PCNA-mediated stabilization of E3 ligase RFWD3 at the replication fork is essential for DNA replication

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Edited by Bruce Stillman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, and approved November 5, 2018 (received for review August 23, 2018)

RING finger and WD repeat domain-containing protein 3 (RFWD3) is an E3 ligase known to facilitate homologous recombination by removing replication protein A (RPA) and RAD51 from DNA damage sites. Further, RPA-mediated recruitment of RFWD3 to stalled replication forks is essential for interstrand cross-link repair. Here, we report that in unperturbed human cells, RFWD3 localizes at replication forks and associates with proliferating cell nuclear antigen (PCNA) via its PCNA-interacting protein (PIP) motif. PCNA association is critical for the stability of RFWD3 and for DNA replication. Cells lacking RFWD3 show slower fork progression, a prolonged S phase, and an increase in the loading of several replication-fork components on the chromatin. These findings all point to increased frequency of stalled forks in the absence of RFWD3. The S-phase defect is rescued by WT RFWD3, but not by the PIP mutant, suggesting that the interaction of RFWD3 with PCNA is critical for DNA replication. Finally, we observe reduced ubiquitination of RPA in cells lacking RFWD3. We conclude that the stabilization of RFWD3 by PCNA at the replication fork enables the polyubiquitination of RPA and its subsequent degradation for proper DNA replication.

DNA replication | PCNA | RFWD3 | RPA | ubiquitination

Maintenance of genome integrity is key to cell survival. Defects in DNA replication and errors in the DNA damage response contribute to genome instability and are key contributing factors in many diseases, including cancer (1). Replication stress is a leading cause of genome instability and occurs when replication forks progress slowly or stall. A huge repertoire of cellular factors can mediate replication stress-induced DNA damage, including deprivation of dNTP pools, defects in DNA replication proteins, and decreased firing of origins due to defects in replication initiation (2). Intricate checkpoint pathways operate to ensure that the entry into or progression through S phase is blocked when the cells encounter DNA damage.

Cells encounter many assaults to their genome that are repaired accurately and efficiently to maintain genome integrity. Ubiquitination is emerging as an important player in DNA replication, repair, and damage-signaling pathways. Nondegradative ubiquitin signaling involving either monoubiquitination or polyubiquitination has been implicated in the maintenance of genome integrity, including in processing of DNA double-strand breaks, repair of interstrand cross-link lesions (ICLs), and bypass of lesion during DNA replication (for review, see ref. 3). The signaling events are triggered by ubiquitin protein ligases that can initiate mono- or polyubiquitination through nonstandard linkage.

DNA ICLs are links between the two strands of DNA with a covalent bond, and many pathways including nucleotide excision repair, structure-specific endonucleases, translesion DNA synthesis (TLS), and homologous recombination (HR) have been implicated in resolving such errors (4). ICLs inhibit DNA replication and transcription, and the dominant mode of ICL repair is believed to happen during S phase and requires converging replication forks (5, 6). Mutations in ICL repair are associated with Fanconi anemia (FA), a rare heritable disorder, and ~21 FA genes reported thus far have been implicated in ICL repair (7, 8). The role of novel factors in ICL repair and the importance of

ubiquitination in the damage response are becoming intense areas of research.

An E3 ubiquitin ligase, RING finger and WD repeat domain-containing protein 3 (RFWD3), was initially identified in a proteomic study as a substrate of ATM/ATR (9). Biallelic mutations in RFWD3 were reported in patients with FA (10). RFWD3 is emerging as an important component in the FA/BRCA pathway and has been assigned the alias FANCW. Previous studies have shown that RFWD3 functions synergistically with Mdm2 to regulate the ubiquitination of tumor suppressor protein p53 in response to DNA damage (11). RFWD3 associates with replication protein A (RPA), the single-stranded binding protein; facilitates the RPA-mediated DNA damage response; and affects HR at stalled replication forks (12–14). Recent studies have shown that RPA-mediated recruitment of RFWD3 is essential for ICL repair (15) and that RFWD3-mediated ubiquitination promotes the removal of RPA and RAD51 from damage sites to allow HR (16). All these findings support the role of RFWD3 in DNA-damage repair.

While RFWD3 is emerging as a new component of the FA pathway, up until now, this role had been thought to be mediated primarily by its interactions with the single-stranded DNA-binding protein RPA that are important for repair of ICL DNA damage. In the present manuscript, we report that the role of RFWD3 in the FA pathway may be related to its role in normal DNA replication via its direct interaction with proliferating cell nuclear antigen (PCNA) at the replication fork. We propose that the association of RFWD3 at the replication fork mediates the ubiquitination of key replication fork components that is essential for efficient progression of DNA replication. This seems to be a fundamentally different way of considering how RFWD3 functions in

Significance

Ubiquitination of several DNA replication–repair proteins is a critical mechanism by which cellular DNA replication and DNA damage repair pathways are controlled. The E3 ligase RING finger and WD repeat domain-containing protein 3 (RFWD3) has emerged as an important regulator of genome stability. We propose that the association of RFWD3 to proliferating cell nuclear antigen stabilizes RFWD3 at the fork, enabling ubiquitination of replication protein A and its subsequent removal to facilitate DNA replication.

Author contributions: Y.-C.L., Y.W., S.G., S.W., K.V.P., and S.G.P. designed research; Y.-C.L., Y.W., R.H., S.G., S.W., M.K.A., and S.G.P. performed research; A.C. contributed new reagents/analytic tools; Y.-C.L., Y.W., S.G., K.V.P., and S.G.P. analyzed data; and Y.-C.L., Y.W., and S.G.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1814521115/-DCSupplemental.

Published online December 10, 2018.

DNA metabolism, as it raises the possibility that a major role of RFWD3 in genome stability occurs via its role in normal, unstressed DNA replication at the replication fork as opposed to solely functioning in DNA repair processes.

Results

RFWD3 Localizes to the Replication Fork and Interacts with PCNA.

RFWD3 is an E3 ligase that is known to play important roles in DNA damage response. A recent nascent chromatin capture proteomics study showed the enrichment of RFWD3 at the replication fork (17), supporting the notion that RFWD3 might play important roles in unperturbed DNA replication. To test this model, we examined the presence of RFWD3 at the fork and monitored the spatiotemporal dynamics of RFWD3 in a quantitative manner by performing the isolation of proteins on nascent DNA (iPOND) assay (18). RFWD3 showed enrichment at the unperturbed replication fork, but not after thymidine chase (matured DNA) (Fig. 1*A*). PCNA, the DNA clamp, was also found to be enriched on the nascent DNA, but not on the mature DNA, confirming the specificity of the iPOND assay (Fig. 1*A*). Histone H4 was found on nascent as well as mature DNA. Further, immunoprecipitation (IP) experiments demonstrated that RFWD3 associated with the fork protein PCNA in unperturbed human cells as well as in DNA-damaged cells (Fig. 1*B* and *SI Appendix, Fig. S1A*). In addition, by using purified proteins, we demonstrated direct interaction between RFWD3 and PCNA (Fig. 1*C*). Lastly, examination of the localization of RFWD3 in asynchronously grown human cells revealed colocalization of RFWD3 with the replication protein PCNA in a subset of cells (Fig. 1*D* and *SI Appendix, Fig. S1B*). These data all strongly support the existence of RFWD3 at the replication fork.

Fig. 1. RFWD3 is at the replication fork and associates with PCNA. (*A*) Cells labeled with 5-ethynyl-2'-deoxyuridine (EdU), processed by iPOND [0 and 60 min thymidine (Thy) chase], and immunoblotted using RFWD3, PCNA, and H4 antibodies. (*B*) IP using HA antibody from U2OS cells stably expressing HA-RFWD3. RFWD3 and PCNA were analyzed by immunoblotting. (*C*) Direct interaction of RFWD3 and PCNA using purified proteins. (*D*) Localization of YFP-RFWD3 with PCNA. (Scale bar: 15 μ m.) (*E*) Schematic representation of RFWD3 protein with different domains. Note the PIP motif at amino acids 620 to 624 within the WD40 domain. (*F*) IP in U2OS cells expressing various HA-RFWD3-WT, HA-RFWD3-PIPm, and HA-RFWD3-I639K mutants using HA antibody and analysis by RFWD3 and PCNA immunoblotting. (*G*) IP in U2OS cells expressing various HA-RFWD3-WT, HA-RFWD3-PIPm, and HA-RFWD3-I639K mutants using HA antibody and analysis by RFWD3 and RPA2 immunoblotting.

PCNA is known to be the master regulator of events at the replication fork (19). PCNA provides a molecular platform that enables protein-protein and protein-DNA interactions at the fork. A large repertoire of proteins associate with PCNA via a general motif, the PCNA-interacting protein (PIP) box sequence [Q-XX-(L/I/M)-XX-(HF/DF/Y)] (20). A close inspection of the sequence of RFWD3 revealed a PIP box consensus toward the C terminus of the protein (Fig. 1*E*). IP experiments using the HA antibody in cell lines stably expressing HA-RFWD3-WT, HA-RFWD3-PIPm, or HA-RFWD3-I639K (the latter a mutant known to abolish RFWD3 interaction with RPA) showed that WT as well as the I639K mutant (albeit weaker than WT) interacted with PCNA (Fig. 1*F*). On the other hand, the PIP box mutant failed to interact with PCNA (Fig. 1*F*). RFWD3 has previously been reported to associate with the single-stranded binding protein RPA. We found that the RPA interaction to RFWD3 was abrogated in the I639K mutant, as expected (Fig. 1*G*). RPA interacted with WT RFWD3 and with the PIP mutant (but to a lesser extent compared with the WT) (Fig. 1*G*). To further investigate the complex assembly of RFWD3, PCNA, and RPA, purified proteins or nuclear extract were fractionated on a Superdex 200 gel filtration column. A significant portion of RFWD3 was found in fraction 7 (~669 kDa), cofractionating with RPA and PCNA, indicating that RFWD3 is in a high-molecular-weight complex containing RPA and PCNA (*SI Appendix, Fig. S1C and D*), in addition to subcomplexes of RFWD3-PCNA and RFWD3-RPA. Further, GST pull-down assays demonstrated that RFWD3, PCNA, and RPA could exist in one single complex, as RPA can bind to RFWD3 without displacing PCNA (*SI Appendix, Fig. S1E*). Our results suggest that RFWD3 associates to the replication fork at S phase and interacts with the fork components PCNA and RPA.

RFWD3 Is Required for S-Phase Progression. Because RFWD3 localized to the replication fork in unperturbed cells, we addressed the role of RFWD3 during the normal cell cycle. Despite numerous efforts, our attempts to knock out RFWD3 in human U2OS cells utilizing the CRISPR/Cas9-mediated genome-editing method were unsuccessful, as we failed to get clones with complete loss of the RFWD3 protein. This is consistent with results from other groups, suggesting that RFWD3 is an essential gene (15, 16). We used two independent siRNAs (RFWD3 si-1 and RFWD3 si-2) to deplete RFWD3 in multiple human cell lines, including U2OS (osteosarcoma), HCT116 (colorectal cancer), WT, p53^{-/-}, and p21^{-/-} cells. Depletion of RFWD3 in most cancer cells, including U2OS and HCT116, showed an increase in the 4C DNA content and accumulation of cells in S phase (Fig. 2*A and B* and *SI Appendix, Fig. S2A and B*).

To address the requirement of RFWD3 in DNA replication, we depleted RFWD3 in serum-starved WI-38 (human diploid fibroblast) cells. The control and RFWD3-depleted cells were released from serum starvation and evaluated by flow cytometry at 0 and 24 h after serum release. While the control cells cycled back efficiently (as observed by increased G2/M population at 24 h release), cells treated with either of the RFWD3 siRNAs showed defects in progressing into S phase (*SI Appendix, Fig. S2C*).

Because we observed RFWD3 localized to the fork, we examined fork progression in RFWD3-depleted cells using a DNA fiber assay. We observed a consistent reduction in replication-fork speed in cells depleted of RFWD3 using either of the siRNAs (Fig. 2*C*). We hypothesize that the reduced fork speed leads to prolonged S phase; hence, more cells are accumulated in S phase. To address the length of S phase, we synchronized control and RFWD3-depleted cells at the G1/S boundary using double thymidine (dT) block and release. At different time points after release from dT block (7, 8, and 9 h), cells were harvested and immunostained for PCNA to evaluate the number of cells in S phase. By 9 h after release, <30% of the control cells showed PCNA

Fig. 2. RFWD3 is required for S-phase progression. (A) Cell cycle profile (PI flow cytometry) of U2OS cells depleted of RFWD3 (RFWD3 si-1 and RFWD3 si-2). (B) Quantification of S-phase population from three independent experiments. (C, Upper) Representative fluorescence image showing fibers from control (control si) and RFWD3-depleted (RFWD3 si-1 and si-2) U2OS cells that were labeled with 5-chloro-2'-deoxyuridine (CldU) and 5-iodo-2'-deoxyuridine (IdU). (C, Lower) Fork speed was calculated and plotted as a box plot. The top and bottom of each box represent the 75th and 25th percentiles, respectively, and the bar in each box represents the median. Dots represent outliers. (D) Schematic of the experimental protocol for RFWD3 depletion in U2OS cells that are synchronized using dT block and release. Immunolocalization of PCNA was performed at 7, 8, and 9 h after release. Percent PCNA-positive cells is calculated. Note that RFWD3-depleted cells continue to show >50% PCNA-positive cells at 9 h. Error bars represent SD. (E) Length of S phase quantitated by live-cell imaging of YFP-PCNA in control and RFWD3-siRNA-treated cells. (F) Cell cycle profile by flow cytometry of various U2OS cell lines (HA-RFWD3-WT, HA-RFWD3-PIpm, HA-RFWD3-C315A, and HA-RFWD3-I639K mutants) depleted of endogenous RFWD3. Note that only WT RFWD3 can rescue the cell cycle defect. (G) DNA fiber assay in HA-RFWD3-WT and HA-RFWD3-PIpm cell lines that are depleted of endogenous RFWD3. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ by unpaired two-tailed Student's t test.

staining, indicating that most of them had already exited S phase. On the other hand, >50% of RFWD3-depleted cells displayed PCNA-positive signals (compare between 7 and 9 h of release) (Fig. 2D). Further, live-cell imaging of YFP-PCNA in control as well as in RFWD3-depleted cells demonstrated that cells lacking RFWD3 displayed an average S-phase length of 16 h, while that of the control was <14 h (Fig. 2E, SI Appendix, Fig. S2D, and Movie S1). These results reveal that the depletion of RFWD3 caused a prolonged S phase.

We next addressed whether the association of RFWD3 with PCNA and/or RPA was required for proper DNA replication. We performed RFWD3 depletion in HA-RFWD3-WT, HA-RFWD3-PIpm, HA-RFWD3-C315A (catalytically inactive), and HA-RFWD3-I639K mutants and tested for cell cycle distribution using propidium iodide (PI) and BrdU-PI flow analyses. Depletion of RFWD3 showed increased population in S phase (Fig. 2F and SI Appendix, Fig. S2E) as well as reduced incorporation of BrdU, with a population of cells accumulating between 2C and 4C DNA content (SI Appendix, Fig. S2F). The siRNA-resistant version

of WT RFWD3 rescued the S-phase accumulation defects in endogenous RFWD3-depleted cells; however, the other mutants did not (Fig. 2F and SI Appendix, Fig. S2E and F). Similarly, WT RFWD3 was able to rescue the defects in DNA fiber length, but the PIP mutant failed to do so (Fig. 2G). These results suggest that the association of RFWD3 and PCNA is important for efficient DNA replication.

Loss of RFWD3 Causes Fork Stalling and Sister Chromatid Cohesion Defects. To gain molecular insight into the cause of DNA replication defects in cells lacking RFWD3, we evaluated the status of the replisome components at the fork. We performed chromatin fractionation to determine the loading of the replisome components in control and RFWD3-depleted cells that had been synchronized in S phase. Immunoblotting using PCNA antibodies demonstrated that RFWD3 knockdown resulted in increased PCNA monoubiquitination, suggesting stalling of the DNA replication fork (Fig. 3A). This modification is known to mediate the switch from replicative DNA polymerases to TLS polymerases (21). We observed an increase in the total as well as chromatin-associated

repair. Interestingly, PCNA is also known to be monoubiquitinated by both the RAD18 E3 ligase in response to ICLs and by CRL4(Cdt2) to promote TLS associated with endogenous replication stress (26, 27). We observed that in the absence of RFWD3, many components of the fork showed increased binding to the chromatin, including RPA, DNA polymerases, Cdc45, and TLS polymerases, all of which point to stalling of the replication fork. Increase in monoubiquitination of PCNA is known to occur instantaneously after fork stalling (28), and we observe robust monoubiquitination of PCNA upon depletion of RFWD3. The uncoupling of the helicase from the stalled polymerase is known to cause an increased accumulation of ssDNA (as evidenced by increased RPA), resulting in ubiquitination of PCNA (29, 30). A recent study has reported the presence of p53 bound to PCNA at stalled forks for suppressing the extension from these forks (31). It is interesting to note that RFWD3 is known to positively regulate p53 stability (11). It is a possibility that in the absence of RFWD3, persistence of stalled forks is because of defects in ubiquitination of substrates like p53 and RPA. Identifying the entire repertoire of RFWD3 substrates during unperturbed DNA replication and during DNA damage would tremendously improve our understanding.

Depletion of RFWD3 has been shown to induce Chk1 and Chk2 phosphorylation in the absence of DNA damage, corroborating our results that RFWD3 has a fundamental role in promoting the stability of unperturbed replication forks (12). We find that PCNA is required for stabilizing RFWD3 to the chromatin. PCNA is known not only to influence the association of a large number of factors to chromatin, but also to affect their activity in many instances (26). The PIP domain within RFWD3 is close to

the I639 moiety, a site that was found to be mutated in an FA patient and has previously been shown to be crucial for RPA binding. Furthermore, it has previously been reported that RPA-mediated recruitment of RFWD3 to stalled forks is critical for ICL repair (15). We find that RFWD3 can be stabilized on chromatin in the absence of RPA, and our results support a model in which RFWD3 associates with PCNA and localizes to the replication fork. The fork-associated RFWD3 is also important for the ubiquitination of replication-fork components, including RPA, to ensure proper fork progression. One model would posit that the ubiquitination of RPA enables the removal of RPA, thus allowing fork progression. Cells lacking RFWD3 show increased accumulation of RPA, increased PCNA monoubiquitination, and, therefore, fork stalling. We propose that RFWD3 plays a key role in resolving the DNA breaks and stalled forks that are natural occurrences during regular DNA replication.

Materials and Methods

A detailed description of all the plasmids, antibodies, and experimental procedures can be found in [SI Appendix, Material and Methods](#). Experimental procedures include the iPOND assay, DNA fiber analysis, co-IP, and the in vivo ubiquitination assay.

ACKNOWLEDGMENTS. We thank members of the S.G.P. and K.V.P. laboratories for discussions and suggestions. We thank Drs. J. Chen, A. Gambus, Z. Gong, A. Maréchal, K. Sato, D. Spector, B. Stillman, M. Takata, Y. Wang, and L. Zou for providing reagents, protocols, and suggestions. We thank Dr. D. Rivier and Ms. S. Adusumilli for critical reading of the paper. This work was supported by NSF Award 1723008 (to K.V.P.) and by NSF Faculty Early Career Development Program Award 1243372, the NSF Award 1818286, and NIH Grants 1R01GM099669 and GM125196 (to S.G.P.).

- Técher H, Koundrioukoff S, Nicolas A, Debatisse M (2017) The impact of replication stress on replication dynamics and DNA damage in vertebrate cells. *Nat Rev Genet* 18: 535–550.
- Toledo L, Neelsen KJ, Lukas J (2017) Replication catastrophe: When a checkpoint fails because of exhaustion. *Mol Cell* 66:735–749.
- Ulrich HD, Walden H (2010) Ubiquitin signalling in DNA replication and repair. *Nat Rev Mol Cell Biol* 11:479–489.
- Hashimoto S, Anai H, Hanada K (2016) Mechanisms of interstrand DNA crosslink repair and human disorders. *Genes Environ* 38:9.
- Akkari YM, Bateman RL, Reifsteck CA, Olson SB, Grompe M (2000) DNA replication is required to elicit cellular responses to psoralen-induced DNA interstrand cross-links. *Mol Cell Biol* 20:8283–8289.
- Taniguchi T, et al. (2002) S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. *Blood* 100:2414–2420.
- Ceccaldi R, Sarangi P, D'Andrea AD (2016) The Fanconi anaemia pathway: New players and new functions. *Nat Rev Mol Cell Biol* 17:337–349.
- Federico MB, Campodonico P, Paviolo NS, Gottifredi V (2017) Beyond interstrand crosslinks repair: Contribution of FANCD2 and other Fanconi anemia proteins to the replication of DNA. *Mutat Res* 808:83–92.
- Matsuoka S, et al. (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316:1160–1166.
- Knies K, et al. (2017) Biallelic mutations in the ubiquitin ligase RFWD3 cause Fanconi anemia. *J Clin Invest* 127:3013–3027.
- Fu X, et al. (2010) RFWD3-Mdm2 ubiquitin ligase complex positively regulates p53 stability in response to DNA damage. *Proc Natl Acad Sci USA* 107:4579–4584.
- Elia AE, et al. (2015) RFWD3-dependent ubiquitination of RPA regulates repair at stalled replication forks. *Mol Cell* 60:280–293.
- Gong Z, Chen J (2011) E3 ligase RFWD3 participates in replication checkpoint control. *J Biol Chem* 286:22308–22313.
- Liu S, et al. (2011) RING finger and WD repeat domain 3 (RFWD3) associates with replication protein A (RPA) and facilitates RPA-mediated DNA damage response. *J Biol Chem* 286:22314–22322.
- Feeney L, et al. (2011) RPA-mediated recruitment of the E3 ligase RFWD3 is vital for interstrand crosslink repair and human health. *Mol Cell* 66:610–621.e4.
- Inano S, et al. (2017) RFWD3-mediated ubiquitination promotes timely removal of both RPA and RAD51 from DNA damage sites to facilitate homologous recombination. *Mol Cell* 66:622–634.e8.
- Alabert C, et al. (2014) Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. *Nat Cell Biol* 16:281–293.
- Sirbu BM, Couch FB, Cortez D (2012) Monitoring the spatiotemporal dynamics of proteins at replication forks and in assembled chromatin using isolation of proteins on nascent DNA. *Nat Protoc* 7:594–605.
- Mailand N, Gibbs-Seymour I, Bekker-Jensen S (2013) Regulation of PCNA-protein interactions for genome stability. *Nat Rev Mol Cell Biol* 14:269–282.
- Varbrick E (1998) PCNA binding through a conserved motif. *BioEssays* 20:195–199.
- Brown S, Niimi A, Lehmann AR (2009) Ubiquitination and deubiquitination of PCNA in response to stalling of the replication fork. *Cell Cycle* 8:689–692.
- Tanaka H, et al. (2009) Replisome progression complex links DNA replication to sister chromatid cohesion in *Xenopus* egg extracts. *Genes Cells* 14:949–963.
- Prasanth SG, Prasanth KV, Siddiqui K, Spector DL, Stillman B (2004) Human Orc2 localizes to centrosomes, centromeres and heterochromatin during chromosome inheritance. *EMBO J* 23:2651–2663.
- Naim V, Rosselli F (2009) The FANCD pathway and mitosis: A replication legacy. *Cell Cycle* 8:2907–2911.
- Prelich G, et al. (1987) Functional identity of proliferating cell nuclear antigen and a DNA polymerase-delta auxiliary protein. *Nature* 326:517–520.
- Choe KN, Moldovan GL (2017) Forging ahead through darkness: PCNA, still the principal conductor at the replication fork. *Mol Cell* 65:380–392.
- Terai K, Abbas T, Jazaeri AA, Dutta A (2010) CRL4(Cdt2) E3 ubiquitin ligase monoubiquitinates PCNA to promote translesion DNA synthesis. *Mol Cell* 37:143–149.
- Hedglin M, Benkovic SJ (2015) Regulation of Rad6/Rad18 activity during DNA damage tolerance. *Annu Rev Biophys* 44:207–228.
- Chang DJ, Lupardus PJ, Cimprich KA (2006) Monoubiquitination of proliferating cell nuclear antigen induced by stalled replication requires uncoupling of DNA polymerase and mini-chromosome maintenance helicase activities. *J Biol Chem* 281: 32081–32088.
- Zhuang Z, et al. (2008) Regulation of polymerase exchange between Pol ϵ and Pol δ by monoubiquitination of PCNA and the movement of DNA polymerase holoenzyme. *Proc Natl Acad Sci USA* 105:5361–5366.
- Hamp S, et al. (2016) DNA damage tolerance pathway involving DNA polymerase ϵ and the tumor suppressor p53 regulates DNA replication fork progression. *Proc Natl Acad Sci USA* 113:E4311–E4319.