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Nucleases Acting at Stalled Forks: How to Reboot the Replication Program with a Few Shortcuts

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Abstract

In a lifetime, a human being synthesizes approximately 2×10^{16} meters of DNA, a distance that corresponds to 130,000 times the distance between the Earth and the Sun. This daunting task is executed by thousands of replication forks, which progress along the chromosomes and frequently stall when they encounter DNA lesions, unusual DNA structures, RNA polymerases, or tightly-bound protein complexes. To complete DNA synthesis before the onset of mitosis, eukaryotic cells have evolved complex mechanisms to process and restart arrested forks through the coordinated action of multiple nucleases, topoisomerases, and helicases. In this review, we discuss recent advances in understanding the role and regulation of nucleases acting at stalled forks with a focus on the nucleolytic degradation of nascent DNA, a process commonly referred to as fork resection. We also discuss the effects of deregulated fork resection on genomic instability and on the unscheduled activation of the interferon response under replication stress conditions.

INTRODUCTION

CMG: Cdc45/MCM2–7/GINS

DSB: DNA double-strand break

ssDNA: single-stranded DNA

HR: homologous recombination

RSR: replication stress response

DNA replication is the basic mechanism used by all living organisms to duplicate their genome and transfer the genetic information between generations. This process is achieved by sophisticated macromolecular machinery termed the replisome (164). DNA replication begins with assembly of a prereplicative complex composed of the origin-recognition complex (ORC), CDC6, CDT1, and a hexameric ring of the mini chromosome maintenance (MCM) proteins MCM2–7 at specific sequence elements termed replication origins (25, 58, 137). Bacteria and archaea are typically characterized by a single replication origin, whereas eukaryotic chromosomes are replicated in a timely fashion by initiating replication at multiple genomic replication origins. DNA synthesis is initiated by cyclin-dependent and DBF4-dependent kinases in a process called origin firing (45, 119, 128, 138). In eukaryotes, the parental DNA duplex is unwound by a replicative helicase composed of the CDC45 protein, the MCM2–7 complex, and the tetrameric GINS complex, which together form the so-called CMG complex (62, 82, 123). Concomitantly, DNA is replicated by the leading and lagging strand polymerases, Pol ε and Pol δ, which are associated with CMG (**Figure 1*a***). Many of the proteins involved in DNA replication are highly conserved between eukaryotes, even though the nature of replication origins can vary significantly between unicellular eukaryotes and metazoans. In this review, we principally refer to human proteins unless otherwise stated.

DNA lesions that arise from either normal metabolism or exposures to natural or artificial agents in the environment constantly challenge replication forks. In addition to DNA lesions, intrinsic replication fork obstacles, such as transcribing RNA polymerases, unusual DNA structures, tightly-bound protein–DNA complexes, and oncogene activation, may also perturb fork progression (19, 170). Replication stress can be defined as a global perturbation of the DNA replication program generally associated with a transient slowing or stalling of replication forks. The effects of replication stress on the structure and the stability of arrested forks can be extremely variable, depending on the nature of the impediment. For instance, small DNA lesions can pass through the CMG helicase and block DNA synthesis, leading to a physical uncoupling between the helicase and DNA polymerases (**Figure 1*b***) (29, 97, 169). In contrast, protein–DNA complexes, interstrand cross-links, or bulky DNA adducts arrest forks by preventing extensive unwinding of parental DNA strands (**Figure 1*c***). However, the notion that roadblocks on the leading strand represent an absolute block to replication fork progression was challenged by recent studies that showed that the replicative machinery can traverse interstrand cross-links in a process that requires the FANCM translocase in complex with the MHF protein (81). DNA double-strand breaks (DSBs) may arise when replication forks collide with lesions or encounter single-stranded DNA (ssDNA) gaps in a process commonly known as replication run-off (**Figure 1*d***) (76). One-ended DSBs might result from endonucleolytic cleavage of the stalled replication intermediates in a process that may contribute to fork restart by homologous recombination (HR) repair (74). Stalled forks can be rescued by converging forks progressing from backup replication origins (**Figure 1*f***). These so-called dormant origins are present in large excess in the human genome and are activated only under replication stress conditions (67). Forks that cannot be restarted or rescued by dormant origins eventually collapse (**Figure 1*e***). Initially, fork collapse was linked to dissociation of the replisome components, but recent results in yeast and human cells indicate that it can more generally be defined as the scenario in which forks are terminally arrested (37, 39, 49, 55, 155).

The mechanisms by which cells deal with replication stress to promote fork recovery depend therefore on the nature of the impediment and on the structure of stressed forks (**Figure 1*f–j***). Defining these different replication stress response (RSR) pathways is paramount for understanding how cells cope with replication challenges and preserve the integrity of their genomes. This

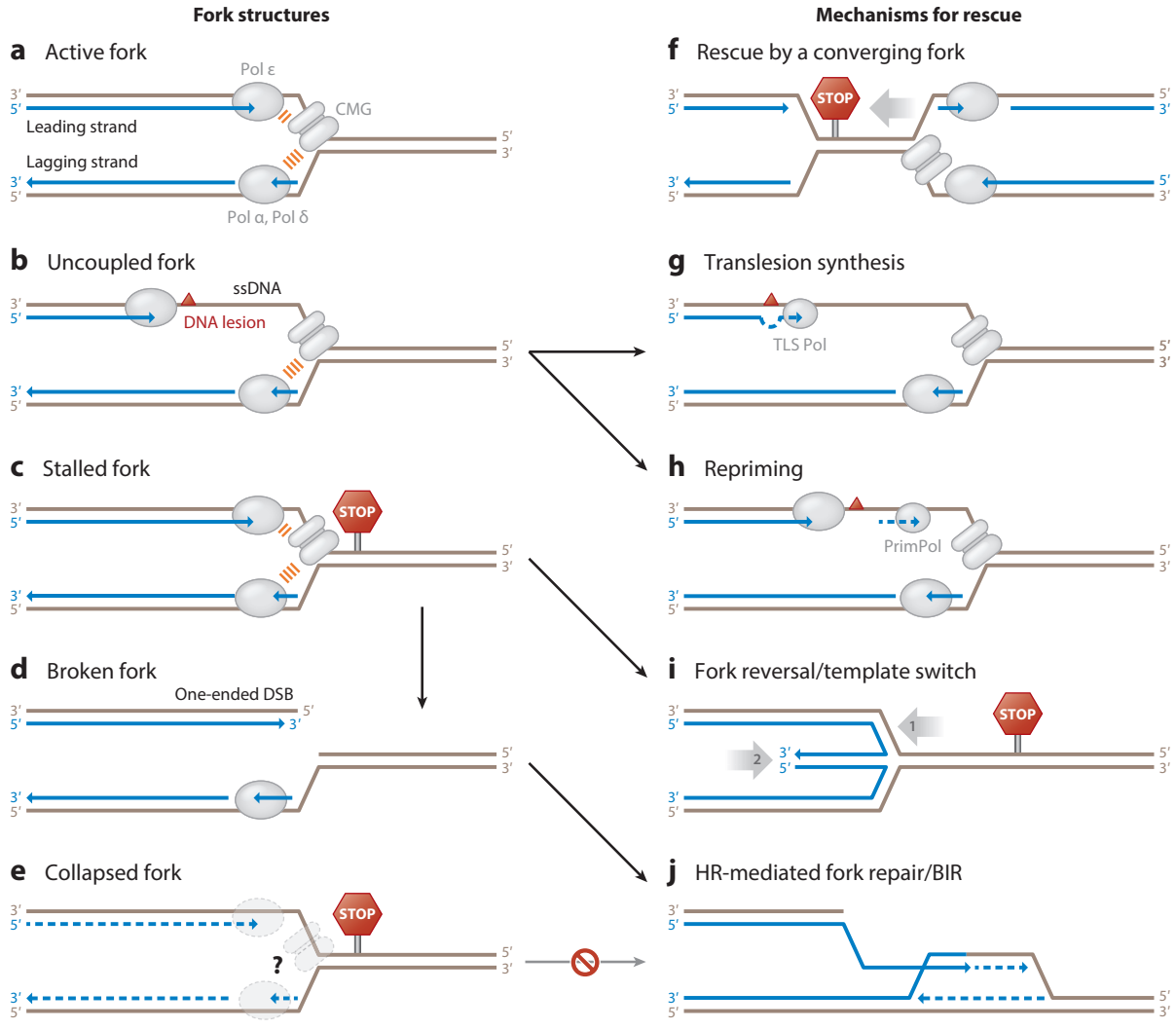


Figure 1

Recovery of stressed replication forks. This figure illustrates the different types of fork structures (*a–e*) observed under replication stress conditions and the mechanisms used by eukaryotic cells to rescue them (*f–j*). (*a*) Simplified representation of a eukaryotic replication fork showing the unwinding of parental DNA strands by the Cdc45/MCM2–7/GINS (CMG) helicase complex and the DNA polymerases acting on the leading and lagging strands. CMG and DNA polymerases associate with many additional factors at replication forks to form the replisome. Orange lines represent physical interactions between the CMG complex and DNA polymerases. (*b*) DNA lesions that are small enough to pass through the CMG channel block the progression of DNA polymerases, leading to uncoupling of polymerase and helicase activities and increased single-stranded DNA (ssDNA) at the fork. (*c*) Replication forks stall when they encounter obstacles impeding the progression of the replisome. (*d*) Replication forks break when they encounter a DNA lesion or an ssDNA gap, and when a stalled fork is cleaved by a structure-specific endonuclease. This leads to the formation of a one-ended DNA double-strand break (DSB) and to replisome run-off. (*e*) Forks that cannot be restarted or rescued by dormant origins in a timely fashion eventually collapse. Fork collapse corresponds to an irreversible inactivation of the fork, with or without loss of replisome components. (*f*) A stalled fork can be rescued by an active fork progressing from a downstream initiation event. (*g*) DNA lesions can be bypassed by translesion DNA synthesis (TLS). (*h*) Lesions on the leading strand can also by bypassed by repriming downstream of the lesion with the human primase PrimPol. (*i*) Newly replicated DNA strands can also anneal at stalled forks, leading to fork reversal or template switching events. (*j*) Broken forks can be repaired by homologous recombination (HR)-mediated mechanisms, including break-induced replication (BIR).

RPA: replication protein A

is particularly important in the context of cancer cells, which display elevated levels of spontaneous DNA damage and rely on RSR mechanisms to proliferate or overcome treatment induced by DNA-damaging chemotherapeutics (1, 4, 73, 98, 102). Therefore, understanding the mechanisms of RSR following genotoxic stress induction is rapidly emerging as a central theme in cell survival and human disease. Nucleases are key factors for processing stalled replication intermediates, and defining the exact function of nucleases in RSR represents a major challenge in investigations of genome stability (50, 165). Nucleases promote the limited degradation of nascent DNA strands required for efficient fork restart. However, they can also promote an extensive and uncontrolled degradation of stalled replication intermediates under pathological conditions through mechanisms yet to be fully understood.

Here, we provide a brief overview of the mechanisms of the RSR. We then focus on the current models of and controversies over the function of nucleases in this process, and on how their uncontrolled activity might lead to the extensive fork degradation phenotypes that underlie the pathological effects observed in cancer cells carrying mutations in key Fanconi anemia (FA)/HR factors.

ATR AND THE SIGNALING OF STALLED FORKS

A characteristic feature of stalled replication forks is the accumulation of ssDNA at replication fork junctions (29, 97, 125, 169). This ssDNA is coated with the heterotrimeric complex RPA, which recruits the sensor kinases of the DNA replication checkpoint, called ATR in vertebrates and Mec1/Rad3 in yeast (**Figure 2*a***) (44, 91, 116, 127, 174). At replication fork junctions, ssDNA might originate from physical uncoupling of the polymerase from the replicative helicase, which continues to unwind the DNA duplex after the leading strand polymerase stalls in response to base damage (**Figure 2*b***) (29, 97, 125, 169). In the presence of DNA polymerase inhibitors, uncoupling may also occur between the replicative helicase and both the leading and lagging strand polymerases in a process generally referred to as hyperunwinding (**Figure 2*c***) (29, 124). Events that physically block helicase movement, such as torsional stress induced by stabilization of the DNA topoisomerase I cleavage complex, are not expected to promote uncoupling. The fact that RPA-coated ssDNA is also detected in the presence of these agents suggests that specific nucleases with the aid of helicases or translocases actively process stalled forks to create ssDNA behind arrested forks (**Figure 2*d***) (100, 129).

Importantly, RPA-coated ssDNA is necessary but not sufficient to activate the DNA replication checkpoint (101). Indeed, 5′ primer junctions are also required to recruit the clamp loader RFC^{Rad17} and load the 9–1–1 (RAD9–RAD1–HUS1) complex (**Figure 2*a***). This in turn allows the binding of the mediator protein topoisomerase-IIβ-binding protein 1 (TopBP1) and the activation of the checkpoint kinase ATR (36) through a process that is conserved in budding yeast (127). The presence of both RPA-coated ssDNA and primer junctions is therefore critical for activating ATR at stalled forks. In addition, recent studies uncovered a novel ATR activation pathway mediated by the Ewing's tumor-associated antigen 1 (ETAA1) protein, which is also recruited to sites of replication stress through its interaction with RPA (**Figure 2*a***) and can activate ATR independently of RAD17, the 9–1–1 complex, and TopBP1 (16, 59, 72, 95).

Once activated, ATR orchestrates different pathways of replication fork restart/stabilization, together with CHK1, the major effector kinase of the DNA replication checkpoint (130). The choice of a specific fork-restart pathway depends on the nature and location of the replication challenge, and on whether a DNA lesion is located on the leading or lagging template strand. For example, lagging strand DNA lesions are normally well tolerated because of the inherently discontinuous nature of Okazaki-fragment synthesis and maturation, whereas leading strand lesions represent a major obstacle for processive DNA synthesis.

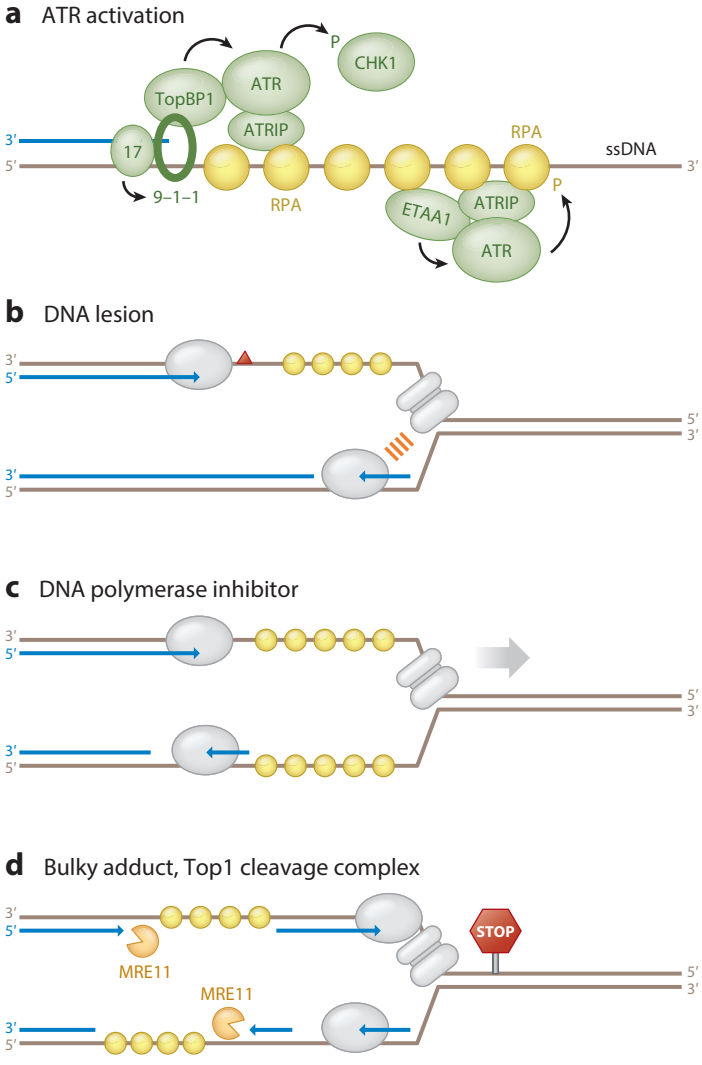


Figure 2

Signaling at arrested forks. Replication stress leads to the accumulation of single-stranded DNA (ssDNA) at arrested forks, which is rapidly coated with a heterotrimeric ssDNA binding protein called RPA and recruits the apical kinase of the DNA replication checkpoint, ATR, through its interaction with ATRIP. (*a*) Two distinct pathways lead to ATR activation at stalled forks. In the canonical pathway, the RFC^{Rad17} complex (here labeled 17) loads the 9–1–1 complex at the junctions of ssDNA with double-stranded DNA and recruits topoisomerase-IIβ-binding protein 1 (TopBP1), which stimulates the activity of the ATR. Activation of ATR can also occur in a TopBP1-independent manner, through the binding of Ewing's tumor-associated antigen 1 (ETAA1) protein to RPA-coated ssDNA. These two pathways may cooperate to phosphorylate different downstream targets, such as RPA or the effector kinase CHK1. (*b–d*) Mechanisms leading to the formation of ssDNA at arrested forks. RPA-coated ssDNA can be formed (*b*) by the uncoupling of leading and lagging strand polymerase activities, (*c*) by the uncoupling of helicase and polymerase activities, or (*d*) by nucleolytic degradation of newly replicated DNA at gapped or reversed forks.

MECHANISMS OF FORK RESTART AND DAMAGE AVOIDANCE

MRE11: meiotic recombination 11

MUS81: methyl methanesulfonate and ultraviolet-sensitive protein 81

CtIP: C-terminal binding protein interacting protein

As discussed above, stalled replication forks can be rescued by activating dormant origins near perturbed replication forks, thus allowing completion of DNA synthesis by an incoming replication fork (**Figure 1*f***) (67, 118). However, if two converging forks stall in regions lacking dormant origins, cells must restart at least one of these forks to ensure full genome duplication. Forks encountering leading strand DNA lesions can bypass them using different damage avoidance pathways—either using specialized polymerases or postponing DNA repair—with minimal effect on fork elongation (27). Fork progression may be facilitated by specialized translesion synthesis polymerases, which have the ability to replicate through a damaged template, albeit with lower fidelity compared to the replicative polymerases (**Figure 1*g***) (140). Alternatively, replisomes might also skip the damaged DNA through the so-called repriming mechanisms that reprime DNA synthesis downstream of a lesion (**Figure 1*h***) (21, 56, 77, 78, 113). These mechanisms operate in all organisms from *E. coli* to human cells (165). They leave unreplicated ssDNA gaps behind damaged forks that would need to be repaired after replication by postreplicative repair pathways (48, 68, 69, 86, 104).

Another important mechanism of fork stabilization and restart involves the annealing of newly replicated DNA strands and the formation of a four-way structure resembling a Holliday junction (HJ) (**Figure 1*i***) (10, 106, 117). This so-called fork reversal process was initially identified in budding yeast as a pathological transition occurring in the absence of the Mec1/ATR pathway (149). However, recent evidence indicates that fork reversal is a remarkably frequent mechanism of RSR in all species that allows replication forks to reverse their course in response to genotoxic insults—including a variety of chemotherapeutic treatments—thereby preventing fork collision with the drug-induced DNA damage (18, 135, 165, 169). Fork reversal can be conceptually divided into two steps: (*a*) formation of reversed forks by the coordinated annealing of the two newly synthesized strands, and (*b*) restart of the reversed forks. Recently, the central recombinase factor RAD51 has been found essential for the first step of reversed fork formation (169), whereas the human RECQ1 helicase is required for the subsequent step of fork restart (18). In addition to RAD51, several DNA translocases—including Rad5 in budding yeast (24), RAD54 (28), SMARCAL1 (20), FANCM (64), ZRANB3 (35, 168), and the Rad5 homolog HLTF (23, 87) in mammalian cells—can potentially promote fork reversal, but their exact function is still under investigation (for details on the factors involved in this process, see 19, 117).

Besides fork reversal, RAD51 has been implicated in the restart and repair of blocked or broken replication forks through a process called HR-mediated fork restart (5, 77, 93). This process operates on forks experiencing replication run-off (**Figure 1*j***) or on uncoupled or reversed forks after cleavage of one strand by structure-specific endonucleases such as the methyl methanesulfonate and ultraviolet (UV)-sensitive protein 81 (MUS81). It has been reported in all species from bacteria to vertebrates (165).

ROLE OF RESECTION IN THE RECOVERY OF STRESSED FORKS

Nucleases have well-defined roles in DSB resection. Two distinct pathways act redundantly to mediate processive DSB resection downstream from the MRE11-RAD50-NBS1 and the C-terminal binding protein interacting protein (CtIP) factors in eukaryotic cells: One requires DNA2 and the other requires EXO1 (70, 109, 120). MRE11 has both a 3′–5′ exonuclease activity and an ssDNA endonuclease activity (152). Previous biochemical studies on the role of MRE11 in DSB resection showed that endonuclease activity is required to initiate the resection process and that CtIP promotes MRE11 endonuclease activity at the 5′ strand (8). Interestingly, this process is promoted

by SAMHD1, a protein initially identified as an HIV restriction factor (47). Resection is then continued by the 5′–3′ exonuclease activity of EXO1 or DNA2 (30, 120, 121, 173). Specifically, EXO1 and DNA2 resect the 5′ ends of DSBs to generate 3′ single-stranded overhangs, which are essential to initiate HR. In yeast, DNA2-dependent double-stranded DNA (dsDNA)-end resection reaction requires the Sgs1 helicase to unwind the DNA from the break (31, 122, 173). This mechanism appears to be largely conserved in mammalian cells, where DNA2 cooperates with the human BLM or WRN helicase to resect dsDNA ends in vitro (121, 153). The same nucleases involved in DSB resection are now emerging as key factors for processing stalled replication intermediates, as detailed below.

SAMHD1: sterile alpha motif and HD-domain containing protein 1

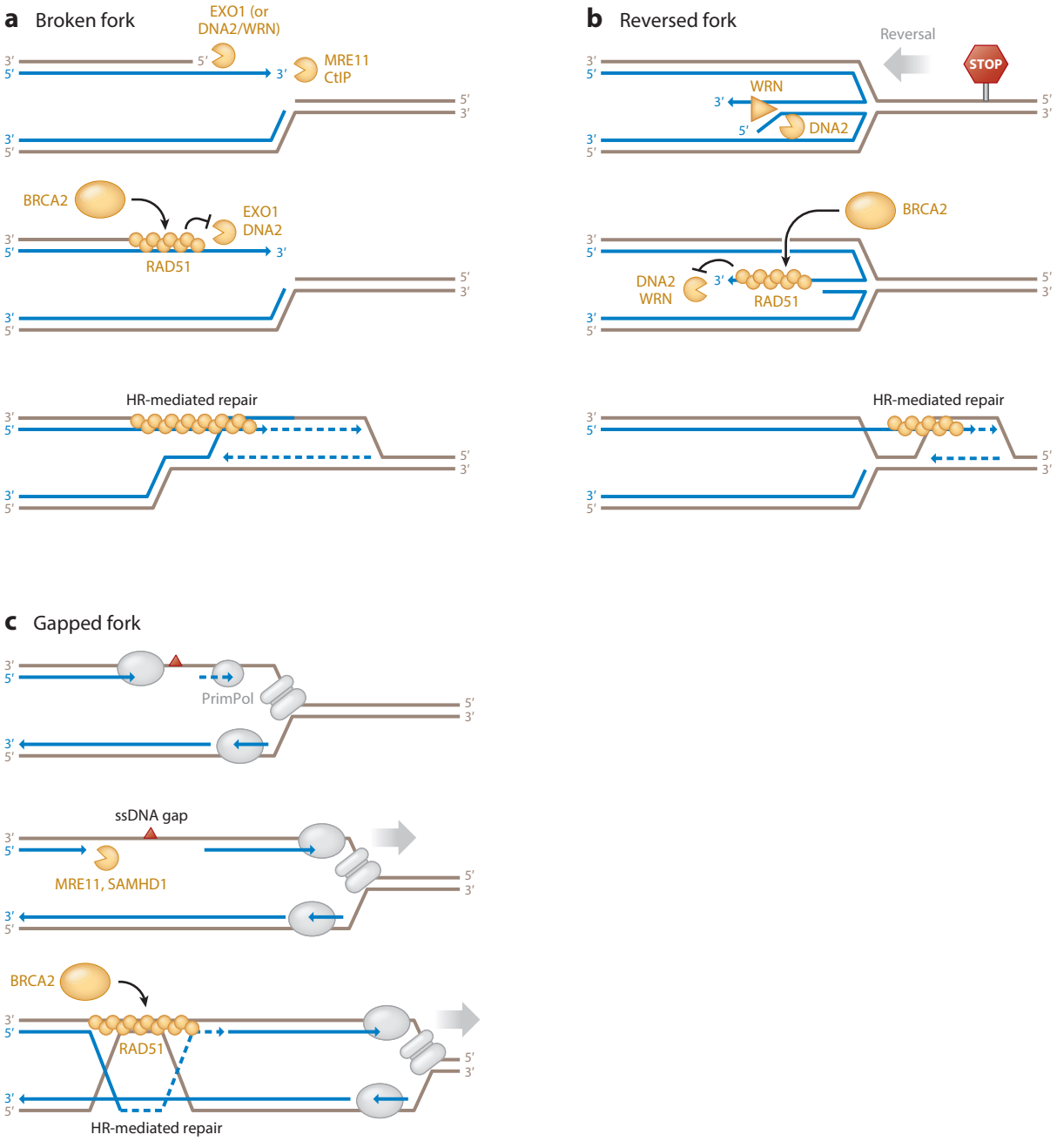
dsDNA: double-stranded DNA

First, the ability of MRE11, CtIP, DNA2, and EXO1 to process dsDNA ends is relevant in the context of DNA replication to prevent the accumulation of replication-associated DSBs by promoting replication-coupled HR repair (**Figure 3*a***) (38, 43, 44, 89, 145, 156, 158, 166). In addition, controlled DNA2-dependent degradation of reversed replication forks is a functionally relevant mechanism to mediate reversed fork restart and provide resistance to prolonged genotoxic treatments (**Figure 3*b***) (156). The DNA2 nuclease cooperates with the WRN helicase in resecting reversed replication forks with a 5′-to-3′ polarity and in mediating fork restart (156). In this context, the partial ssDNA structures generated by DNA2/WRN-dependent resection may activate an HR-like mechanism of reversed-fork restart. The 3′ overhang on the regressed arm might be coated by RAD51 to invade donor sequences ahead of the reversed fork (133). Alternatively, the 3′ tail generated by DNA2/WRN-dependent resection of the reversed arm may be specifically recognized by a motor protein that drives branch migration–assisted reestablishment of a functional replication fork. For example, the SWI/SNF-related SMARCAL1 DNA translocase efficiently converts four-way junctions into functional replication forks and displays a preference for reversed forks with a 3′ ssDNA tail coated by RPA (20).

The same nucleases have also been shown to promote recovery of replication fork blockage by processing different replication fork structures, both in yeast and in vertebrates (6, 77). For example, MRE11 was shown to prevent DSB formation upon replication stress, and the limited MRE11-dependent degradation of nascent strands may reflect a role for MRE11 in removing stalled polymerases and promoting repriming past the lesion (**Figure 3*c***) (41, 42). Repriming mechanisms lead to the formation of ssDNA gaps opposite the damage on the daughter strand, also known as daughter-strand gaps. Repriming mechanisms were originally identified in bacteria, where synthesis downstream of a leading strand lesion can be reinitiated by recycling or exchange of stalled replicative polymerases (78, 165). This mechanism also appears to efficiently restart replication in eukaryotes, and the human primase PrimPol was recently identified in vertebrates as an enzyme required to resume DNA synthesis after UV irradiation and under conditions of deoxynucleoside triphosphate (dNTP) shortage (**Figure 3*c***) (21, 56, 63, 113). This mechanism leads to ssDNA gap formation, and these gaps need to be filled or repaired postreplicatively by gap filling or by postreplication HR-like mechanisms (21, 56, 63, 113). Postreplication HR-like mechanisms would entail the formation of a D-loop and a double HJ structure that could be resolved by specific resolvases or dissolved by the combined action of a RecQ helicase (BLM in human, Sgs1 in budding yeast, and Rqh1 in fission yeast) and the type I topoisomerase TOP3 (22). In the context of HR-mediated gap repair, the limited resection activity of MRE11 at ssDNA gaps could be required to create the proper DNA structure for RAD51 loading. At the same time, RAD51 loading might be needed to limit the MRE11-dependent resection of internal ssDNA gaps that form when the fork moves beyond persistent DNA lesions to be repaired postreplicatively (76).

Other nucleases have been implicated in recovery from replication fork blockage, but the exact structures of the stalled replication intermediates targeted by these nucleases are unknown. For

example, the Fanconi-associated nuclease FAN1 cooperates with the central FA protein FANCD2 in promoting fork restart in response to replication stress (33). Similarly, CtIP is also crucial for fork restart, when properly controlled by FANCD2 (166). CtIP acts together with MRE11 in this process to remove the end-binding protein Ku from one-ended DSBs present at broken or reversed forks (32, 94). At internal gaps, other proteins could act as cofactors for MRE11.



A candidate for this function is SAMHD1, a dNTP hydrolase involved in HIV-1 restriction and frequently mutated in the Aicardi-Goutières syndrome (13, 92). SAMHD1 promotes the resection of DNA ends independently of its dNTPase activity (47). Like CtIP, SAMHD1 is phosphorylated by cyclin A–CDK2 upon entry into S phase, and this phosphorylation is essential for the resection of stalled forks (F. Coquel, Y.-L. Lin & P. Pasero, unpublished data). SAMHD1 also shows a 3′–5′ exonuclease activity in vitro (17), but recent evidence indicates that this activity could be due to a contaminating nuclease (144). SAMHD1 could therefore act as a cofactor of MRE11 to promote the resection of gapped forks, rather than act as a bona fide exonuclease.

ENDONUCLEASES ACTING AT STALLED FORKS

Several structure-specific nucleases have been associated with fork restart upon genotoxic stress induction (50). MUS81 is a structure-specific nuclease that preferentially cleaves 3′ flap substrates, fork-like structures, and nicked HJs (132, 161). Increasing evidence suggests that the nuclease activity of MUS81 is required for fork restart following a variety of replication challenges, even though there are still several open questions regarding its exact function in this process (61, 74, 131, 136, 148). For example, MUS81 forms a heterodimeric complex with either the essential meiotic endonuclease 1 (EME1) or EME2. However, it is still unclear which of the two EME proteins is required in order for MUS81 to function during DNA replication (132, 162). Moreover, several factors can regulate MUS81 activity at replication forks—e.g., the human BLM helicase (148, 171), SLX4 (54, 65, 114, 141), and different S-phase checkpoint proteins (26, 60, 84, 154)—suggesting that its cleavage activity needs to be tightly regulated by a mechanism that remains unclear. Finally, researchers have proposed that MUS81 promotes DNA synthesis both during S phase and in mitosis to resolve persistent replication intermediates and to safeguard chromosome segregation (54, 110). The exact structure cleaved by MUS81 at stalled forks has also been subject to debate. Recent studies have suggested that MUS81 cleaves reversed fork substrates that form in response to a variety of replication challenges (61, 74, 131, 136, 148). However, biochemical data show that MUS81 has negligible activity toward intact HJs, which resemble an intact reversed fork, thereby suggesting that reversed forks might need to be processed by other nucleases prior to MUS81 cleavage (132, 161). In this regard, recent electron microscopy studies provided the first clear determination of the structural changes that occur at the level of reversed replication forks, making them prone to MUS81 cleavage (96). These electron microscopy experiments showed that the substrate cleaved by MUS81 in breast cancer susceptibility factor 2 (*BRCA2*)-deficient cells is a resected reversed fork with a 3′ ssDNA flap, matching previous biochemical observations

Figure 3

Role of resection in fork repair. (*a*) The MRE11, CtIP, DNA2, and EXO1 exonucleases process one-ended DNA double-strand breaks to generate a 3′ ssDNA overhang that can be used as a substrate for HR-mediated fork repair. MRE11 and CtIP promote this function by removing the end-binding protein Ku from the DNA end. The BRCA2-dependent loading of RAD51 on ssDNA is important for initiating the recombination process, but also for preventing excessive nucleolytic degradation. Strand invasion depends on RAD52 in human cells. (*b*) DNA2 and WRN functionally interact to process reversed forks, leading to formation of a 3′ ssDNA overhang. DNA2 degrades reversed forks with a 5′–3′ polarity. WRN ATPase activity assists DNA2 degradation, possibly by promoting the opening of the reversed arm of the fork. The resulting 3′ overhang might in turn be necessary to engage HR-mediated fork restart. Alternatively, binding of SMARCAL1 to the resected reversed fork can mediate branch migration and reversed fork resolution. (*c*) ssDNA gaps can also be generated behind stalled forks by MRE11 in a SAMHD1-dependent manner. These internal gaps can be repaired in a postreplicative manner by HR-mediated gap repair. Abbreviations: BRCA2, breast cancer susceptibility factor 2; CtIP, C-terminal binding protein interacting protein; HR, homologous recombination; SAMHD1, sterile alpha motif and HD-domain containing protein 1; ssDNA, single-stranded DNA.

BRCA1: breast cancer susceptibility factor 1

BRCA2: breast cancer susceptibility factor 2

(132, 161). Interestingly, MUS81 is required for completion of DNA replication at common fragile sites (110, 115) and telomeric loci (51) via a specialized form of DNA repair originally characterized in yeast termed break-induced replication (BIR) (9, 40, 150). In this context, MUS81 cleavage of stalled replication forks produces a migrating bubble that in turn drives DNA polymerase δ-dependent DNA synthesis to repair the broken forks. The same BIR mechanism seems to be promoted by MUS81 in *BRCA2*-deficient cells (96), suggesting that MUS81 activity is important for rescuing replication forks that face intrinsic replication challenges or that are degraded by nucleases under specific genetic backgrounds. More details on the mechanisms of fork degradation in the absence of FA and HR factors are provided in the following section. Finally, it is worth noting that BIR is a highly mutagenic process occurring by an unusually conservative DNA synthesis (in contrast to the usual semiconservative mode of DNA synthesis) at a migrating D-loop (40, 53, 139). In this context, cleavage of the D-loop by MUS81 helps to restore a normal DNA replication fork and to suppress the genomic instability promoted by BIR (105). Together, these data suggest that MUS81 performs multiple roles at stalled replication forks and acts on different substrates to resolve HJs, cleave reversed forks, and convert D-loops into canonical replication forks (**Figure 4**).

Besides MUS81, Metnase and the endonuclease/exonuclease/phosphatase family domain containing 1 protein (EEPD1) are other two structure-specific endonucleases that have gained increasing attention for their functions during DNA replication (88, 89). Metnase is a human protein with a methylase (SET) and nuclease domain that also possesses unique—yet poorly defined—fork cleavage activity necessary for its function in replication restart. Interestingly, Metnase function seems to play additional roles at replication forks that are uncoupled with Metnase endonuclease activity. Indeed, its SET domain is somehow required for recovery from hydroxyurea-induced DNA damage, and recent studies suggested that Metnase is also required for EXO1 loading on stalled replication forks (89). Similarly, EEPD1 is a 5′ endonuclease that cleaves replication forks at the junction between the lagging parental strand and the unreplicated DNA parental double strands to create a structure that EXO1 requires for 5′-end resection and HR initiation (88). Finally, FEN1 is another endonuclease well known for its flap-cleavage function during Okazaki-fragment maturation (12) that was recently shown to be involved in processing DNA secondary structures upon replication fork stalling (146, 172). This novel role of FEN1 in RSR requires its recently described gap endonuclease activity, which is needed to cleave stalled replication forks containing bubble-like structures (172). Interestingly, the gap endonuclease activity of FEN1 is stimulated by the human WRN helicase upon replication stress induction, whereas the association of FEN1 with the WD40 protein WUHO is required to stimulate the flap-cleavage activity of FEN1 while inhibiting gap endonuclease activity in the absence of replication stress (34).

PATHOLOGICAL FORK RESECTION

Nucleolytic resection of stalled replication forks is a tightly regulated process that must ensure a limited degradation of the nascent DNA strands required for efficient fork restart. In budding yeast, DNA end resection is downregulated by the DNA replication checkpoint (7, 15), and the Rad53 kinase represses Exo1 in response to excessive resection (112). In *rad53* mutants, the deregulated activity of Exo1 leads to the formation of long ssDNA gaps and contributes to fork collapse (43, 111, 145, 149). In addition, according to a recent report, uncontrolled resection of nascent DNA by Exo1 at a paused replication fork in fission yeast generates a structure that cannot be rescued by a converging fork and that leads to the formation of an anaphase bridge in mitosis (6). Fork protection in this system depends on Rad52 and on Rad51 in a recombination-independent manner (6). In mammalian cells, several FA and HR factors, including BRCA1, BRCA2, and FANCD2, are

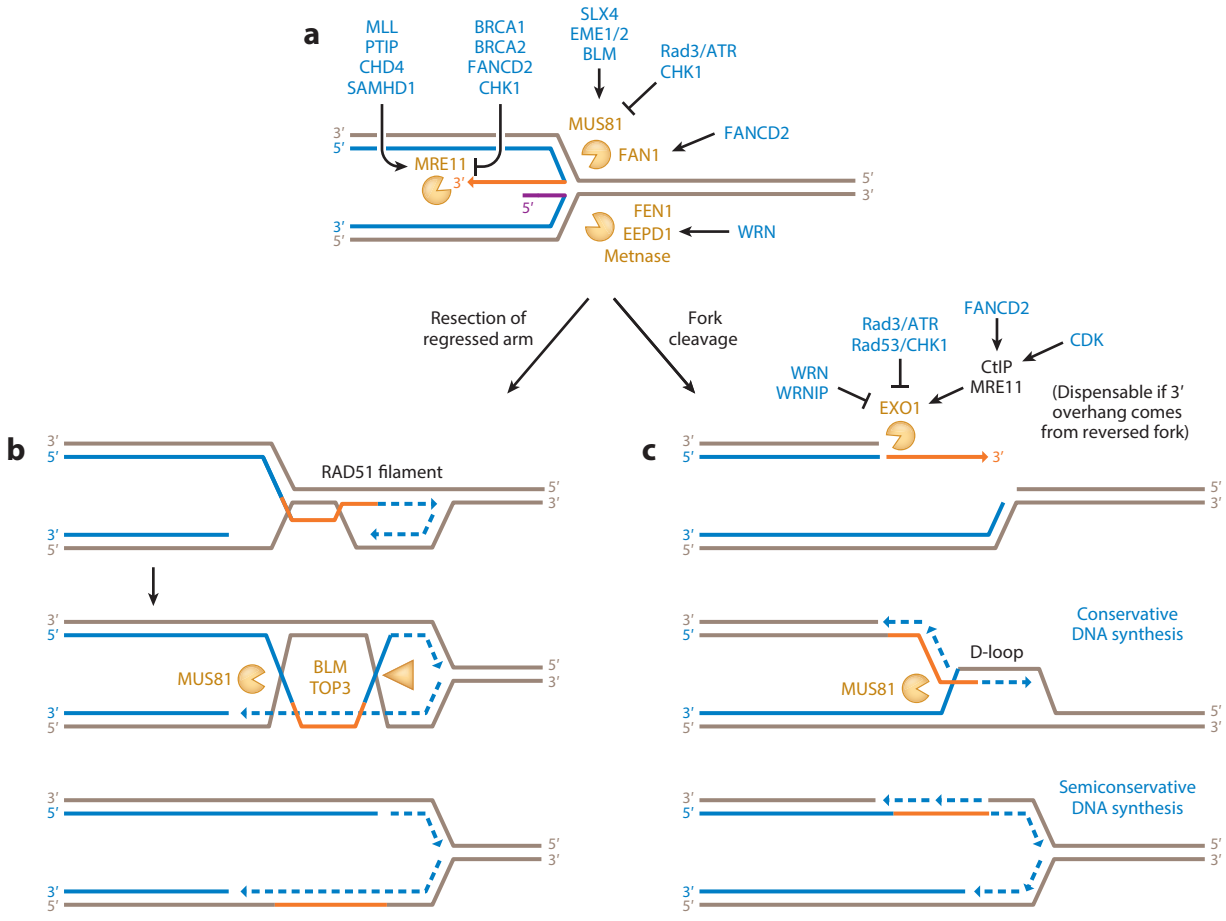


Figure 4

Coordinated action of endo- and exonucleases at arrested forks. (*a*) Reversed replication forks can be restarted by two distinct mechanisms involving either endo- or exonucleases. The factors that regulate these nucleases are indicated in blue. Digestion of the 5′ strand of the regressed fork (*purple*) by MRE11 generates a 3′ overhang (*orange*) that can be used to engage homologous recombination (HR)-mediated repair of the reversed fork. (*b*) HR leads to the formation of Holliday junctions, which can be resolved by MUS81 or dissolved by the combined action of TOP3 and BLM. (*c*) Cleavage of the partially resected reversed fork containing a 3′ ssDNA flap by MUS81 or another endonuclease generates a one-ended DNA double-strand break (DSB) with a 3′ overhang. If the one-ended DSB arises from replication run-off at a single-stranded DNA gap or from the cleavage of a stalled fork, the DNA end is processed by MRE11 and EXO1 to generate a 3′ overhang. This overhang is next used to engage break-induced replication (conservative DNA synthesis, low fidelity). The D-loop can be cleaved by MUS81 to restore a normal replication fork (semiconservative DNA synthesis, high fidelity). Abbreviations: CtIP, C-terminal binding protein interacting protein; EEPD1, endonuclease/exonuclease/phosphatase family domain containing 1 protein; EME1/2, essential meiotic endonuclease 1/2; EXO1, exonuclease 1; MRE11, meiotic recombination 11; MUS81, methyl methanesulfonate and ultraviolet-sensitive protein 81; PTIP, Pax2 transactivation domain-interacting protein; TOP, topoisomerase.

emerging as key regulators of these resection processes, and increasing evidence supports the idea that the extensive degradation of stalled replication intermediates underlies the pathological effects observed in FA- and *BRCA*-deficient cancer cells (76, 85, 107, 134, 142, 143, 167). In the absence of these key regulatory factors, uncontrolled nuclease activity may lead to extended and pathological degradation of stalled replication intermediates and create large stretches of ssDNA on the stalled

replication forks. For example, replication forks are extensively degraded by the MRE11 and EXO1 nucleases in the absence of BRCA proteins (96, 134, 142, 143, 167). Recent studies suggest that BRCA proteins protect forks from extended nucleolytic degradation by stabilizing the RAD51 filament on the regressed arms of replication forks that have reversed upon drug treatment. In other words, these studies suggest that the one-ended DSBs of the regressed arms of reversed replication forks are entry points for nucleases in *BRCA1*- and *BRCA2*-deficient cells (90, 96, 108, 153a). The CtIP protein initiates MRE11-dependent degradation of the regressed arms, which is then extended by the EXO1 nuclease (96). RAD51 has two distinct functions in this process: (*a*) a BRCA2-independent function in promoting the initial step of reversed fork formation (169), and (*b*) a BRCA2-dependent function whereby BRCA proteins protect the already formed reversed forks from nucleolytic degradation by stabilizing the RAD51 filament on the regressed arm. In *BRCA2*-deficient cells, this second function is lost. This causes the nascent strand degradation phenotype observed with *BRCA2* mutants that are unable to stabilize RAD51 on ssDNA (142) or with RAD51 mutants that destabilize the RAD51 nucleofilament (90, 96, 108, 159) (**Figure 5*a***).

Of note, the extended degradation observed in FA- and *BRCA*-mutated cells does not represent a terminal event because the extensively resected forks can restart upon drug removal, suggesting that cells can somehow cope with forks containing long stretches of ssDNA (96, 134, 142). However, this restart mechanism leads to increased chromosomal aberrations and genomic instability (134, 142). In this regard, recent studies suggested that the rescue of the resected forks and cell survival in *BRCA2*-deficient cells largely relies on a BIR-like mechanism whereby MUS81 cleavage of partially resected reversed forks promotes DNA polymerase δ-dependent DNA fork rescue. This could explain the increased chromosomal instability of *BRCA2*-deficient cells (96). If the partially resected reversed forks are not promptly restarted by MUS81, nucleolytic degradation might quickly proceed to degrade nascent strands beyond the length of the regressed arm, leading to extensively degraded fork structures (**Figure 5*a***). The resulting nuclease-dependent ssDNA gaps that form behind the forks could promote reannealing of the parental strands and fork backtracking. As a consequence of fork backtracking, a new reversal event may easily occur, possibly promoting a new MUS81 cleavage event, and repeated cycles of this sequence of events might ultimately rescue most resected forks (96). Resection could also occur at internal gaps due to the destabilization of the RAD51 filament (**Figure 5*b***). For example, recent studies suggested that FANCD2 interacts with the MCM2–7 complex to restrain DNA synthesis in the presence of reduced nucleotide pools and that this interaction is important for preventing accumulation of ssDNA gaps that would otherwise provide an entry point for nucleolytic degradation (99). Whether the same BIR-like mechanism described in *BRCA2* mutant cells rescues the resected forks in *FANCD2* mutant cells remains to the determined. Interestingly, this mechanism does not appear to be required to rescue resected forks in *BRCA1*-mutant cells (96), suggesting that different pathways might mediate the restart of the resected forks depending on the particular genetic background.

Other factors are emerging as key regulators of replication fork processing in addition to the HR and FA factors mentioned above. For example, the biorientation defect 1-like protein (BOD1L) is another important component for replication fork protection from extended nucleolytic degradation (79). In particular, BOD1L is required to stabilize the RAD51 filament at stalled replication forks. In its absence, the human BLM and FBH1 helicases destabilize the RAD51 filament, and the human DNA2 helicase/nuclease promotes overresection of the damaged forks (79). Interestingly, inhibition of MRE11 by the chemical Mirin does not rescue nucleolytic fork degradation in the absence of BOD1L, in contrast to the results obtained with *BRCA1/2*- and *FANCD2*-deficient cells, suggesting that different nucleases can promote the overresection of stalled/damaged replication intermediates depending on the particular genetic background of

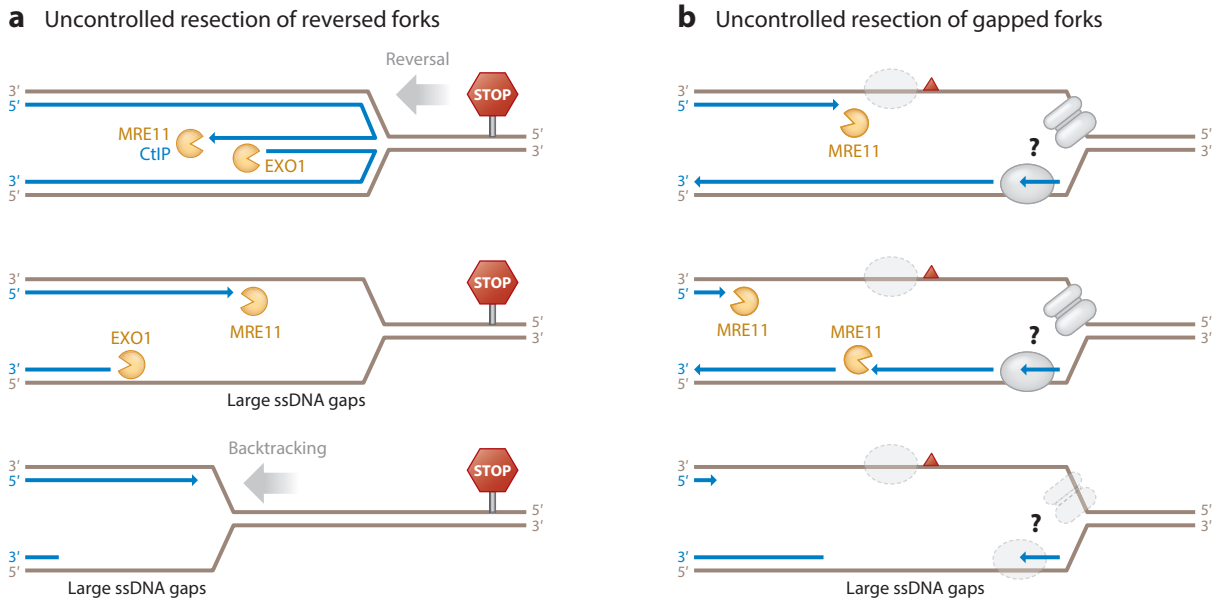


Figure 5

Pathological resection of arrested forks in the absence of breast cancer susceptibility factor 1 (BRCA1) or BRCA2. (*a*) Extensive resection mediated by meiotic recombination 11 (MRE11) and exonuclease 1 (EXO1) occurs on both strands of reversed forks in BRCA-deficient cells. C-terminal binding protein interacting protein (CtIP) stimulates the 5′ to 3′ endonuclease activity of MRE11 to initiate the degradation of the regressed arms, which is then extended by the EXO1 nuclease. MRE11 might also use its 3′ to 5′ exonuclease activity to degrade the opposite strand, possibly in conjunction with a yet to be identified 3′ to 5′ nuclease. This leads to the accumulation of large single-stranded DNA (ssDNA) gaps. The resulting nuclease-dependent ssDNA gaps that form behind the forks could promote reannealing of the parental strands and fork backtracking. (*b*) Similar mechanisms operate at gapped forks, potentially leading to irreversible fork collapse.

the individual. Another mechanism that promotes RAD51 destabilization and DNA2-dependent fork degradation is never-in-mitosis A-related kinase 1 (NEK1) mediated by phosphorylation of RAD54. This suggests that RAD54 is another important factor for replication fork stability (151).

Of note, the extended degradation of replication forks observed in the absence of the factors described above is emerging as one of the leading causes of the sensitivity to therapies that target DNA or inhibit specific repair pathways, such as poly(ADP-ribose) polymerase (PARP) inhibitors (134). The efficacy of these therapies is, however, hampered by the development of chemoresistance. These findings led to the recent discovery that chemoresistance is associated with the recovered ability of FA- or HR-deficient cells to protect replication forks from nucleolytic degradation (134). Aside from genetic reversion, which might occur in a limited subset of *BRCA2*-mutant breast cancers, the mechanisms that lead to the recovered ability of FA- or HR-deficient cells to protect forks from degradation and that mediate resistance to therapy are largely unknown. In an effort to shed light onto the possible mechanisms of chemoresistance, recent studies have focused on identifying the factors directly involved in nuclease recruitment at stalled/damaged replication forks. These studies led to the identification of the Pax2 transactivation domain-interacting protein (PTIP) and the histone methyltransferases MLL3 and MLL4 as key factors involved in MRE11 recruitment at stalled replication forks in *BRCA1/2*-deficient mouse cells (134) (**Figure 4*a***). Similarly, loss of the nucleosome remodeling factor CHD4 and inhibition of the poly(ADP-ribosyl)ation activity of PARP also confer fork protection in *BRCA*-deficient cells,

suggesting that CHD4 and PARP are two other factors involved in MRE11 recruitment (52, 71). These studies lead to the provocative idea that deficiencies in PTIP, CHD4, and PARP1 could confer drug resistance in *BRCA*-deficient cells by limiting MRE11 loading to stalled replication forks. Although the mechanisms of MRE11 recruitment at stalled forks have been widely investigated, little is known about the mechanisms that control the recruitment of other nucleases involved in fork degradation. It will be key to define the mechanisms by which the constantly growing number of nucleases found at replication forks are recruited onto the stalled replication intermediates in order to achieve a full understanding of the mechanisms of genome stability. We envision that these studies will lead to breakthrough discoveries on the molecular basis of chemosensitivity and on the chemoresistance of FA- and HR-deficient tumors.

FORK PROCESSING AND THE INDUCTION OF THE INTERFERON RESPONSE

Another consequence of the processing of stalled replication forks by nucleases and helicases is the production of DNA fragments that are released from the nucleus and accumulate in the cytosol. Cytosolic DNA is sensed by a variety of pattern recognition receptors (PRRs), which recognize microbial nucleic acids as non-self to activate the innate immune system (66, 126). One of the best characterized PRRs is the cyclic GMP–AMP synthase (cGAS), a cytosolic DNA receptor that activates STING (stimulator of interferon genes) in response to microbial infections and promotes the expression of type I interferons (IFNs) and other proinflammatory cytokines (2, 14). However, the STING pathway can also sense endogenous DNA accidentally released in the cytosol, and this can lead to autoimmune and inflammatory disorders, such as systemic lupus erythematosus and Aicardi-Goutières syndrome (46). Interestingly, most of the genes mutated in Aicardi-Goutières syndrome are implicated in the metabolism of nucleic acids. For instance, mutations that impair ribonuclease H2 chronically induce type I IFN genes in a STING-dependent manner (103), and mutations affecting the cytoplasmic nuclease TREX1 induce the accumulation of cytosolic ssDNA fragments and activate type I IFNs (3, 163). The origin of these DNA fragments is currently unknown, but a large body of evidence suggests that they represent by-products of faulty DNA replication or repair reactions. For instance, short ssDNA fragments are generated by DNA2 during the processing of Okazaki fragments (11) and are produced by MRE11 during DSB repair (83). In TREX1-deficient cells, it has been recently reported that cytoplasmic ssDNA is generated by the long-range resection pathways mediated by BLM and EXO1 (57). Finally, DNA damage activates the interferon response in ATM-deficient cells (75), supporting the view that both DNA damage and microbial infections trigger a common immune response through the STING pathway.

Interestingly, the presence of cytosolic DNA was recently reported in various cancer cell lines and was shown to further increase upon treatment with the replication inhibitor Ara-C (147), suggesting that cytosolic DNA is generated by stressed forks. The mechanism by which ssDNA is released from arrested forks was recently characterized in cells depleted for SAMHD1. As discussed above, SAMHD1 acts together with MRE11 to promote fork resection (**Figure 6**). In the absence of SAMHD1, arrested forks are processed by an alternative pathway, leading to the RECQ1-dependent release of ssDNA fragments and the induction of type I IFNs (F. Coquel, Y.-L. Lin & P. Pasero, unpublished data). The mechanism by which ssDNA fragments diffuse to the cytoplasm is currently unknown, but recent evidence indicates that they are sequestered in the nucleus by RPA and RAD51 (160). Because RPA levels are limiting (157), it is tempting to speculate that cells exposed to mild replication stress could buffer the IFN response by sequestering ssDNA fragments in the nucleus. However, RPA exhaustion induced by massive replication fork collapse would allow the release of ssDNA and the production of inflammatory cytokines. This

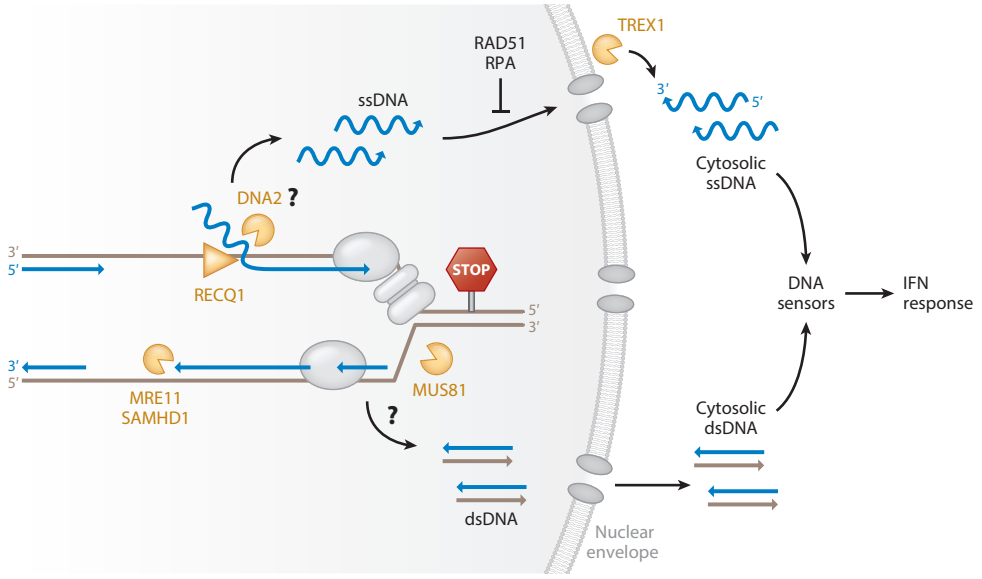


Figure 6

Defective processing of arrested forks activates the type I interferon (IFN) response. The deoxynucleoside triphosphate hydrolase SAMHD1 acts together with MRE11 to degrade nascent DNA at arrested forks. In SAMHD1-depleted cells, single-stranded DNA (ssDNA) fragments are released from forks in a RECQ1-dependent manner and accumulate in the cytoplasm, where they are detected by the STING pathway and induce type I IFN genes. Diffusion of ssDNA to the cytosol is prevented by RPA and RAD51. Double-stranded DNA (dsDNA) fragments are also generated by MUS81 to trigger type I IFNs and promote the rejection of cancer cells, but the mechanisms involved in the production of these fragments are currently unknown. Abbreviations: MRE11, meiotic recombination 11; MUS81, methyl methanesulfonate and ultraviolet-sensitive protein 81; RPA, replication protein A; SAMHD1, sterile alpha motif and HD-domain containing protein 1; STING, stimulator of interferon genes; TREX1, three prime repair exonuclease 1.

cell-autonomous activation of the type I IFN response can be seen as an extension of the RSR that signals the presence of abnormal cells to the microenvironment in order to promote their elimination by the innate immune system. This view is supported by a recent report showing that cytosolic DNA fragments are generated by the MUS81 resolvase in prostate cancer cells and contribute to their rejection by the immune system in a STING pathway–dependent manner (80).

CONCLUSION AND PERSPECTIVES

In conclusion, the picture that emerges from recent studies is that nucleases are central players of the RSR. They contribute to the rapid signaling of arrested forks through the production of RPA-coated ssDNA and promote fork restart through the cleavage of stalled or reversed forks. They are also involved in the resolution of branched structures formed during HR-mediated fork repair and in the conversion of mutagenic D-loop structures formed during BIR into proper replication forks. However, playing with knives and scissors near arrested forks is a dangerous game, and a tight control by checkpoint kinases and HR factors is essential for maintaining genome integrity during DNA replication. In cells mutated for these factors, unrestrained nucleolytic degradation leads to increased genomic instability and cancer development. More unexpectedly,

fork processing also contributes to the accumulation of cytosolic DNA fragments that activate IFNs and proinflammatory cytokines, connecting the RSR to innate immunity. Further work is needed to decipher the complex interplay between all of these factors, but a better understanding of these processes will undoubtedly contribute to the development of novel therapeutic strategies against chronic inflammation, cancer, and aging.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Abbas T, Keaton MA, Dutta A. 2013. Genomic instability in cancer. *Cold Spring Harb. Perspect. Biol.* 5:a012914
2. Abe T, Harashima A, Xia T, Konno H, Konno K, et al. 2013. STING recognition of cytoplasmic DNA instigates cellular defense. *Mol. Cell* 50:5–15
3. Ablasser A, Hemmerling I, Schmid-Burgk JL, Behrendt R, Roers A, Hornung V. 2014. TREX1 deficiency triggers cell-autonomous immunity in a cGAS-dependent manner. *J. Immunol.* 192:5993–97
4. Aguilera A, García-Muse T. 2013. Causes of genome instability. *Annu. Rev. Genet.* 47:1–32
5. Ahn JS, Osman F, Whitby MC. 2005. Replication fork blockage by *RTS1* at an ectopic site promotes recombination in fission yeast. *EMBO J.* 24:2011–23
6. Ait Saada A, Teixeira-Silva A, Iraqui I, Costes A, Hardy J, et al. 2017. Unprotected replication forks are converted into mitotic sister chromatid bridges. *Mol. Cell* 66:398–410.e4
7. Alabert C, Bianco JN, Pasero P. 2009. Differential regulation of homologous recombination at DNA breaks and replication forks by the Mrc1 branch of the S-phase checkpoint. *EMBO J.* 28:1131–41
8. Anand R, Ranjha L, Cannavo E, Cejka P. 2016. Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. *Mol. Cell* 64:940–50
9. Anand RP, Lovett ST, Haber JE. 2013. Break-induced DNA replication. *Cold Spring Harb. Perspect. Biol.* 5:a010397
10. Atkinson J, McGlynn P. 2009. Replication fork reversal and the maintenance of genome stability. *Nucleic Acids Res.* 37:3475–92
11. Bae S-H, Bae K-H, Kim J-A, Seo Y-S. 2001. RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. *Nature* 412:456–61
12. Balakrishnan L, Bambara RA. 2013. Flap endonuclease 1. *Annu. Rev. Biochem.* 82:119–38
13. Ballana E, Este JA. 2015. SAMHD1: at the crossroads of cell proliferation, immune responses, and virus restriction. *Trends Microbiol.* 23:680–92
14. Barber GN. 2015. STING: infection, inflammation and cancer. *Nat. Rev. Immunol.* 15:760–70
15. Barlow JH, Rothstein R. 2009. Rad52 recruitment is DNA replication independent and regulated by Cdc28 and the Mec1 kinase. *EMBO J.* 28:1121–30
16. Bass TE, Luzwick JW, Kavanaugh G, Carroll C, Dungrawala H, et al. 2016. ETAA1 acts at stalled replication forks to maintain genome integrity. *Nat. Cell Biol.* 18:1185–95

17. Beloglazova N, Flick R, Tchigvintsev A, Brown G, Popovic A, et al. 2013. Nuclease activity of the human SAMHD1 protein implicated in the Aicardi-Goutières Syndrome and HIV-1 restriction. *J. Biol. Chem.* 288:8101–10
18. Berti M, Ray Chaudhuri A, Thangavel S, Gomathinayagam S, Kenig S, et al. 2013. Human RECQ1 promotes restart of replication forks reversed by DNA topoisomerase I inhibition. *Nat. Struct. Mol. Biol.* 20:347–54
19. Berti M, Vindigni A. 2016. Replication stress: getting back on track. *Nat. Struct. Mol. Biol.* 23:103–9
20. Betous R, Couch FB, Mason AC, Eichman BF, Manosas M, Cortez D. 2013. Substrate-selective repair and restart of replication forks by DNA translocases. *Cell Rep.* 3:1958–69
21. Bianchi J, Rudd SG, Jozwiakowski SK, Bailey LJ, Soura V, et al. 2013. PrimPol bypasses UV photoproducts during eukaryotic chromosomal DNA replication. *Mol. Cell* 52:566–73
22. Bizard AH, Hickson ID. 2014. The dissolution of double Holliday junctions. *Cold Spring Harb. Perspect. Biol.* 6:a016477
23. Blastyák A, Hajdú I, Unk I, Haracska L. 2010. Role of double-stranded DNA translocase activity of human HLTF in replication of damaged DNA. *Mol. Cell. Biol.* 30:684–93
24. Blastyák A, Pintér L, Unk I, Prakash L, Prakash S, Haracska L. 2007. Yeast Rad5 protein required for postreplication repair has a DNA helicase activity specific for replication fork regression. *Mol. Cell* 28:167–75
25. Bleichert F, Botchan MR, Berger JM. 2017. Mechanisms for initiating cellular DNA replication. *Science* 355:eaah6317
26. Boddy MN, Lopez-Girona A, Shanahan P, Interthal H, Heyer WD, Russell P. 2000. Damage tolerance protein Mus81 associates with the FHA1 domain of checkpoint kinase Cds1. *Mol. Cell. Biol.* 20:8758–66
27. Branzei D, Psakhye I. 2016. DNA damage tolerance. *Curr. Opin. Cell Biol.* 40:137–44
28. Bugreev DV, Rossi MJ, Mazin AV. 2011. Cooperation of RAD51 and RAD54 in regression of a model replication fork. *Nucleic Acids Res.* 39:2153–64
29. Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. 2005. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev.* 19:1040–52
30. Cannavo E, Cejka P, Kowalczykowski SC. 2013. Relationship of DNA degradation by *Saccharomyces cerevisiae* Exonuclease 1 and its stimulation by RPA and Mre11-Rad50-Xrs2 to DNA end resection. *PNAS* 110:E1661–68
31. Cejka P, Cannavo E, Polaczek P, Masuda-Sasa T, Pokharel S, et al. 2010. DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. *Nature* 467:112–16
32. Chanut P, Britton S, Coates J, Jackson SP, Calsou P. 2016. Coordinated nuclease activities counteract Ku at single-ended DNA double-strand breaks. *Nat. Commun.* 7:12889
33. Chaudhury I, Stroik DR, Sobeck A. 2014. FANCD2-controlled chromatin access of the Fanconi-associated nuclease FAN1 is crucial for the recovery of stalled replication forks. *Mol. Cell. Biol.* 34:3939–54
34. Cheng IC, Chen BC, Shuai HH, Chien FC, Chen P, Hsieh TS. 2016. Wuho is a new member in maintaining genome stability through its interaction with flap endonuclease 1. *PLOS Biol.* 14:e1002349
35. Ciccia A, Nimonkar Amitabh V, Hu Y, Hajdu I, Achar Yathish J, et al. 2012. Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress. *Mol. Cell* 47:396–409
36. Cimprich KA, Cortez D. 2008. ATR: an essential regulator of genome integrity. *Nat. Rev. Mol. Cell Biol.* 9:616–27
37. Cobb JA, Schleker T, Rojas V, Bjergbaek L, Tercero JA, Gasser SM. 2005. Replisome instability, fork collapse, and gross chromosomal rearrangements arise synergistically from Mec1 kinase and RecQ helicase mutations. *Genes Dev.* 19:3055–69
38. Colosio A, Frattini C, Pellicanò G, Villa-Hernández S, Bermejo R. 2016. Nucleolytic processing of aberrant replication intermediates by an Exo1-Dna2-Sae2 axis counteracts fork collapse-driven chromosome instability. *Nucleic Acids Res.* 44:10676–90
39. Cortez D. 2015. Preventing replication fork collapse to maintain genome integrity. *DNA Repair* 32:149–57

40. Costantino L, Sotiriou SK, Rantala JK, Magin S, Mladenov E, et al. 2014. Break-induced replication repair of damaged forks induces genomic duplications in human cells. *Science* 343:88–91
41. Costanzo V. 2011. Brca2, Rad51 and Mre11: performing balancing acts on replication forks. *DNA Repair* 10:1060–65
42. Costanzo V, Robertson K, Bibikova M, Kim E, Grieco D, et al. 2001. Mre11 protein complex prevents double-strand break accumulation during chromosomal DNA replication. *Mol. Cell* 8:137–47
43. Cotta-Ramusino C, Fachinetti D, Lucca C, Doksani Y, Lopes M, et al. 2005. Exo1 processes stalled replication forks and counteracts fork reversal in checkpoint-defective cells. *Mol. Cell* 17:153–59
44. Couch FB, Bansbach CE, Driscoll R, Luzwick JW, Glick GG, et al. 2013. ATR phosphorylates SMARCAL1 to prevent replication fork collapse. *Genes Dev*. 27:1610–23
45. Coverley D, Laman H, Laskey RA. 2002. Distinct roles for cyclins E and A during DNA replication complex assembly and activation. *Nat. Cell Biol.* 4:523–28
46. Crow YJ, Manel N. 2015. Aicardi-Goutières syndrome and the type I interferonopathies. *Nat. Rev. Immunol.* 15:429–40
47. Daddacha W, Koyen AE, Bastien AJ, Head PS, Dhere VR, et al. 2017. SAMHD1 promotes DNA end resection to facilitate DNA repair by homologous recombination. *Cell Rep*. 20:1921–35
48. Daigaku Y, Davies AA, Ulrich HD. 2010. Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature* 465:951–55
49. De Piccoli G Katou Y, Itoh T, Nakato R, Shirahige K, Labib K. 2012. Replisome stability at defective DNA replication forks is independent of S phase checkpoint kinases. *Mol. Cell* 45:696–704
50. Dehe PM, Gaillard PH. 2017. Control of structure-specific endonucleases to maintain genome stability. *Nat. Rev. Mol. Cell Biol.* 18:315–30
51. Dilley RL, Verma P, Cho NW, Winters HD, Wondisford AR, Greenberg RA. 2016. Break-induced telomere synthesis underlies alternative telomere maintenance. *Nature* 539:54–58
52. Ding X, Ray Chaudhuri A, Callen E, Pang Y, Biswas K, et al. 2016. Synthetic viability by BRCA2 and PARP1/ARTD1 deficiencies. *Nat. Commun.* 7:12425
53. Donnianni RA, Symington LS. 2013. Break-induced replication occurs by conservative DNA synthesis. *PNAS* 110:13475–80
54. Duda H, Arter M, Gloggnitzer J, Teloni F, Wild P, et al. 2016. A mechanism for controlled breakage of under-replicated chromosomes during mitosis. *Dev. Cell* 39:740–55
55. Dungrawala H, Rose KL, Bhat KP, Mohni KN, Glick GG, et al. 2015. The replication checkpoint prevents two types of fork collapse without regulating replisome stability. *Mol. Cell* 59:998–1010
56. Elvers I, Johansson F, Groth P, Erixon K, Helleday T. 2011. UV stalled replication forks restart by re-priming in human fibroblasts. *Nucleic Acids Res*. 39:7049–57
57. Erdal E, Haider S, Rehwinkel J, Harris AL, McHugh PJ. 2017. A prosurvival DNA damage-induced cytoplasmic interferon response is mediated by end resection factors and is limited by Trex1. *Genes Dev*. 31:353–69
58. Evrin C, Clarke P, Zech J, Lurz R, Sun J, et al. 2009. A double-hexameric MCM2–7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication. *PNAS* 106:20240–45
59. Feng S, Zhao Y, Xu Y, Ning S, Huo W, et al. 2016. Ewing tumor-associated antigen 1 interacts with replication protein A to promote restart of stalled replication forks. *J. Biol. Chem.* 291:21956–62
60. Froget B, Blaisonneau J, Lambert S, Baldacci G. 2008. Cleavage of stalled forks by fission yeast Mus81/Eme1 in absence of DNA replication checkpoint. *Mol. Biol. Cell* 19:445–56
61. Fu H, Martin MM, Regairaz M, Huang L, You Y, et al. 2015. The DNA repair endonuclease Mus81 facilitates fast DNA replication in the absence of exogenous damage. *Nat. Commun.* 6:6746
62. Gambus A, Jones RC, Sanchez-Diaz A, Kanemaki M, van Deursen F, et al. 2006. GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat. Cell Biol.* 8:358–66
63. Garcia-Gómez S, Reyes A, Martinez-Jiménez MI, Chocrón ES, Mourón S, et al. 2013. PrimPol, an archaic primase/polymerase operating in human cells. *Mol. Cell* 52:541–53
64. Gari K, Décaillet C, Delannoy M, Wu L, Constantinou A. 2008. Remodeling of DNA replication structures by the branch point translocase FANCM. *PNAS* 105:16107–12

65. Garner E, Kim Y, Lach FP, Kottemann MC, Smogorzewska A. 2013. Human GEN1 and the SLX4-associated nucleases MUS81 and SLX1 are essential for the resolution of replication-induced Holliday junctions. *Cell Rep*. 5:207–15
66. Gasser S, Zhang WYL, Tan NYJ, Tripathi S, Suter MA, et al. 2017. Sensing of dangerous DNA. *Mech. Ageing Dev.* 165:33–46
67. Ge XQ, Blow JJ. 2010. Chk1 inhibits replication factory activation but allows dormant origin firing in existing factories. *J. Cell Biol.* 191:1285–97
68. Ghosal G, Chen J. 2013. DNA damage tolerance: a double-edged sword guarding the genome. *Transl. Cancer Res.* 2:107–29
69. González-Prieto R, Muñoz-Cabello AM, Cabello-Lobato MJ, Prado F. 2013. Rad51 replication fork recruitment is required for DNA damage tolerance. *EMBO J.* 32:1307–21
70. Gravel S, Chapman JR, Magill C, Jackson SP. 2008. DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev*. 22:2767–72
71. Guillemette S, Serra RW, Peng M, Hayes JA, Konstantinopoulos PA, et al. 2015. Resistance to therapy in *BRCA2* mutant cells due to loss of the nucleosome remodeling factor CHD4. *Genes Dev*. 29:489–94
72. Haahr P, Hoffmann S, Tollenaere MA, Ho T, Toledo LI, et al. 2016. Activation of the ATR kinase by the RPA-binding protein ETAA1. *Nat. Cell Biol.* 18:1196–207
73. Halazonetis TD, Gorgoulis VG, Bartek J. 2008. An oncogene-induced DNA damage model for cancer development. *Science* 319:1352–55
74. Hanada K, Budzowska M, Davies SL, van Drunen E, Onizawa H, et al. 2007. The structure-specific endonuclease Mus81 contributes to replication restart by generating double-strand DNA breaks. *Nat. Struct. Mol. Biol.* 14:1096–104
75. Härtlova A, Erttmann SF, Raffi FAM, Schmalz AM, Resch U, et al. 2015. DNA damage primes the type I interferon system via the cytosolic DNA sensor STING to promote anti-microbial innate immunity. *Immunity* 42:332–43
76. Hashimoto Y, Ray Chaudhuri A, Lopes M, Costanzo V. 2010. Rad51 protects nascent DNA from Mre11-dependent degradation and promotes continuous DNA synthesis. *Nat. Struct. Mol. Biol.* 17:1305–11
77. Hashimoto Y, Puddu F, Costanzo V. 2012. RAD51- and MRE11-dependent reassembly of uncoupled CMG helicase complex at collapsed replication forks. *Nat. Struct. Mol. Biol.* 19:17–24
78. Heller RC, Marians KJ. 2006. Replication fork reactivation downstream of a blocked nascent leading strand. *Nature* 439:557–62
79. Higgs MR, Reynolds JJ, Winczura A, Blackford AN, Borel V, et al. 2015. BOD1L is required to suppress deleterious resection of stressed replication forks. *Mol. Cell* 59:462–77
80. Ho SSW, Zhang WYL, Tan NYJ, Khatoo M, Suter MA, et al. 2016. The DNA structure-specific endonuclease MUS81 mediates DNA sensor STING-dependent host rejection of prostate cancer cells. *Immunity* 44:1177–89
81. Huang J, Liu S, Bellani MA, Thazhathveetil AK, Ling C, et al. 2013. The DNA translocase FANCM/MHF promotes replication traverse of DNA interstrand crosslinks. *Mol. Cell* 52:434–46
82. Ilves I, Petojevic T, Pesavento JJ, Botchan MR. 2010. Activation of the MCM2–7 helicase by association with Cdc45 and GINS proteins. *Mol. Cell* 37:247–58
83. Jazayeri A, Balestrini A, Garner E, Haber JE, Costanzo V. 2008. Mre11–Rad50–Nbs1-dependent processing of DNA breaks generates oligonucleotides that stimulate ATM activity. *EMBO J.* 27:1953–62
84. Kai M, Boddy MN, Russell P, Wang TS. 2005. Replication checkpoint kinase Cds1 regulates Mus81 to preserve genome integrity during replication stress. *Genes Dev*. 19:919–32
85. Kais Z, Rondinelli B, Holmes A, O'Leary C, Kozono D, et al. 2016. FANCD2 maintains fork stability in BRCA1/2-deficient tumors and promotes alternative end-joining DNA repair. *Cell Rep*. 15:2488–99
86. Karras GI, Jentsch S. 2010. The *RAD6* DNA damage tolerance pathway operates uncoupled from the replication fork and is functional beyond S phase. *Cell* 141:255–67
87. Kile AC, Chavez DA, Bacal J, Eldirany S, Korzhnev DM, et al. 2015. HLTF's ancient HIRAN domain binds 3′ DNA ends to drive replication fork reversal. *Mol. Cell* 58:1090–100
88. Kim H-S, Nickoloff JA, Wu Y, Williamson EA, Sidhu GS, et al. 2017. Endonuclease EEPD1 is a gatekeeper for repair of stressed replication forks. *J. Biol. Chem.* 292:2795–804

89. Kim H-S, Williamson EA, Nickoloff JA, Hromas RA, Lee S-H. 2017. Metnase mediates loading of exonuclease 1 onto single-strand overhang DNA for end resection at stalled replication forks. *J. Biol. Chem.* 292:1414–25
90. Kolinjivadi AM, Sannino V, De Antoni A Zadorozhny K, Kikenny M, et al. 2017. Smarcal1-mediated fork reversal triggers Mre11-dependent degradation of nascent DNA in the absence of Brca2 and Stable Rad51 nucleofilaments. *Mol. Cell.* 67:867–81.e7
91. Labib K, De Piccoli G. 2011. Surviving chromosome replication: the many roles of the S-phase checkpoint pathway. *Philos. Trans. R. Soc. B* 366:3554–61
92. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, et al. 2011. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474:654–57
93. Lambert S, Mizuno K, Blaisonneau J, Martineau S, Chanet R, et al. 2010. Homologous recombination restarts blocked replication forks at the expense of genome rearrangements by template exchange. *Mol. Cell* 39:346–59
94. Langerak P, Mejia-Ramirez E, Limbo O, Russell P. 2011. Release of Ku and MRN from DNA ends by Mre11 nuclease activity and Ctp1 is required for homologous recombination repair of double-strand breaks. *PLOS Genet.* 7:e1002271
95. Lee Y-C, Zhou Q, Chen J, Yuan J. 2016. RPA-binding protein ETAA1 is an ATR activator involved in DNA replication stress response. *Curr. Biol.* 26:3257–68
96. Lemaçon D, Jackson J, Quinet A, Brickner JR, Li S, et al. 2017. MRE11 and EXO1 nucleases degrade reversed forks and elicit MUS81-dependent fork rescue in BRCA2-deficient cells. *Nat. Commun.* 8:860
97. Lopes M, Foiani M, Sogo JM. 2006. Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Mol. Cell* 21:15–27
98. Lopez-Contreras AJ, Fernandez-Capetillo O. 2010. The ATR barrier to replication-born DNA damage. *DNA Repair* 9:1249–55
99. Lossaint G, Larroque M, Ribeyre C, Bec N, Larroque C, et al. 2013. FANCD2 binds MCM proteins and controls replisome function upon activation of S phase checkpoint signaling. *Mol. Cell* 51:678–90
100. Luke-Glaser S, Luke B, Grossi S, Constantinou A. 2010. FANCM regulates DNA chain elongation and is stabilized by S-phase checkpoint signalling. *EMBO J.* 29:795–805
101. MacDougall CA, Byun TS, Van C, Yee M-c, Cimprich KA. 2007. The structural determinants of checkpoint activation. *Genes Dev.* 21:898–903
102. Macheret M, Halazonetis TD. 2015. DNA replication stress as a hallmark of cancer. *Annu. Rev. Pathol. Mech. Dis.* 10:425–48
103. Mackenzie KJ, Carroll P, Lettice L, Tarnauskaitė Ž, Reddy K, et al. 2016. Ribonuclease H2 mutations induce a cGAS/STING-dependent innate immune response. *EMBO J.* 35:831–44
104. Mailand N, Gibbs-Seymour I, Bekker-Jensen S. 2013. Regulation of PCNA–protein interactions for genome stability. *Nat. Rev. Mol. Cell Biol.* 14:269–82
105. Mayle R, Campbell IM, Beck CR, Yu Y, Wilson M, et al. 2015. Mus81 and converging forks limit the mutagenicity of replication fork breakage. *Science* 349:742–47
106. Meng X, Zhao X. 2017. Replication fork regression and its regulation. *FEMS Yeast Res.* 17:fow110
107. Michl J, Zimmer J, Buffa FM, McDermott U, Tarsounas M. 2016. FANCD2 limits replication stress and genome instability in cells lacking BRCA2. *Nat. Struct. Mol. Biol.* 23:755–57
108. Mijic S, Zellweger R, Chappidi N, Berti M, Jacobs K, et al. 2017. Replication fork reversal triggers fork degradation in *BRCA2*-defective cells. *Nat. Commun.* 8:859
109. Mimitou EP, Symington LS. 2008. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455:770–74
110. Minocherhomji S, Ying S, Bjerregaard VA, Bursomanno S, Aleliunaite A, et al. 2015. Replication stress activates DNA repair synthesis in mitosis. *Nature* 528:286–90
111. Morafraile EC, Diffley JF, Tercero JA, Segurado M. 2015. Checkpoint-dependent RNR induction promotes fork restart after replicative stress. *Sci. Rep.* 5:7886
112. Morin I, Ngo H-P, Greenall A, Zubko MK, Morrice N, Lydall D. 2008. Checkpoint-dependent phosphorylation of Exo1 modulates the DNA damage response. *EMBO J.* 27:2400–10

113. Mourón S, Rodriguez-Acebes S, Martinez-Jiménez MI, Garcia-Gómez S, Chocrón S, et al. 2013. Repriming of DNA synthesis at stalled replication forks by human PrimPol. *Nat. Struct. Mol. Biol.* 20:1383–89
114. Muñoz-Galván S, Tous C, Blanco MG, Schwartz EK, Ehmsen KT, et al. 2012. Distinct roles of Mus81, Yen1, Slx1-Slx4, and Rad1 nucleases in the repair of replication-born double-strand breaks by sister chromatid exchange. *Mol. Cell. Biol.* 32:1592–603
115. Naim V, Wilhelm T, Debatisse M, Rosselli F. 2013. ERCC1 and MUS81–EME1 promote sister chromatid separation by processing late replication intermediates at common fragile sites during mitosis. *Nat. Cell Biol.* 15:1008–15
116. Nam EA, Cortez D. 2011. ATR signalling: more than meeting at the fork. *Biochem. J.* 436:527–36
117. Neelsen KJ, Lopes M. 2015. Replication fork reversal in eukaryotes: from dead end to dynamic response. *Nat. Rev. Mol. Cell Biol.* 16:207–20
118. Nguyen MO, Jalan M, Morrow CA, Osman F, Whitby MC. 2015. Recombination occurs within minutes of replication blockage by *RTS1* producing restarted forks that are prone to collapse. *eLife* 4:e04539
119. Nguyen VQ, Co C, Li JJ. 2001. Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature* 411:1068–73
120. Nicolette ML, Lee K, Guo Z, Rani M, Chow JM, et al. 2010. Mre11–Rad50–Xrs2 and Sae2 promote 5′ strand resection of DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 17:1478–85
121. Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, et al. 2011. BLM-DNA2–RPA–MRN and EXO1–BLM–RPA–MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev.* 25:350–62
122. Niu H, Chung WH, Zhu Z, Kwon Y, Zhao W, et al. 2010. Mechanism of the ATP-dependent DNA end-resection machinery from *S. cerevisiae*. *Nature* 467:108–11
123. Pacek M, Tutter AV, Kubota Y, Takisawa H, Walter JC. 2006. Localization of MCM2–7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication. *Mol. Cell* 21:581–87
124. Pacek M, Walter JC. 2004. A requirement for MCM7 and Cdc45 in chromosome unwinding during eukaryotic DNA replication. *EMBO J.* 23:3667–76
125. Pages V, Fuchs RP. 2003. Uncoupling of leading- and lagging-strand DNA replication during lesion bypass in vivo. *Science* 300:1300–3
126. Paludan SR. 2015. Activation and regulation of DNA-driven immune responses. *Microbiol. Mol. Biol. Rev.* 79:225–41
127. Pardo B, Crabbé L, Pasero P. 2017. Signaling pathways of replication stress in yeast. *FEMS Yeast Res.* 17:fow101
128. Pasero P, Braguglia D, Gasser SM. 1997. ORC-dependent and origin-specific initiation of DNA replication at defined foci in isolated yeast nuclei. *Genes Dev.* 11:1504–18
129. Patro BS, Frøhlich R, Bohr VA, Stevnsner T. 2011. WRN helicase regulates the ATR–CHK1-induced S-phase checkpoint pathway in response to topoisomerase-I–DNA covalent complexes. *J. Cell Sci.* 124:3967–79
130. Paulsen RD, Cimprich KA. 2007. The ATR pathway: fine-tuning the fork. *DNA Repair* 6:953–66
131. Pepe A, West SC. 2014. MUS81–EME2 promotes replication fork restart. *Cell Rep.* 7:1048–55
132. Pepe A, West SC. 2014. Substrate specificity of the MUS81-EME2 structure selective endonuclease. *Nucleic Acids Res.* 42:3833–45
133. Petermann E, Orta ML, Issaeva N, Schultz N, Helleday T. 2010. Hydroxyurea-stalled replication forks become progressively inactivated and require two different RAD51-mediated pathways for restart and repair. *Mol. Cell* 37:492–502
134. Ray Chaudhuri A, Callen E, Ding X, Gogola E, Duarte AA, et al. 2016. Replication fork stability confers chemoresistance in BRCA-deficient cells. *Nature* 535:382–87
135. Ray Chaudhuri A, Hashimoto Y, Herrador R, Neelsen KJ, Fachinetti D, et al. 2012. Topoisomerase I poisoning results in PARP-mediated replication fork reversal. *Nat. Struct. Mol. Biol.* 19:417–23
136. Regairaz M, Zhang YW, Fu H, Agama KK, Tata N, et al. 2011. Mus81-mediated DNA cleavage resolves replication forks stalled by topoisomerase I–DNA complexes. *J. Cell Biol.* 195:739–49
137. Remus D, Beuron F, Tolun G, Griffith JD, Morris EP, Diffley JF. 2009. Concerted loading of Mcm2–7 double hexamers around DNA during DNA replication origin licensing. *Cell* 139:719–30

138. Remus D, Blanchette M, Rio DC, Botchan MR. 2005. CDK phosphorylation inhibits the DNA-binding and ATP-hydrolysis activities of the *Drosophila* origin recognition complex. *J. Biol. Chem.* 280:39740–51
139. Saini N, Ramakrishnan S, Elango R, Ayyar S, Zhang Y, et al. 2013. Migrating bubble during break-induced replication drives conservative DNA synthesis. *Nature* 502:389–92
140. Sale JE, Lehmann AR, Woodgate R. 2012. Y-family DNA polymerases and their role in tolerance of cellular DNA damage. *Nat. Rev. Mol. Cell Biol.* 13:141–52
141. Sarbajna S, Davies D, West SC. 2014. Roles of SLX1–SLX4, MUS81–EME1, and GEN1 in avoiding genome instability and mitotic catastrophe. *Genes Dev.* 28:1124–36
142. Schlacher K, Christ N, Siaud N, Egashira A, Wu H, Jasin M. 2011. Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* 145:529–42
143. Schlacher K, Wu H, Jasin M. 2012. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell* 22:106–16
144. Seamon KJ, Sun Z, Shlyakhtenko LS, Lyubchenko YL, Stivers JT. 2015. SAMHD1 is a single-stranded nucleic acid binding protein with no active site-associated nuclease activity. *Nucleic Acids Res.* 43:6486–99
145. Segurado M, Diffley JFX. 2008. Separate roles for the DNA damage checkpoint protein kinases in stabilizing DNA replication forks. *Genes Dev.* 22:1816–27
146. Sharma S, Sommers JA, Gary RK, Friedrich-Heineken E, Hübscher U, Brosh RM Jr. 2005. The interaction site of Flap Endonuclease-1 with WRN helicase suggests a coordination of WRN and PCNA. *Nucleic Acids Res.* 33:6769–81
147. Shen YJ, Le Bert N, Chitre AA, Koo CX, Nga XH, et al. 2015. Genome-derived cytosolic DNA mediates type I interferon-dependent rejection of B cell lymphoma cells. *Cell Rep.* 11:460–73
148. Shimura T, Torres MJ, Martin MM, Rao VA, Pommier Y, et al. 2008. Bloom's syndrome helicase and Mus81 are required to induce transient double-strand DNA breaks in response to DNA replication stress. *J. Mol. Biol.* 375:1152–64
149. Sogo JM, Lopes M, Foiani M. 2002. Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. *Science* 297:599–602
150. Sotiriou SK, Kamileri I, Lugli N, Evangelou K, Da-Re C, et al. 2016. Mammalian RAD52 functions in break-induced replication repair of collapsed DNA replication forks. *Mol. Cell* 64:1127–34
151. Spies J, Waizenegger A, Barton O, Sürder M, Wright WD, et al. 2016. Nek1 regulates Rad54 to orchestrate homologous recombination and replication fork stability. *Mol. Cell* 62:903–17
152. Stracker TH, Petrini JH. 2011. The MRE11 complex: starting from the ends. *Nat. Rev. Mol. Cell Biol.* 12:90–103
153. Sturzenegger A, Burdova K, Kanagaraj R, Levikova M, Pinto C, et al. 2014. DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA end resection in human cells. *J. Biol. Chem.* 289:27314–26
153a. Taglialatela A, Alvarez S, Leuzzi G, Sannino V, Ranjha L, et al. 2017. Restoration of replication fork stability and genome integrity in BRCA1- and BRCA2-deficient cells by inactivation of SNF2-family fork remodelers. *Mol. Cell* 68:414–430.e8
154. Técher H, Koundrioukoff S, Carignon S, Wilhelm T, Millot GA, et al. 2016. Signaling from Mus81-Eme2-dependent DNA damage elicited by Chk1 deficiency modulates replication fork speed and origin usage. *Cell Rep.* 14:1114–27
155. Tercero JA, Diffley JF. 2001. Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint. *Nature* 412:553–57
156. Thangavel S, Berti M, Levikova M, Pinto C, Gomathinayagam S, et al. 2015. DNA2 drives processing and restart of reversed replication forks in human cells. *J. Cell Biol.* 208:545–62
157. Toledo LI, Altmeyer M, Rask M-B, Lukas C, Larsen DH, et al. 2013. ATR prohibits replication catastrophe by preventing global exhaustion of RPA. *Cell* 155:1088–103
158. Tsang E, Miyabe I, Iraqui I, Zheng J, Lambert SAE, Carr AM. 2014. The extent of error-prone replication restart by homologous recombination is controlled by Exo1 and checkpoint proteins. *J. Cell Sci.* 127:2983–94

159. Wang AT, Kim T, Wagner JE, Conti BA, Lach FP, et al. 2015. A dominant mutation in human RAD51 reveals its function in DNA interstrand crosslink repair independent of homologous recombination. *Mol. Cell* 59:478–90
160. Wolf C, Rapp A, Berndt N, Staroske W, Schuster M, et al. 2016. RPA and Rad51 constitute a cell intrinsic mechanism to protect the cytosol from self DNA. *Nat. Commun.* 7:11752
161. Wyatt HD, Sarbajna S, Matos J, West SC. 2013. Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. *Mol. Cell* 52:234–47
162. Xing M, Wang X, Palmai-Pallag T, Shen H, Helleday T, et al. 2015. Acute MUS81 depletion leads to replication fork slowing and a constitutive DNA damage response. *Oncotarget* 6:37638–46
163. Yang Y-G, Lindahl T, Barnes DE. 2007. Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. *Cell* 131:873–86
164. Yao NY, O'Donnell M. 2009. Replisome structure and conformational dynamics underlie fork progression past obstacles. *Curr. Opin. Cell Biol.* 21:336–43
165. Yeeles JT, Poli J, Marians KJ, Pasero P. 2013. Rescuing stalled or damaged replication forks. *Cold Spring Harb. Perspect. Biol.* 5:a012815
166. Yeo JE, Lee EH, Hendrickson EA, Sobeck A. 2014. CtIP mediates replication fork recovery in a FANCD2-regulated manner. *Hum. Mol. Genet.* 23:3695–705
167. Ying S, Hamdy FC, Helleday T. 2012. Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1. *Cancer Res.* 72:2814–21
168. Yuan J, Ghosal G, Chen J. 2012. The HARP-like domain-containing protein AH2/ZRANB3 binds to PCNA and participates in cellular response to replication stress. *Mol. Cell* 47:410–21
169. Zellweger R, Dalcher D, Mutreja K, Berti M, Schmid JA, et al. 2015. Rad51-mediated replication fork reversal is a global response to genotoxic treatments in human cells. *J. Cell Biol.* 208:563–79
170. Zeman MK, Cimprich KA. 2014. Causes and consequences of replication stress. *Nat. Cell Biol.* 16:2–9
171. Zhang R, Sengupta S, Yang Q, Linke SP, Yanaihara N, et al. 2005. BLM helicase facilitates Mus81 endonuclease activity in human cells. *Cancer Res.* 65:2526–31
172. Zheng L, Zhou M, Chai Q, Parrish J, Xue D, et al. 2005. Novel function of the flap endonuclease 1 complex in processing stalled DNA replication forks. *EMBO Rep.* 6:83–89
173. Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. 2008. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134:981–94
174. Zou L, Elledge SJ. 2003. Sensing DNA damage through ATRIP recognition of RPA–ssDNA complexes. *Science* 300:1542–48