Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Biochem Soc Trans. Author manuscript; available in PMC 2019 December 17.

Published in final edited form as:

Biochem Soc Trans. 2018 December 17; 46(6): 1643–1651. doi:10.1042/BST20180308.

A tough row to hoe: when replication forks encounter DNA damage

Darshil R. Patel and **Robert S. Weiss**

Department of Biomedical Sciences, Cornell University, Ithaca, NY 14853

Abstract

Eukaryotic cells continuously experience DNA damage that can perturb key molecular processes like DNA replication. DNA replication forks that encounter DNA lesions typically slow and may stall, which can lead to highly detrimental fork collapse if appropriate protective measures are not executed. Stabilization and protection of stalled replication forks ensures the possibility of effective fork restart and prevents genomic instability. Recent efforts from multiple laboratories have highlighted several proteins involved in replication fork remodeling and DNA damage response pathways as key regulators of fork stability. Homologous recombination factors such as RAD51, BRCA1, and BRCA2, along with components of the Fanconi Anemia pathway, are now known to be crucial for stabilizing stalled replication forks and preventing nascent strand degradation. Several checkpoint proteins have additionally been implicated in fork protection. Ongoing work in this area continues to shed light on a sophisticated molecular pathway that balances the action of DNA resection and fork protection to maintain genomic integrity, with important implications for the fate of both normal and malignant cells following replication stress.

Introduction

Faithful transmission of genetic information depends on accurate duplication of the genome by the DNA replication machinery. However, DNA lesions that arise spontaneously or due to endogenous or exogenous DNA damaging agents pose a challenge to replication fork (RF) progression (1). DNA replication is conducted by a multi-protein molecular machine comprised of DNA polymerases, a DNA helicase complex, and a multitude of accessory proteins that ensure the accurate duplication of all genomic sequences precisely once each cell cycle. Replication stress perturbs DNA replication, often resulting in RF slowing or stalling (2). Blockage of a replicative DNA polymerase by a lesion on the leading strand template often results in helicase uncoupling, causing single stranded DNA (ssDNA) accumulation that triggers a DNA damage response (DDR) (3). Subsequently, either the processive polymerase is temporarily replaced with a translesion synthesis (TLS) polymerase to bypass the lesion, or replication is resumed downstream of the lesion by repriming and/or recombinational mechanisms (4). A major determinant of pathway choice

*Correspondent footnote: Robert S. Weiss, Department of Biomedical Sciences, Cornell University, T2-006C Veterinary Research Tower, Ithaca, New York 14853, Telephone: (607) 253-4443, Fax: (607) 253-4212, rsw26@cornell.edu.

Declaration of Interest

The authors declare that they have no conflicts of interest with the contents of this article.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in such situations involves the ubiquitination of PCNA on K164, with potentially error-prone TLS requiring PCNA mono-ubiquitination, and PCNA poly-ubiquitination instead promoting error-free template switching (5). Certain barriers represent an even greater challenge to DNA replication, such as in the case of interstrand DNA crosslinks (ICLs), which involve covalent linkage of the two DNA strands. Some mechanistic models suggest that RF stalling at ICLs is followed by lesion unhooking and subsequent DNA repair prior to the resumption of DNA synthesis, whereas others invoke fork traverse of the ICL followed by post-replicative repair (6–8). For the RF to successfully negotiate impediments and for replication to proceed, cells rely on accessory proteins to stabilize and remodel the blocked RF (1). Resolving stalled RFs requires a complex signaling pathway to coordinate lesion repair and fork processing in order to avoid DNA double-strand breaks (DSBs) and other undesirable outcomes associated with RF destabilization and collapse (Figure 1).

Accumulation of ssDNA due to RF stalling activates ATR-mediated checkpoint signaling, which also requires the PCNA-like RAD9A-RAD1-HUS1 (9–1–1) complex and the adaptor and ATR activator TOPBP1 (9). Upon activation, ATR phosphorylates a plethora of substrates, including the transducer kinase CHK1, that trigger protective responses such as cell cycle arrest and DNA repair (10). With respect to DNA replication, ATR activation halts new origin firing and promotes RF stability by regulating several key downstream proteins in fork reversal, protection, and restart (11). In response to RF stalling, phosphorylation by ATR of the Bloom Syndrome (BLM) and Werner Syndrome (WRN) helicases is required for accurate fork restart (12). Checkpoint kinases also regulate the activity of several nucleases that participate in fork restart (13). Apart from the checkpoint machinery, several additional proteins, including homologous recombination (HR) proteins, nucleases, translocases, and many others, play significant roles in overseeing the resolution of stalled RFs.

Roles for homologous recombination proteins in protecting stalled replication forks against nascent strand nucleolytic degradation

HR is a primary cellular mechanism for error-free DSB repair. Remarkably, growing evidence also highlights key roles for several HR pathway members, such as RAD51 and BRCA1/2, in protecting stalled RFs and orchestrating a delicate balance between necessary resection events and excessive nascent strand degradation (14–18). Upon binding ssDNA at a conventional resected DSB, RAD51 promotes homology search and strand exchange, essential steps in HR-mediated DSB repair (19). At stalled RFs, BRCA2 and accessory proteins promote the stabilization of the RAD51 nucleoprotein filament along ssDNA, protecting newly synthesized DNA strands from nucleolytic degradation (20, 21).

Nucleases such as MRE11, EXO1, DNA2 (DNA replication helicase/nuclease 2), and CtIP have traditionally been studied in the context of DSB repair, where they contribute to DSB end resection as a critical early step in HR. However, key roles for these nucleases in stalled RF remodeling have also been identified in recent years (16). For example, MRE11, in coordination with CtIP, can initiate DNA resection and generate ssDNA gaps at stalled forks, enabling RAD51 nucleofilament formation that facilitates fork reversal and template switching (15). On the other hand, excessive nuclease activity can result in the degradation

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of stalled RFs and fork collapse (15, 22–24). Hence, maintaining tight control over nucleases that are attracted to stalled RFs is crucial for successful, mutation-free outcomes.

One key element for the regulation of nuclease activity at RFs involves HR proteins, such as RAD51, which protect newly synthesized DNA from MRE11-mediated degradation (25). Based in part on analysis of DNA replication using single molecule DNA fiber assays, Schlacher, Jasin and colleagues proposed that *BRCA2* deficiency leads to destabilization and degradation of the stalled forks by MRE11, resulting in genomic instability (20, 21). Subsequently, crosstalk between HR proteins and members of the Fanconi Anemia (FA) pathway was implicated in modulating stalled RFs. FA is an inherited genomic instability syndrome caused by mutation in any of several proteins that function to coordinate multiple repair processes, including HR, and checkpoint signaling, particularly in the context of ICL repair (26). Included among the FA proteins are BRCA1 (FANCS), BRCA2 (FANCD1), RAD51 (FANCR), and RAD51C (FANCO). Other FA pathway components, most notably FANCD2, have also been implicated in fork protection (27).

Recent work established that FA/BRCA proteins stabilize RAD51 at stalled RFs to protect nascent strands from MRE11-dependent fork degradation (21, 28). Meanwhile, several other factors have been tabbed as facilitators of nascent strand degradation by MRE11. For instance, it was recently shown that RAD52 promotes stalled RF degradation in *BRCA2*-deficient cells by priming MRE11-dependent resection (18). The histone methyltransferases MLL3/4 as well as PTIP, CHD4, and PARP1, additionally modulate MRE11 recruitment at stalled RFs (17, 29, 30). Interestingly, MLL3/4 or PTIP loss restored RF stability and chemoresistance without correcting HR defects in *BRCA1/2* deficient cells. These data reveal a novel mechanism in which protecting stalled RFs can promote viability and drug resistance in *BRCA1/2*-deficient cells irrespective of their HR capacity. In parallel, D'Andrea and colleagues delineated another pathway involving EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) and MUS81 that impacts fork stability as well as PARP inhibitor (PARPi) sensitivity in *BRCA2*-deficient cancers (23). EZH2, a histone lysine methyltransferase, methylates H3K27 at stalled forks, promoting MUS81 endonuclease recruitment and subsequent MUS81-mediated fork degradation. Loss of EZH2 promotes fork stability and confers PARPi resistance in *BRCA*2-deficient tumors. Similar results have been observed for MUS81 inactivation in *BRCA2* mutant cells, although there are conflicting reports with respect to its effects on PARPi sensitivity (11, 19). Interestingly, the EZH2-MUS81 pathway does not regulate fork protection in *BRCA1*-deficient tumors, suggesting distinct functions for BRCA1 and BRCA2 in fork protection (23). These and other studies raise an important point regarding the multiple, separable roles for BRCA1/2 and RAD51 in HR, fork protection, and stress responses. For instance, Nussenzweig and colleagues observed that although 53BP1 loss rescues the HR defects and PARPi sensitivity of *BRCA1* mutant cells, it does not restore normal sensitivity to ICL-inducing agents, suggesting a requirement for BRCA1 in ICL repair that is independent of HR (31). Recent studies describing separation of function mutants of RAD51 and BRCA2 provide further evidence that their fork protection activities can be distinguished mechanistically from HR, as discussed below (24, 32).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Accurate fork reversal is essential for stabilization of stalled replication forks

Restart and resolution of stalled RFs often requires RF remodeling and formation of a "chicken foot" structure known as a reversed fork (RVF) (33). RVFs reflect the remodeling of a typical three-way junction at a RF into a four-way junction, created by annealing the two newly synthesized DNA strands to generate an additional regressed arm (34). Foiani and colleagues first visualized RVFs using electron microscopy, and this remains the primary method for detecting such structures (1, 33, 35, 36). RVFs are frequently observed in response to oncogene-induced replication stress, underscoring the relevance of RVFs in cancer (34). Restructuring a stalled RF into a RVF not only stabilizes the fork, but also promotes accurate fork restart. Optimally, RF reversal promotes the transient pausing of replication to restrict extensive ssDNA accumulation, allow sufficient time for DNA repair, and promote completion of replication by a second incoming fork during ICL repair (34). However, it has become clear that faulty RF reversal can lead to adverse pathological consequences. For example, fork remodeling can promote DNA strand misalignment, contributing to genomic instability (37). Without adequate fork protection, RVFs can be subject to extensive nuclease activity, leading to fork degradation and the formation of aberrant DNA structures (15, 16). Recent work indicates that DDR factors such as RNF168 and 53BP1 enable efficient DNA replication and suppress chromosomal instability by preventing the excessive accumulation and nucleolytic processing of RVFs at difficult-to-replicate genomic regions, even in the absence of exogenous stressors (38).

Several SNF2-family translocases, such as SMARCAL1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1), HLTF (helicase-like transcription factor), and ZRANB3 (zinc finger RANBP2-type containing 3), have been implicated in initiating fork reversal. Initially, *SMARCAL1* was identified as a gene of interest in patients with Schimke immuno-osseous dysplasia (39). Subsequent work from the Elledge and Cortez labs demonstrated the importance of SMARCAL1 in regulating fork reversal to maintain genomic stability (40, 41). Both loss and overexpression of SMARCAL1 can be deleterious for the genome, highlighting the importance of SMARCAL1 regulation, which occurs via interactions with RPA and phosphorylation by ATR (37). The concentration and DNA-binding orientation of RPA dictates its interaction with SMARCAL1, thereby regulating SMARCAL1 engagement at RFs. Once at the fork, SMARCAL1 is subject to ATR-mediated phosphorylation, which suppresses SMARCAL1-dependent fork reversal activity (37).

ZRANB3 was uncovered by the Elledge group as a PCNA interacting protein that promotes fork reversal upon its interaction with polyubiquitinated PCNA (42). PCNA polyubiquitination is regulated in part by the yeast RAD5 homolog HLTF, and the HIRAN domain of HLTF can also stimulate fork reversal activity (37). Other DNA translocases, such as RAD54 and FANCM, have been implicated in fork reversal as well (43). More recently, studies have revealed that the loss of SMARCAL1 and other translocases such as ZRANB3 and HLTF rescues fork degradation in *BRCA1/2*-deficient cells in response to replication stress, presumably by limiting the generation of DNA structures that are substrates for fork

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

degrading nucleases (15, 18, 22, 36, 42). Unlike PTIP or MLL3/4 depletion, SMARCAL1 or ZRANB3 depletion does not confer chemoresistance in *BRCA1*-deficient cells, even though genomic stability is restored (22). Although the detailed mechanisms of these SNF2-family fork remodelers are still being resolved, the recent work underscores the importance of fork-remodeling enzymes in genome maintenance.

RAD51 is required to stabilize reversed forks

Upon fork remodeling by nucleases and translocases, RAD51 coats exposed ssDNA at the RVF, protecting against unregulated nuclease-mediated fork degradation (44). RAD51 loading during fork reversal can occur independently of its association with BRCA2 (14). Interestingly, other HR mediators, such as RAD54, the RAD51 paralogs, and MMS22L–TONSL (MMS22-like, DNA repair protein–tonsoku-like, DNA repair protein), support BRCA2-independent RAD51 loading during fork reversal (19, 45, 46). Unlike BRCA2 deficiency, RAD51 loss reduces fork reversal, inhibits MRE11-dependent fork degradation, and restores fork stability in *Brca2*-deficient cells (18). Together, these data highlight the importance of RAD51 in fork reversal and degradation, but also as a fork protector. Unlike the initial loading of RAD51 at reversed forks, subsequent RAD51 nucleoprotein filament stabilization and successful HR require BRCA2. Notably, the roles of RAD51 in protection of RVFs against nuclease-dependent fork degradation and in HR are separable, revealed by analyses of a patient-derived RAD51^{T131P} mutant cell line characterized by Smogorzewska and colleagues (32). The RAD51^{T131P} mutant protein interacts with DNA and supports HR; however, it fails to form a stable nucleofilament even in the presence of BRCA2. Consequently, RAD51^{T131P} mutant cells exhibit increased ssDNA accumulation, RPA exhaustion, and increased DNA2 and WRN activity, resulting in defective fork protection and ICL repair. Furthermore, the Costanzo lab showed that RAD51^{T131P} mutant cells are competent for fork reversal, but fail to protect RVFs from MRE11-dependent degradation (36). Similarly, BRCA2 separation-of-function mutants have been identified that are defective for fork protection but competent for HR (16, 20).

Intriguingly, RAD51 overexpression promotes increased fork reversal, leading to fork degradation and replication-associated DSBs (47, 48), perturbs replication elongation, and triggers unscheduled origin firing (49). Recent proteomic analyses of stalled RFs revealed a novel factor, RADX, that competes with RAD51 for ssDNA binding at the stalled RFs, thereby suppressing aberrant fork remodeling (48). Consistent with a role for RADX as a RAD51 antagonist, RADX overexpression promotes MRE11- and DNA2-mediated fork degradation, and its loss restores fork protection in cells lacking BRCA1, BRCA2, or FANCD2 (50). The RAD51 ubiquitylating factor, FBH1, also acts as a negative regulator of RAD51 function, with loss of RAD51 ubiquitylation resulting in replication stress and hyper-recombination (51). In sum, RAD51 plays multiple roles in the context of RF stability, including in fork protection, fork reversal, and HR, and is subject to both positive and negative regulation at stalled RFs to ensure the maintenance of genomic stability.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Additional DDR factors besides canonical HR/FA proteins also regulate replication fork stability

In addition to the HR/FA proteins noted above, several other DDR factors have been implicated in fork protection. These include the Abraxas paralog ABRO1 (abraxas 2, BRISC complex subunit) as well as BOD1L (biorientation of chromosomes in cell division 1–like 1), which protect stalled RFs against DNA2- and EXO1-dependent fork degradation (52, 53). Our own laboratory has identified 9-1-1 as another piece in the replication fork protection puzzle. The 9-1-1 complex is a heterotrimeric DNA binding clamp that stimulates ATR-mediated checkpoint signaling and DNA repair (54). 9-1-1 dysfunction has been linked to several of the hallmarks of fork protection defects, including S-phase specific DNA damage accumulation (55), hypersensitivity to replication stress-inducing agents (56, 57), and predisposition to radial chromosome formation (58). Indeed, our recent studies have identified a requirement for the 9-1-1 complex in protecting stalled RFs against MRE11-dependent nascent strand degradation following replication stress (unpublished results from Weiss lab). Although 9-1-1 has multiple, separable functions in the DDR (59), it is ATR activation by 9-1-1 that is particularly important for fork protection, a finding that is consistent with prior studies linking ATR to replication fork stability (13, 60–62). A challenge for the field moving forward is to determine how these new players, including ABRO1, BOD1L, and 9-1-1, fit into the existing landscape of RF protection and repair. Common phenotypes observed in several of the corresponding mutants are suggestive of potential functional interactions, and with comprehensive screens and detailed analysis of the replication forks, the molecular intricacies governing the pathways responsible for protecting and degrading RFs will be uncovered.

RecQ family helicases resolve reversed forks to promote accurate fork restart

The RecQ family helicases RECQ1, BLM, and WRN have central roles in RF restoration and replication restart (34). RECQ1 is an ATP-dependent DNA helicase that interacts with RVFs, unwinding the leading strand at the stalled RF and promoting branch migration (63). RECQ1 additionally inhibits DNA2 activity in an ATPase-independent manner (64). PARP1-mediated ADP ribosylation keeps RECQ1 activity at stalled RFs in check, and RECQ1 activity remains inhibited by activated PARP1 until replication stress is relieved. This mechanism prevents premature RVF restoration (34). RECQ1 also has been implicated in the generation and release of DNA fragments during RF resection. Pasero and colleagues observed this role during studies of SAMHD1 (SAM and HD domain–containing dNTP triphosphohydrolase 1), a dNTP hydrolase that activates MRE11, promoting gapped fork resection and checkpoint activation. In the absence of SAMHD1, processing of stalled forks by RECQ1 results in ssDNA release into the cytosol and subsequent induction of interferon signaling, linking cancer-associated genomic instability to inflammatory responses (65).

Independent of RECQ1, WRN and BLM, other members of the RecQ helicase family, can also promote fork restart activity in a HR-dependent manner (16). Interestingly, unlike other RecQ helicases, WRN has 3′ to 5′ exonuclease activity, making it a prime candidate for

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

restoring forks containing gaps in the leading strand (3). Another helicase/nuclease, DNA2, is aided by WRN/BLM ATPase activity and resects RVFs to promote the production of 3′ ssDNA overhangs (34, 66). Upon controlled resection by DNA2, RPA and RAD51 bind ssDNA, leading to strand invasion and formation of Holliday Junctions (HJs) that are subsequently resolved by the HR machinery (67). However, WRN and BLM have both pro- and anti-recombinogenic activity, and instead of promoting HR can also promote fork restart by dissolving HJs (68).

Importance of replication fork stability and restart in promoting chemoresistance in *BRCA* deficient tumors

Despite sharing similar names, related disease associations, and clear linkages to DSB repair, *BRCA1*- and *BRCA2*-deficient tumors are associated with distinct mechanisms of chemoresistance. Until recently, restoration of HR was the only known mechanism for promoting cell viability and chemoresistance in *BRCA1/2*-deficient tumors (69, 70). As mentioned earlier, Nussenzweig and colleagues determined that eliminating non-homologous end joining protein 53BP1 in *BRCA1*-deficient cells restores HR, rescuing the embryonic lethality of *Brca1* mutant mice (71). Parallel work in the Ashworth laboratory showed restoration of cell viability in *BRCA2*-deficient tumors by restoring HR (70). However, multiple groups have gone on to show that independently of HR reinstatement, restoring stalled replication forks and promoting fork protection and stability enables chemoresistance in *BRCA1/2*-deficient tumors (15, 17, 18, 23, 24, 27, 50, 62). In *BRCA1*-deficient cancer cells, acquisition of PARPi resistance is associated with the sequential bypass of the requirements for BRCA1 for both HR and fork protection through mechanisms that are dependent upon ATR signaling (62). While fork protection activity contributes to chemoresistance, it is less certain that it is necessary for tumor suppression. In assessing the functions of the BRCA1/BARD1 complex, Billing et al. recently found that BARD1 mutants that are defective for fork protection but competent for HR cause chromosomal instability but not tumor predisposition (72).

BRCA2 deficiency leads to increased nascent strand degradation but is associated with relatively normal fork restart activity (20). Until recently, the mechanisms that facilitate fork restart in *BRCA2*-deficient cells remained unclear. Lemacon et al. (11) determined that in *BRCA2*-deficient cells, MUS81 cleaves the partially resected RVF with a ssDNA flap to ensure POLD3-dependent fork restart and cell survival, suggesting a possible synthetic lethal effect in cells upon BRCA2 and MUS81/POLD3 depletion. On the other hand, D'Andrea and colleagues demonstrated that MUS81 disruption in *BRCA2*-deficient cells restores fork stability and confers resistance to PARPi, which Lemacon et al. did not observe (15, 23). This discrepancy regarding the role of MUS81 in regulating fork stability could be related to its ability to process diverse substrates. Interestingly, *MUS81* loss in *BRCA1*-deficient cells did not rescue fork stability as observed upon *BRCA2* deficiency (15). Whereas BRCA1 promotes fork restart by promoting the cleavage of stalled RFs by the SLX-MUS endonuclease complex, BRCA2 suppresses fork breakage, leading to the suggestion that BRCA2 may participate in a cleavage-free pathway for fork restart, possibly in conjunction with 53BP1, which counteracts BRCA1-mediated cleavage-coupled restart

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(73). Despite these distinctions between BRCA1 and BRCA2 functions, restoration of RF stability and cell survival is observed in both *BRCA1/2*-deficient cells upon inactivation of SNF2-family fork remodelers (22, 36). Given that the pathways critical for cell viability differ under particular stress conditions and with *BRCA1* vs. *BRCA2* deficiency, it is essential to fully resolve the underlying molecular details and leverage this knowledge to generate potent cancer therapies.

Conclusions

A clear take home message from the above-mentioned studies is that RF remodeling and stability involves a tightly regulated balancing act among several proteins from different pathways. A tug-of-war between fork protection proteins and fork degrading nucleases ultimately determines outcomes related to cell survival and genomic stability. Importantly, fork remodeling has come to the forefront as a major contributor to chemoresistance and cancer. With many factors already linked to RF reversal, protection, and restart, and with more likely to be discovered, one of the challenges that remains in the field is the integration of these various components into a cohesive model for what happens when RFs encounter DNA lesions. Given that many of the factors involved in fork protection have been implicated in cancer and that many commonly used anticancer therapies cause replication stress or target critical DNA repair proteins, fully resolving the molecular mechanisms in action at stalled RFs holds great promise for yielding new therapeutic approaches.

Acknowledgements

The authors thank Pei Xin Lim and Elizabeth Moore for helpful discussions and comments on the manuscript.

Funding Information

Research in the Weiss laboratory is funded by NYSTEM grant DOH01-C32571GG-3450000 and NIH grants R03 HD083621-02 and R01 HD 095296-01. DRP received funding from NIH training grant T32 GM07273 and the Cornell Center for Vertebrate Genomics Scholars Program.

Abbreviations List:

9-1-1	RAD9-HUS1-RAD1
ABRO1	Abraxas 2, BRISC complex subunit
ATR	Ataxia telangiectasia and Rad3-related protein
BLM	Bloom helicase
BOD1L	Biorientation of chromosomes in cell division 1–like 1
BRCA1/2	Breast Cancer 1/2
CHK1	Checkpoint kinase 1
CtIP	CtBP1-interacting protein
DDR	DNA damage response

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

DSB	Double-stranded break
EXO1	Exonuclease 1
EZH2	Enhancer of zeste homolog 2
FA	Fanconi Anemia
HR	Homologous recombination
ICL	Interstrand crosslinks
HLTF	Helicase-like transcription factor
ZRANB3	Zinc finger RANBP2-type containing 3
MLL3/4	Mixed-lineage leukemia protein 3/4
MMS22L–TONSL	MMS22-like, DNA repair protein–tonsoku-like, DNA repair protein
MRE11	Meiotic recombination 11
PARP	Poly [ADP-ribose] polymerase
PCNA	Proliferating cell nuclear antigen
POLD3	DNA polymerase subunit D
PTIP	Pax interacting protein
RADX	RPA-related and RAD51-antagonist X chromosome
RECQ1	RecQ-like helicase
RF	Replication fork
RPA	Replication protein A
RVF	Reversed fork
SAMHD1	SAM and HD domain–containing dNTP triphosphohydrolase 1
ssDNA	Single-stranded DNA
SMARCAL1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like
TLS	Translesion synthesis
WRN	Werner syndrome ATP-dependent helicase

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

References

1. Lopes M, Cotta-Ramusino C, Pellicioli A, Liberi G, Plevani P, Muzi-Falconi M, et al. The DNA replication checkpoint response stabilizes stalled replication forks. Nature 2001;412(6846):557–61. [PubMed: 11484058]
2. Johnson A, O'Donnell M. Cellular DNA replicases: components and dynamics at the replication fork. Annual review of biochemistry 2005;74:283–315.
3. Atkinson J, McGlynn P. Replication fork reversal and the maintenance of genome stability. Nucleic acids research 2009;37(11):3475–92. [PubMed: 19406929]
4. Branzei D, Foiani M. Maintaining genome stability at the replication fork. Nature reviews Molecular cell biology 2010;11(3):208–19. [PubMed: 20177396]
5. Kanao R, Masutani C. Regulation of DNA damage tolerance in mammalian cells by post-translational modifications of PCNA. Mutation research 2017;803–805:82–8.
6. Zhang J, Walter JC. Mechanism and regulation of incisions during DNA interstrand crosslink repair. DNA Repair (Amst) 2014;19:135–42. [PubMed: 24768452]
7. Huang J, Liu S, Bellani MA, Thazhathveetil AK, Ling C, de Winter JP, et al. The DNA translocase FANCM/MHF promotes replication traverse of DNA interstrand crosslinks. Molecular cell 2013;52(3):434–46. [PubMed: 24207054]
8. Mutreja K, Krietsch J, Hess J, Ursich S, Berti M, Roessler FK, et al. ATR-Mediated Global Fork Slowing and Reversal Assist Fork Traverse and Prevent Chromosomal Breakage at DNA Interstrand Cross-Links. Cell reports 2018;24(10):2629–42.e5. [PubMed: 30184498]
9. Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. Genes & development 2007;21(12):1472–7. [PubMed: 17575048]
10. Zhao H, Piwnica-Worms H. ATR-Mediated Checkpoint Pathways Regulate Phosphorylation and Activation of Human Chk1. Molecular and cellular biology 2001;21(13):4129–39. [PubMed: 11390642]
11. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Molecular cell 2017;66(6):801–17. [PubMed: 28622525]
12. Friedel AM, Pike BL, Gasser SM. ATR/Mec1: coordinating fork stability and repair. Current opinion in cell biology 2009;21(2):237–44. [PubMed: 19230642]
13. Trenz K, Smith E, Smith S, Costanzo V. ATM and ATR promote Mre11 dependent restart of collapsed replication forks and prevent accumulation of DNA breaks. The EMBO Journal 2006;25(8):1764–74. [PubMed: 16601701]
14. Zellweger R, Dalcher D, Mutreja K, Berti M, Schmid JA, Herrador R, et al. Rad51-mediated replication fork reversal is a global response to genotoxic treatments in human cells. The Journal of cell biology 2015;208(5):563–79. [PubMed: 25733714]
15. Lemacon D, Jackson J, Quinet A, Brickner JR, Li S, Yazinski S, et al. MRE11 and EXO1 nucleases degrade reversed forks and elicit MUS81-dependent fork rescue in BRCA2-deficient cells. Nature communications 2017;8(1):860.
16. Pasero P, Vindigni A. Nucleases Acting at Stalled Forks: How to Reboot the Replication Program with a Few Shortcuts. Annual review of genetics 2017;51:477–99.
17. Ray Chaudhuri A, Callen E, Ding X, Gogola E, Duarte AA, Lee JE, et al. Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature 2016;535(7612):382–7. [PubMed: 27443740]
18. Mijic S, Zellweger R, Chappidi N, Berti M, Jacobs K, Mutreja K, et al. Replication fork reversal triggers fork degradation in BRCA2-defective cells. Nature communications 2017;8(1):859.
19. Krejci L, Altmannova V, Spirek M, Zhao X. Homologous recombination and its regulation. Nucleic acids research 2012;40(13):5795–818. [PubMed: 22467216]
20. Schlacher K, Christ N, Siaud N, Egashira A, Wu H, Jasin M. Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell 2011;145(4):529–42. [PubMed: 21565612]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Schlacher K, Wu H, Jasin M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer cell 2012;22(1):106–16. [PubMed: 22789542]
22. Taglialatela A, Alvarez S, Leuzzi G, Sannino V, Ranjha L, Huang JW, et al. Restoration of Replication Fork Stability in BRCA1- and BRCA2-Deficient Cells by Inactivation of SNF2-Family Fork Remodelers. Molecular cell 2017;68(2):414–30.e8. [PubMed: 29053959]
23. Rondinelli B, Gogola E, Yucel H, Duarte AA, van de Ven M, van der Sluijs R, et al. EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. Nature cell biology 2017;19(11):1371–8. [PubMed: 29035360]
24. Feng W, Jasin M. BRCA2 suppresses replication stress-induced mitotic and G1 abnormalities through homologous recombination. Nature communications 2017;8(1):525.
25. Hashimoto Y, Ray Chaudhuri A, Lopes M, Costanzo V. Rad51 protects nascent DNA from Mre11-dependent degradation and promotes continuous DNA synthesis. Nature structural & molecular biology 2010;17(11):1305–11.
26. Ceccaldi R, Sarangi P, D'Andrea AD. The Fanconi anaemia pathway: new players and new functions. Nature reviews Molecular cell biology 2016;17(6):337–49. [PubMed: 27145721]
27. Kais Z, Rondinelli B, Holmes A, O'Leary C, Kozono D, D'Andrea AD, et al. FANCD2 Maintains Fork Stability in BRCA1/2-Deficient Tumors and Promotes Alternative End-Joining DNA Repair. Cell reports 2016;15(11):2488–99. [PubMed: 27264184]
28. Lopez-Martinez D, Liang C-C, Cohn MA. Cellular response to DNA interstrand crosslinks: the Fanconi anemia pathway. Cellular and Molecular Life Sciences 2016;73:3097–114. [PubMed: 27094386]
29. Guillemette S, Serra RW, Peng M, Hayes JA, Konstantinopoulos PA, Green MR, et al. Resistance to therapy in BRCA2 mutant cells due to loss of the nucleosome remodeling factor CHD4. Genes & development 2015;29(5):489–94. [PubMed: 25737278]
30. Ding X, Ray Chaudhuri A, Callen E, Pang Y, Biswas K, Klarmann KD, et al. Synthetic viability by BRCA2 and PARP1/ARTD1 deficiencies. Nature communications 2016;7:12425.
31. Bunting SF, Callen E, Kozak ML, Kim JM, Wong N, Lopez-Contreras AJ, et al. BRCA1 functions independently of homologous recombination in DNA interstrand crosslink repair. Molecular cell 2012;46(2):125–35. [PubMed: 22445484]
32. Wang AT, Kim T, Wagner JE, Conti BA, Lach FP, Huang AL, et al. A Dominant Mutation in Human RAD51 Reveals Its Function in DNA Interstrand Crosslink Repair Independent of Homologous Recombination. Molecular cell 2015;59(3):478–90. [PubMed: 26253028]
33. Sogo JM, Lopes M, Foiani M. Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. Science (New York, NY) 2002;297(5581):599–602.
34. Neelsen KJ, Lopes M. Replication fork reversal in eukaryotes: from dead end to dynamic response. Nature Reviews Molecular Cell Biology 2015;16:207. [PubMed: 25714681]
35. Vindigni A, Lopes M. Combining electron microscopy with single molecule DNA fiber approaches to study DNA replication dynamics. Biophysical chemistry 2017;225:3–9. [PubMed: 27939387]
36. Kolinjivadi AM, Sannino V, De Antoni A, Zadorozhny K, Kilkenny M, Techer H, et al. Smarcal1-Mediated Fork Reversal Triggers Mre11-Dependent Degradation of Nascent DNA in the Absence of Brca2 and Stable Rad51 Nucleofilaments. Molecular cell 2017;67(5):867–81.e7. [PubMed: 28757209]
37. Poole LA, Cortez D. Functions of SMARCAL1, ZRANB3, and HLTF in maintaining genome stability. Critical reviews in biochemistry and molecular biology 2017;52(6):696–714. [PubMed: 28954549]
38. Schmid JA, Berti M, Walser F, Raso MC, Schmid F, Krietsch J, et al. Histone Ubiquitination by the DNA Damage Response Is Required for Efficient DNA Replication in Unperturbed S Phase. Molecular cell 2018;71(6):897–910.e8. [PubMed: 30122534]
39. Elizondo LI, Cho KS, Zhang W, Yan J, Huang C, Huang Y, et al. Schimke immuno-osseous dysplasia: SMARCAL1 loss-of-function and phenotypic correlation. Journal of medical genetics 2009;46(1):49–59. [PubMed: 18805831]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

40. Bansbach CE, Betous R, Lovejoy CA, Glick GG, Cortez D. The annealing helicase SMARCAL1 maintains genome integrity at stalled replication forks. Genes & development 2009;23(20):2405–14. [PubMed: 19793861]
41. Ciccia A, Bredemeyer AL, Sowa ME, Terret ME, Jallepalli PV, Harper JW, et al. The SIOD disorder protein SMARCAL1 is an RPA-interacting protein involved in replication fork restart. Genes & development 2009;23(20):2415–25. [PubMed: 19793862]
42. Ciccia A, Nimonkar AV, Hu Y, Hajdu I, Achar YJ, Izhar L, et al. Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress. Molecular cell 2012;47(3):396–409. [PubMed: 22704558]
43. Quinet A, Lemacon D, Vindigni A. Replication Fork Reversal: Players and Guardians. Molecular cell 2017;68(5):830–3. [PubMed: 29220651]
44. Burkovics P, Sebesta M, Balogh D, Haracska L, Krejci L. Strand invasion by HLTF as a mechanism for template switch in fork rescue. Nucleic acids research 2014;42(3):1711–20. [PubMed: 24198246]
45. Somyajit K, Saxena S, Babu S, Mishra A, Nagaraju G. Mammalian RAD51 paralogs protect nascent DNA at stalled forks and mediate replication restart. Nucleic acids research 2015;43(20): 9835–55. [PubMed: 26354865]
46. Piwko W, Mlejnkova LJ, Mutreja K, Ranjha L, Stafa D, Smirnov A, et al. The MMS22L-TONSL heterodimer directly promotes RAD51-dependent recombination upon replication stress. Embo j 2016;35(23):2584–601. [PubMed: 27797818]
47. Couch FB, Bansbach CE, Driscoll R, Luzwick JW, Glick GG, Betous R, et al. ATR phosphorylates SMARCAL1 to prevent replication fork collapse. Genes & development 2013;27(14):1610–23. [PubMed: 23873943]
48. Dungrawala H, Bhat KP, Le Meur R, Chazin WJ, Ding X, Sharan SK, et al. RADX Promotes Genome Stability and Modulates Chemosensitivity by Regulating RAD51 at Replication Forks. Molecular cell 2017;67(3):374–86.e5. [PubMed: 28735897]
49. Parplys AC, Seelbach JI, Becker S, Behr M, Wrona A, Jend C, et al. High levels of RAD51 perturb DNA replication elongation and cause unscheduled origin firing due to impaired CHK1 activation. Cell Cycle 2015;14(19):3190–202. [PubMed: 26317153]
50. Bhat KP, Krishnamoorthy A, Dungrawala H, Garcin EB, Modesti M, Cortez D. RADX Modulates RAD51 Activity to Control Replication Fork Protection. Cell reports 2018;24(3):538–45. [PubMed: 30021152]
51. Chu WK, Payne MJ, Beli P, Hanada K, Choudhary C, Hickson ID. FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51. Nature communications 2015;6:5931.
52. Higgs MR, Reynolds JJ, Winczura A, Blackford AN, Borel V, Miller ES, et al. BOD1L Is Required to Suppress Deleterious Resection of Stressed Replication Forks. Molecular cell 2015;59(3):462–77. [PubMed: 26166705]
53. Xu S, Wu X, Wu L, Castillo A, Liu J, Atkinson E, et al. Abro1 maintains genome stability and limits replication stress by protecting replication fork stability. Genes & development 2017;31(14): 1469–82. [PubMed: 28860160]
54. Wang X, Hu B, Weiss RS, Wang Y. The effect of Hus1 on ionizing radiation sensitivity is associated with homologous recombination repair but is independent of nonhomologous end-joining. Oncogene 2006;25(13):1980–3. [PubMed: 16278671]
55. Zhu M, Weiss RS. Increased common fragile site expression, cell proliferation defects, and apoptosis following conditional inactivation of mouse Hus1 in primary cultured cells. Molecular biology of the cell 2007;18(3):1044–55. [PubMed: 17215515]
56. Weiss RS, Enoch T, Leder P. Inactivation of mouse Hus1 results in genomic instability and impaired responses to genotoxic stress. Genes & development 2000;14(15):1886–98. [PubMed: 10921903]
57. Balmus G, Lim PX, Oswald A, Hume KR, Cassano A, Pierre J, et al. HUS1 regulates in vivo responses to genotoxic chemotherapies. Oncogene 2016;35(5):662–9. [PubMed: 25915840]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

58. Levitt PS, Zhu M, Cassano A, Yazinski SA, Liu H, Darfler J, et al. Genome maintenance defects in cultured cells and mice following partial inactivation of the essential cell cycle checkpoint gene Hus1. Molecular and cellular biology 2007;27(6):2189–201. [PubMed: 17220276]
59. Lim PX, Patel DR, Poisson KE, Basuita M, Tsai C, Lyndaker AM, et al. Genome Protection by the 9-1-1 Complex Subunit HUS1 Requires Clamp Formation, DNA Contacts, and ATR Signaling-independent Effector Functions. The Journal of biological chemistry 2015;290(24):14826–40. [PubMed: 25911100]
60. Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. Nature reviews Molecular cell biology 2008;9(8):616–27. [PubMed: 18594563]
61. Dungrawala H, Rose KL, Bhat KP, Mohni KN, Glick GG, Couch FB, et al. The Replication Checkpoint Prevents Two Types of Fork Collapse without Regulating Replisome Stability. Molecular cell 2015;59(6):998–1010. [PubMed: 26365379]
62. Yazinski SA, Comaills V, Buisson R, Genois MM, Nguyen HD, Ho CK, et al. ATR inhibition disrupts rewired homologous recombination and fork protection pathways in PARP inhibitor-resistant BRCA-deficient cancer cells. Genes & development 2017;31(3):318–32. [PubMed: 28242626]
63. Berti M, Ray Chaudhuri A, Thangavel S, Gomathinayagam S, Kenig S, Vujanovic M, et al. Human RECQ1 promotes restart of replication forks reversed by DNA topoisomerase I inhibition. Nature structural & molecular biology 2013;20(3):347–54.
64. Thangavel S, Berti M, Levikova M, Pinto C, Gomathinayagam S, Vujanovic M, et al. DNA2 drives processing and restart of reversed replication forks in human cells. The Journal of cell biology 2015;208(5):545–62. [PubMed: 25733713]
65. Coquel F, Silva MJ, Techer H, Zadorozhny K, Sharma S, Nieminuszczy J, et al. SAMHD1 acts at stalled replication forks to prevent interferon induction. Nature 2018;557(7703):57–61. [PubMed: 29670289]
66. Hu J, Sun L, Shen F, Chen Y, Hua Y, Liu Y, et al. The intra-S phase checkpoint targets Dna2 to prevent stalled replication forks from reversing. Cell 2012;149(6):1221–32. [PubMed: 22682245]
67. Popuri V, Bachrati CZ, Muzzolini L, Mosedale G, Costantini S, Giacomini E, et al. The Human RecQ helicases, BLM and RECQ1, display distinct DNA substrate specificities. The Journal of biological chemistry 2008;283(26):17766–76. [PubMed: 18448429]
68. Patel DS, Misenko SM, Her J, Bunting SF. BLM helicase regulates DNA repair by counteracting RAD51 loading at DNA double-strand break sites. The Journal of cell biology 2017;216(11): 3521–34. [PubMed: 28912125]
69. Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 2008;451:1116. [PubMed: 18264087]
70. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. The New England journal of medicine 2009;361(2):123–34. [PubMed: 19553641]
71. Bunting SF, Callén E, Wong N, Chen H-T, Polato F, Gunn A, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 2010;141(2): 243–54. [PubMed: 20362325]
72. Billing D, Horiguchi M, Wu-Baer F, Taglialatela A, Leuzzi G, Nanez SA, et al. The BRCT Domains of the BRCA1 and BARD1 Tumor Suppressors Differentially Regulate Homology-Directed Repair and Stalled Fork Protection. Molecular cell 2018.
73. Xu Y, Ning S, Wei Z, Xu R, Xu X, Xing M, et al. 53BP1 and BRCA1 control pathway choice for stalled replication restart. eLife 2017;6.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

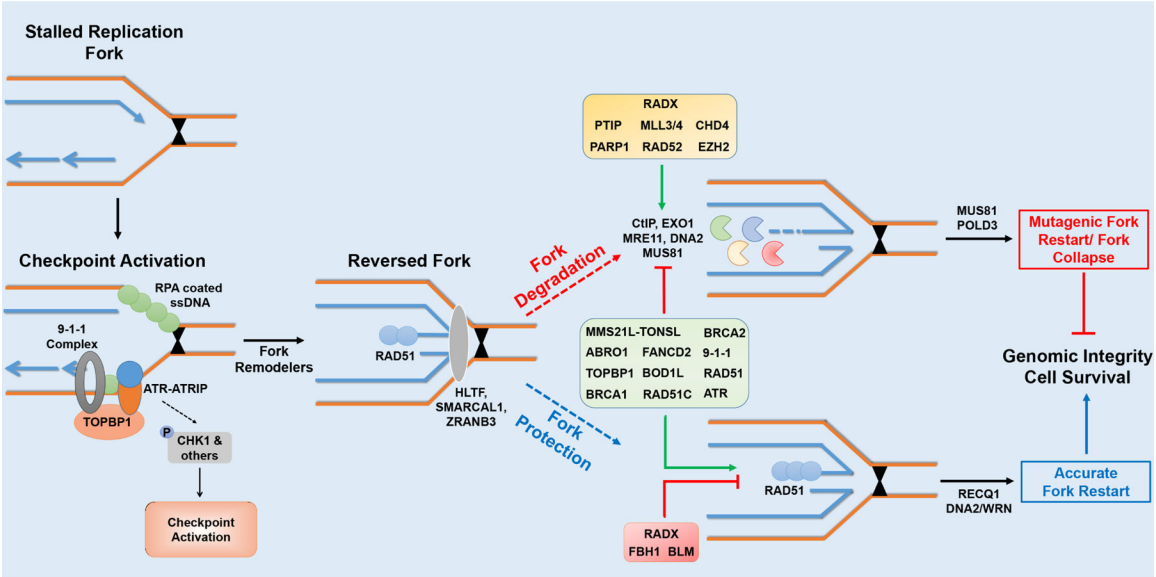

Figure 1. Molecular mechanisms of replication fork protection and degradation.

DNA replication is constantly threatened by ongoing replication stress. Stabilization of stalled replication forks is essential for genomic integrity. Fork protection proteins (RAD51, BRCA1/2, FANCD2, and others) stabilize the stalled fork against nascent strand nucleolytic degradation. Fork reversal also is a critically important process that is required for accurate fork restart. Reversal of stalled replication forks is mediated by DNA translocase enzymes (HLTF, SMARCAL1, ZRANB3 and RAD54) in conjunction with RAD51. RECQ1 and BLM/WRN helicases promote effective fork restart of reversed forks. Along with these HR/FA proteins, several other novel proteins, ABRO1, BOD1L, RADX and checkpoint proteins, such as ATR and the 9-1-1 complex, have also been implicated in regulating fork stability.