RAD18 are aberrantly overexpressed in a broad spectrum of cancers. Further investigation into the mechanisms underlying replication fork stabilization will facilitate a more comprehensive understanding of susceptibility and resistance when developing novel chemotherapeutic strategies.

Competing Interests

The authors declare that there are no competing interests associated with this manuscript.

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Donghui Xia: Writing—original draft, Writing—review & editing. **Xuefei Zhu:** Writing—original draft, Writing—review & editing. **Ying Wang:** Writing—review & editing. **Peng Gong:** Writing—review & editing. **Hong-Shu Su:** Writing—review & editing. **Xingzhi Xu:** Writing—review & editing.

Abbreviations

ATM, ataxia telangiectasia mutated; ATR, ataxia-telangiectasia and RAD3-related kinase; CDC48, cell division control protein 48; CDT1, CDC10-dependent transcript 1; CHK1, checkpoint kinase 1; CMG, Cdc45-MCM-GINS; CtIP, C-terminal binding protein interacting protein; DDB1, DNA damage-binding protein 1; DPC, DNA–protein cross-link; DUBTACs , deubiquitinase-targeting chimeras; ETAA1, Ewing's tumor-associated antigen 1; EXO1, exonuclease 1; FA, Fanconi anemia; FBH1, F-box DNA helicase 1; GC, gastric cancer; HLTF, helicase-like transcription factor; HR, homologous recombination; ICL, DNA interstrand cross-link; MCL, mantle cell lymphoma; MCM2-7, minichromosome maintenance 2-7; MM, multiple myeloma; MRE11, meiotic recombination 11 homolog 1; NSCLC, non-small cell lung cancer; ORC, origin recognition complex; PCNA, proliferating cell nuclear antigen; PRIMPOL, primase/polymerase; PROTACs , proteolysis-targeting chimeras; RPA, replication protein A; SCF, SKP1-cullin1-F-box protein; SHPRH, SNF2 histone-linker PHD-finger RING-finger helicase; SMARCAL1, SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily a like 1; TIPIN, TIMELESS-interacting protein; TLS, translesion synthesis; TNBC, triple-negative breast cancer; TopBP1, DNA topoisomerase 2-binding protein 1; TS, template-switching; UPS, ubiquitin–proteasome system; USP7, ubiquitin carboxyl-terminal hydrolase 7; WRNIP1, werner helicase interacting protein 1; ZRANB3, zinc finger RANBP2-type containing 3.

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