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DNA End Resection: Mechanism and Control

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Abstract

DNA double-strand breaks (DSBs) are cytotoxic lesions that threaten genome integrity and cell viability. Typically, cells repair DSBs by either nonhomologous end joining (NHEJ) or homologous recombination (HR). The relative use of these two pathways depends on many factors, including cell cycle stage and the nature of the DNA ends. A critical determinant of repair pathway selection is the initiation of 5′→3′ nucleolytic degradation of DNA ends, a process referred to as DNA end resection. End resection is essential to create single-stranded DNA overhangs, which serve as the substrate for the Rad51 recombinase to initiate HR and are refractory to NHEJ repair. Here, we review recent insights into the mechanisms of end resection, how it is regulated, and the pathological consequences of its dysregulation.

INTRODUCTION

DNA double-strand breaks (DSBs) form when both strands of the DNA duplex are severed. DSBs can result from exposure of cells to exogenous agents, such as ionizing radiation (IR) or some chemotherapeutic drugs, and can arise spontaneously under conditions of DNA replication stress. Despite their potential toxicity, DSBs can be programmed by cells and are essential intermediates during meiosis to ensure accurate pairing and segregation of chromosome homologs (14). Meiotic recombination also contributes to the genetic diversity of germ cells. In addition, adaptive immunity relies on programmed DSBs to increase the diversity of antibodies and T cell receptors and to switch antibody isotypes (14). Failure to repair DSBs results in loss of genetic information, whereas inappropriate repair can cause mutations or chromosome rearrangements. Genomic instability stemming from defective DSB repair contributes to a number of human pathologies, including immunodeficiency, neurodegeneration, infertility, premature aging, and cancer (42, 108).

DSBs are repaired by one of two main mechanisms: nonhomologous end joining (NHEJ) or homologous recombination (HR) (128). NHEJ involves the direct ligation of DNA ends and is generally considered to be error prone because gain or loss of sequence can occur prior to the ligation of ends. By contrast, HR tends to be more accurate because a homologous DNA sequence, generally the identical sister chromatid, is used as a repair template. The relative use of these two pathways depends on many factors, including the cell cycle phase and nature of the DNA ends. The 5′→3′ nucleolytic degradation of DNA ends, a process referred to as DNA end resection, plays a pivotal role in dictating which mechanism is used to repair DSBs (151). DNA end resection is essential to create 3′ single-stranded DNA (ssDNA) overhangs, which serve as the substrate for the HR machinery (**Figure 1**). The resected tracts are initially bound by the ssDNA-binding protein, replication protein A (RPA), which is then replaced by Rad51 (and Dmc1 in meiotic cells)

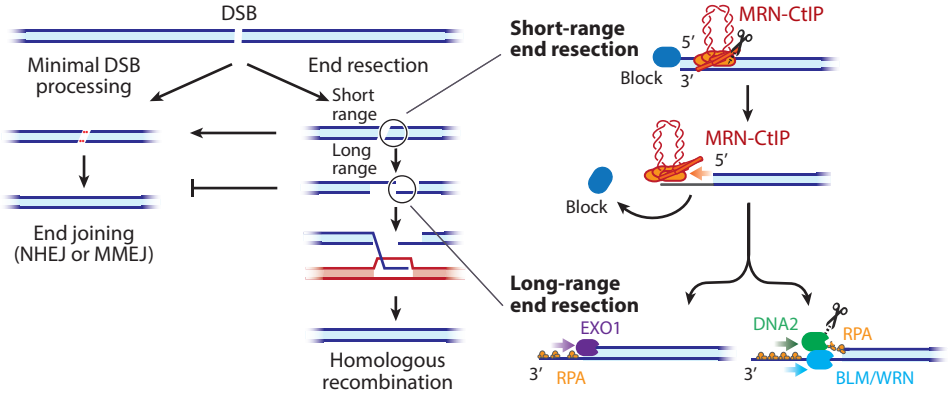

Figure 1

Schematic of the two main pathways, NHEJ and HR, used to repair DSBs. DNA end resection, which is essential for HR, occurs by a two-step mechanism. In the first step, the MRN/X complex and its cofactor CtIP/Sae2 nick the 5′-terminated strand internal to the end. The nick is an entry site for Mre11 nuclease to degrade the 3′ strand back to the break end, dislodging bound proteins or other blocks, while more extensive resection of the 5′-terminated strands is catalyzed by EXO1 or DNA2 (in conjunction with BLM, WRN, or the budding yeast BLM homolog Sgs1). Abbreviations: BLM, Bloom; CtIP, C-terminal binding protein 1 interacting protein; DNA2, DNA synthesis defective 2; DSB, DNA double-strand break; EXO1, exonuclease 1; HR, homologous recombination; MMEJ, microhomology-mediated end joining; Mre11, meiotic recombination 11; MRN/X complex, MRE11-RAD50-NBS1/Xrs2; NHEJ, nonhomologous end joining; RPA, replication protein A; Sae2, sporulation in the absence of Spo eleven 2; Sgs1, slow growth suppressor 1; WRN, Werner. Figure adapted from Reference 128 with permission.

in a reaction facilitated by mediator proteins (128). The Rad51 nucleoprotein filament initiates pairing and strand exchange with a homologous donor sequence to form a displacement loop (D-loop). The 3′ invading end within the D-loop can then be extended by DNA polymerases to replace sequences lost at the break site and by end resection. At the same time, DNA ends with long ssDNA overhangs are poor substrates for repair by NHEJ. Thus, end resection commits cells to repair by HR and limits the use of NHEJ. Whereas unprocessed DSBs are generally sensed by ATM/Tel1 (hereafter we use the notation human/yeast), the ssDNA generated by end resection is an essential intermediate for activation of the ATR/Mec1-dependent DNA damage checkpoint (145, 174).

DNA end resection has been most extensively studied in the context of two-ended DSBs generated by IR; endonucleases; stalled type II topoisomerases; or Spo11, the topoisomerase-like protein that initiates meiotic recombination. End resection also functions at one-ended DSBs, such as those formed at broken or reversed replication forks and at telomeres, the natural ends of linear chromosomes. The current view of end resection is a two-step mechanism, in which the MRE11-RAD50-NBS1/Xrs2 (MRN/X) complex together with its cofactor CtIP/Sae2 catalyzes the initial processing of DNA ends (i.e., short-range resection), and, in a second step, one of the long-range resection nucleases, EXO1 or DNA2, extends the resected tracts, the latter in conjunction with a RecQ-family helicase: Bloom (BLM; called Sgs1 in budding yeast) or Werner (WRN). Here, we review recent studies on the mechanism of DNA end resection, how it is regulated, and the pathological consequences of uncontrolled end resection.

MECHANISM OF DNA END RESECTION

The end resection machineries must process DSBs with the right polarity, targeting DNA degradation to the 5′-terminated DNA strand, while protecting the 3′ end to prevent loss of genetic information. Resection needs to be highly efficient and at the same time flexible to enable processing of DNA ends with noncanonical structures such as protein adducts or secondary DNA structures. Finally, resection has a built-in control system that allows a cell cycle stage-specific activation only in the S and G2 phases, when sister chromatids are available as templates for HR-based repair. A two-step resection mechanism, consisting of short-range and long-range resection pathways, has evolved to fulfill all of these modalities (**Figure 1**). Whereas short-range resection is slow and generally limited to the vicinity of the break end, it is very versatile and capable of processing DNA ends with noncanonical structures. As the initiating and committing step, short-range resection is under a strict cell cycle stage control. Long-range resection is instead fast but generally inefficient in processing DNA ends with large adducts. The combination of the distinct resection mechanisms guarantees optimal processing of DSBs in most conditions.

Short-Range DNA End Resection by the MRN/X Complex and CtIP/Sae2

The key nuclease responsible for short-range DNA end resection is MRE11, which forms the Mre11-Rad50-Xrs2 (MRX) complex in *Saccharomyces cerevisiae* and the MRE11-RAD50-NBS1 (MRN) complex in human cells. In budding yeast, the nuclease activity of Mre11 is essential only for processing DSBs with noncanonical structures such as DNA hairpins or protein adducts, including meiotic DNA breaks with covalently attached Spo11, and is largely dispensable for the processing of clean breaks (100). In contrast to HR in lower eukaryotes, short-range resection by the MRN complex is indispensable for HR in mammals, even for the repair of clean breaks (136, 143). The possible reason for this difference is discussed at the end of this section. In the absence of long-range resection in yeast, the short-range pathway can resect DNA up to

~300–400 nucleotides (nt) away from the end (102, 179, 183), similar to the estimated length of short-range resection in mammalian cells (121).

MRE11 is a globular protein that binds RAD50, forming a heterotetramer (M_2R_2). Adjacent to MRE11, the N- and C-terminal regions of RAD50 together form a globular ATPase domain at the base of the complex, from which protrudes a long coiled coil culminating with a zinc hook at the apex of the structure (73, 168). The zinc hook is a dimerization module that is necessary for all activities of the MRN/X complex; likewise, the coiled coil is essential, although mutations with a less severe phenotype have also been described (71, 72, 154). NBS1/Xrs2 has no intrinsic enzymatic activity per se, likely forming the $M_2R_2N_2/X_2$ complex (167).

MRE11 in isolation is a manganese-dependent exonuclease that degrades ssDNA strands with a 3′→5′ polarity within double-stranded DNA (dsDNA) (123). This polarity of DNA degradation stands in contrast to the DSB repair model and direct observation in cells, which indicate that resection occurs in a 5′→3′ direction (100). This apparent nuclease polarity paradox can be explained by the action of MRE11 cofactors, which turn MRE11 into an endonuclease that preferentially cuts the 5′-terminated DNA strand (4, 22, 50). Single-molecule studies demonstrated that MRN/X can diffuse along DNA, even on nucleosome-coated DNA (110). Questions remain about the mechanism by which MRN/X identifies the end, in particular when it is bound and likely obscured by protein adducts. Once the end is identified, the endonuclease of MRN/X is engaged and nicks preferentially the 5′-terminated strand some distance away from the break end (22). Limited DNA cleavage of the 3′ strand was also observed in vitro, as well as cutting of the 3′ strand opposite to a nick on the 5′ strand (49). However, experiments with yeast cells suggest that 3′-strand degradation, which could result in a loss of genetic information, is minimal (35, 148, 183). In the case of a DNA end covalently bound by a protein, or containing a secondary DNA structure, the MRN/X endonuclease cuts the 5′-terminated DNA strand at sites internal from the block some distance away from the DSB end (132, 162). This cutting-from-a-flank mechanism explains the versatility of the complex, which is thus capable of bypassing even covalently bound obstacles at the DNA end and initiating recombination at DSBs with noncanonical structures.

Depending on the nature of the protein block, MRE11-dependent nicking sites closest to the DNA end were found ~15–45 nt away in vitro (4, 50, 132, 162), although DNA cleavage at positions up to ~300–400 nt from the end was also observed in cells (58). It remains to be clarified whether these more distant sites are cleaved in a stepwise fashion that proceeds gradually away from the DNA end or whether the MRN/X complex has a capacity to initiate cleavage at the more distant sites (25). The endonucleolytic cleavage sites then serve as entry points for the long-range resection nucleases acting downstream. At the same time, the 3′→5′ exonuclease activity of MRE11, presumably acting within the MRN/X complex, may proceed from these nicking sites in the opposite direction back toward the DNA end (**Figure 1**). The model in which the internal sites resulting from the endonucleolytic cleavage by the MRN/X complex serve as initiating points for both 5′→3′ degradation (EXO1 or DNA2) and 3′→5′ degradation (MRN/X exonuclease) is termed bidirectional resection (58, 143). The 3′→5′ exonucleolytic DNA degradation by MRN/X back toward the end, possibly coupled with DNA melting (26) and in some cases limited cleavage of the 3′ end (49), then helps release the protein obstacle from the DNA end, leading to a clean 3′ overhang required for the downstream steps in HR.

What are the functions of the MRE11 partners in short-range resection? RAD50 and its ATPase are necessary for the endonuclease activity. ATP hydrolysis is required, and structural studies have illustrated that the MRE11-RAD50 (MR) complex undergoes a notable conformational change upon ATP binding and hydrolysis (51, 79). In the ATP-bound state, RAD50 blocks the access of MRE11 to dsDNA and therefore prevents its nuclease activity. Upon ATP hydrolysis, a large conformational change leads to the relocation of RAD50, which makes the DNA

accessible, thus licensing endonucleolytic DNA cleavage by MRE11. Xrs2 is responsible for the nuclear entry of the complex and is consequently essential for all of its functions in vivo (159). Biochemical reconstitutions, where nuclear import is irrelevant, and in vivo studies with cells expressing mutant alleles engineered with the nuclear localization signal sequence artificially positioned on Mre11 instead of Xrs2 revealed that the third subunit of the complex functions differently in DNA end resection in lower versus higher eukaryotes. In yeast, Xrs2 is partially dispensable for resection, while, in contrast, mammalian NBS1 is much more important because its physical interaction with MRE11 is specifically required to activate the endonuclease activity (3, 4, 48, 81, 120).

A critical additional cofactor of the MRN/X complex is CtIP/Sae2/Ctp1, which enables cell-cycle-dependent regulation of the endonucleolytic activity upon its phosphorylation and, hence, initiation of DNA end resection and HR (74, 75, 136). Phosphorylation of CtIP/Sae2/Ctp1 is required for this regulatory action. The key phosphorylation events are catalyzed by cyclin-dependent kinases (CDKs) in the S/G2 phase of the cell cycle at Ser267 in Sae2 and Thr847 in CtIP, although other kinases, such as casein kinase II of *Schizosaccharomyces pombe*, are also involved (180). In human cells, DNA-damage-dependent CtIP phosphorylation by ATM is required for resection, while in yeast cells, phosphorylation of Sae2 by the ATM ortholog Tel1 is largely dispensable (24, 50, 102, 161, 175). In yeast, phosphorylated Sae2 interacts with Rad50 to activate the endonuclease of the MR/MRX complex (24), whereas phosphorylated CtIP is instead primarily sensed by NBS1 in human cells, which in turn enhances the endonuclease activity of the MRN complex (3, 49, 161). However, physical and functional interactions between CtIP and RAD50 are also likely involved (3).

Bacterial cells lack CtIP/Sae2/Ctp1 and NBS1/Xrs2 equivalents, but homologs of RAD50 and MRE11 (termed SbcCD in *Escherichia coli*) are present, and they show a remarkable degree of structural and functional similarity to their eukaryotic counterparts (43). A recent structural study identified a domain in the bacterial $SbcD^{Mre11}$ subunit, termed a fastener loop, which needs to interact with a surface on $SbcC^{Rad50}$ (79). Mutations in the fastener loop that disrupt this interaction impair the nuclease activity of the complex. Interestingly, the putative equivalent surface residues on *S. cerevisiae* Rad50 correspond to the positions of *rad50S* mutations (79). Yeast *rad50S* mutants are deficient in meiotic DSB processing, while the same mutations cause only limited impairment of mitotic HR (100). Biochemical studies demonstrated that the prototypical Rad50S mutant (K81I) fails to interact physically and functionally with phosphorylated Sae2 (24). Eukaryotic MRE11 proteins lack such a fastener loop. Possibly, that function is carried out by Sae2/CtIP (79), allowing cells to couple activation of the MRE11 endonuclease with cell-cycle-dependent regulation by phosphorylation.

In accord with the more stringent requirement for short-range resection and the MRE11 nuclease activity in mammalian cells compared to yeast, Sae2 is only necessary for the resection of blocked DNA ends such as occurs in meiosis (80, 92). In contrast, CtIP is needed for most DNA end resection events in human cells, including for nuclease-induced clean breaks (136). Resection in yeast cells defective for Mre11 nuclease or Sae2 is mainly the result of Sgs1-Dna2 activity because Exo1 is inhibited at DNA ends by the Ku70-Ku80 heterodimer (hereafter referred to as Ku), an essential NHEJ factor (101). Consequently, deletion of Ku rescues some phenotypes of *sae2*Δ or *mre11* nuclease-deficient mutants, while Ku overexpression has the opposite effect (101). Beyond processing meiotic Spo11 adducts, hairpin-capped DSBs, and aberrant topoisomerase-DNA cleavage complexes, short-range resection may have an important function in counteracting Ku. Ku is highly abundant in the nucleus and is recruited to DSBs within seconds. In cases when Ku-dependent NHEJ repair is not possible, resection proteins need to gain access to the Ku-bound DSBs to channel the repair away from NHEJ towards HR. Accordingly, biochemical and single-molecule studies demonstrated that MRX-Sae2 and MRN-CtIP ensembles efficiently

cleave DNA past Ku-blocked ends, suggesting that short-range resection may help to actively remove Ku from DSBs and thus channel repair to HR (30, 50, 110, 132, 162). The higher concentrations of Ku in human cells compared to yeast may contribute to the enhanced requirement for short-range resection in human cells. In support of this idea, resection of clean breaks in murine embryonic cells becomes independent of CtIP upon the deletion of Ku (96). More studies are needed to clarify the impact of Ku on short-range resection and to ascertain whether there are additional reasons for the more stringent requirement for short-range resection in mammalian HR.

Long-Range DNA End Resection by DNA2 or EXO1

Long-range DNA end resection is typically catalyzed by either EXO1/Exo1 or the DNA2/Dna2 nuclease (64, 99, 183). EXO1 and DNA2 partially overlap in function, as indicated by synergistic defects in resection upon inactivation of both pathways. However, the EXO1- and DNA2-dependent pathways differ in their capacities to overcome various base lesions, which is why the relative usage of the two nucleases seems to vary depending on cellular conditions (47). In yeast meiotic cells, the majority of long-range resection is dependent on Exo1, while in mouse cells the nuclease activity of EXO1 is largely dispensable in meiosis (121, 179).

Components of the short-range pathway also have structural roles promoting long-range resection, which helps to make resection overall more efficient. Yeast *mre11*Δ cells display a more severe resection defect compared to *mre11* nuclease-deficient mutants, indicating that the MRX complex can promote resection even without its nuclease function (91, 101, 144). Similar relationships between MRN, CtIP, and long-range resection also exist in the human system (29, 110, 117). The long-range resection pathways were observed to resect DNA tens of kilobases in length (99, 183). However, resection of such long lengths was mostly observed in artificially constrained experimental systems where HR repair was not possible. It is believed that physiological resection lengths on average are much shorter, on the order of 1 kb (102, 121, 172, 179). Long-range resection appears to be largely dispensable for meiotic recombination and for sister chromatid recombination in response to IR-induced DSBs in yeast (165, 179). However, one caveat to this interpretation is that the extent of resection catalyzed by MRX-Sae2 in the *exo1*Δ *sgs1*Δ background may be greater than that which occurs in cells that are proficient for long-range resection; i.e., Exo1 and Dna2 might initiate degradation at the nick formed by the first MRX-Sae2-induced DNA cleavage and in so doing attenuate additional nicking by MRX-Sae2.

If long-range resection is not essential for HR, then what is its purpose? In organisms with abundant repeated DNA sequences, exposing long tracts of ssDNA could be important to ensure that pairing occurs with the correct partner (a sister chromatid) instead of an ectopic sequence that might occur upon the exposure of a relatively short resected tract (38). In addition, Sgs1 and Exo1 are required for full activation of the Mec1/ATR kinase, which is recruited to RPA-coated tracts of ssDNA (64, 183). Failure to activate the checkpoint could result in progression through mitosis, with unrepaired chromosome fragments causing genomic instability or lethality of haploid cells. Several studies have shown that chromatin becomes more mobile in response to DNA damage, both at the damaged locus and globally (54, 103). The increase in chromatin mobility is proposed to enhance repair between chromosome homologs or dispersed repeats in the yeast genome (34). Chromatin mobility is regulated by the DNA damage checkpoint and end resection (54, 103, 140).

EXO1/Exo1 belongs to the XPG/Rad2 nuclease family. It degrades 5′-terminated DNA strands within dsDNA (157), with a preference for DSBs with 3′ overhangs, and it is least efficient on substrates with 5′ overhangs (23, 157). EXO1 can also efficiently initiate DNA degradation from nicks within dsDNA that could result from the endonuclease activity of the MRN/X

complex (132, 163). Depending on in vitro assay conditions, RPA can either stimulate or inhibit resection by Exo1 (23, 111). In *S. cerevisiae*, depletion of RPA results in reduced long-range resection in *sgs1*Δ mutant cells, suggesting a positive effect of RPA on resection by Exo1, even though the intensity of Exo1 foci at DSBs is increased in the absence of RPA (35). In human cells, RPA also competes with EXO1 for access to ssDNA, and resection by EXO1 may be instead stimulated by the sensor of ssDNA complex 1 (SOSS1) (111). MRX, through employing its DNA melting capacity or acting as a recruitment or processivity factor, can facilitate DNA degradation by Exo1 (62, 116, 144). Similar stimulation of human EXO1 by MRN was observed with human recombinant proteins (110, 117).

DNA2/Dna2 is a bifunctional enzyme possessing domains with similarities to both RecB-like nucleases and superfamily I helicases. Unlike Mre11 and Exo1, which degrade single strands within dsDNA, Dna2 only degrades ssDNA (78). RPA directs DNA degradation by Dna2 to the 5′-terminated strand, which guarantees the correct polarity of resection (5, 28, 118). The Dna2 helicase domain cannot unwind dsDNA unless it is unleashed by mutations inactivating the nuclease activity (89, 124). Therefore, for Dna2 to function in the resection of dsDNA, it must act together with a lead helicase. In yeast, the partner of Dna2 is Sgs1, whereas in human cells, it appears to be either the BLM or WRN helicase; all known DNA2 helicase partners belong to the RecQ helicase family (28, 64, 99, 118, 150, 183). BLM and WRN helicases might function in a redundant manner, although their employment differs depending on cell type and conditions (150). The motor activity of DNA2 functions downstream of BLM/WRN as an ssDNA translocase to accelerate the movement of DNA2 along RPA-coated ssDNA, which aids degradation (29, 90, 98). The nuclease domain of Dna2 is embedded in a narrow tunnel through which the DNA has to thread; this explains why Dna2 is blocked by bulky adducts at the ends and why RPA needs to be removed before the DNA strand can fit and DNA degradation can take place (182). The Dna2 pathway is also regulated by CDK (37); however, this control appears to be less strict than for short-range resection by Sae2/CtIP phosphorylation. Similar to Exo1, the Dna2 pathway is stimulated by components of the short-range resection machinery. Yeast MRX and human MRN promote Sgs1 and BLM activities, respectively, while CtIP stimulates BLM and the motor activity of DNA2 (28, 29, 117, 118, 144).

REGULATION OF END RESECTION

Regulation of Resection Initiation

As initiation of end resection is a critical step in repair pathway selection, it is no surprise that the process is regulated by multiple mechanisms. Positive regulation is exerted by the cell cycle to ensure that end resection is coordinated with the presence of a sister chromatid, the favored template for repair in somatic cells. As described above, CDK-phosphorylated CtIP/Sae2 activates the Mre11 endonuclease to initiate end resection (22, 74, 75).

SAMHD1 and FANCJ both positively regulate end resection by promoting recruitment and retention of CtIP to DSBs, resulting in more efficient HR-mediated repair and DNA damage resistance (46, 114). Additionally, SAMHD1 was reported to directly stimulate MRE11 exonuclease activity in vitro (44). Several other factors have been shown to promote or restrict MRN binding to DSBs in cells or to directly regulate its enzymatic activities in vitro. C1QBP directly binds to MRE11, stabilizing the soluble pool of MR in cells and inhibiting MRE11 binding to dsDNA (6). Following DNA damage, ATM phosphorylates C1QBP, resulting in its dissociation from the MR complex and the subsequent recruitment of MRN to chromatin. Thus, C1QBP1 acts in a positive manner to maintain pools of MR but in a negative fashion to ensure the availability of MR for chromatin binding only after DNA damage. UBQLN4, a proteasomal shuttle

factor, is also phosphorylated by ATM in response to DNA damage and associates with ubiquitylated MRE11 (77). In the absence of UBQLN4, MRE11 persists at DSBs for a longer time, resulting in higher frequencies of HR at the expense of NHEJ repair, while overexpression of UBQLN4 has the opposite effect. The MRN-interacting protein, MRNIP, promotes MRN association with chromatin and full activation of ATM in response to IR (149). Although MRNIP promotes end resection and HR at DSBs, in the context of stalled replication forks it suppresses nascent strand degradation by directly inhibiting the MRE11 3′→5′ exonuclease activity without affecting the endonuclease activity (13). DYNLL1, which regulates multiple cellular functions, including 53BP1 oligomerization (11), reduces MRE11 focal accumulation and end resection at DSBs (68). DYNLL1 physically interacts with MRE11, BLM, and DNA2, and in biochemically reconstituted systems was also found to reduce MRE11 3′→5′ exonuclease activity (68). Given the multitude of factors that regulate assembly and/or activity of MRN and CtIP, it will be interesting to determine whether the cofactors function independently or synergize with each other.

Interplay Between BRCA1 and 53BP1 in Controlling End Resection

53BP1 and BRCA1 exhibit antagonistic roles in DSB repair and occupy mutually exclusive domains within damaged chromatin (32, 55). 53BP1 localizes to DSBs in G1 phase cells and promotes repair by NHEJ, whereas BRCA1 counteracts 53BP1 in the S/G2 phase to promote end resection and HR. 53BP1 is not a canonical NHEJ factor but is required for class switch recombination (CSR) in B lymphocytes and for fusion of telomeres resulting from deprotection of chromosome ends (104). 53BP1 is recruited to chromatin via dually modified nucleosomes containing histone H4, methylated on K20, and histone H2A, ubiquitylated at K15 (141). The H4 methylation mark is constitutive, whereas H2A ubiquitylation via RNF8 and RNF168 is induced by DNA damage. Once bound to damaged chromatin, 53BP1 is phosphorylated by ATM on multiple sites within the N-terminal region (141). BRCA1 recruitment to chromatin is mediated by its obligate binding partner, BARD1, and involves the same histone residues contacted by 53BP1. Parental modified histones are equally distributed to the two daughter strands of replicated chromatin alongside newly synthesized unmodified histones. BARD1 is recruited to unmethylated H4 K20 in newly replicated chromatin via its ankyrin repeat domain, effectively competing with 53BP1 binding to nucleosomes before they become fully methylated in the G2 phase (113). The tandem BRCT domain ubiquitin-dependent recruitment motif (BUDR) of BARD1 is responsible for the recognition of ubiquitylated H2A K15 in the vicinity of a DSB (10). BARD1 ankyrin repeat mutations that prevent the recognition of unmethylated H4 K20 or BUDR mutations that prevent binding to H2A K15ub result in 53BP1 accumulation at DSBs in S/G2 phase cells, inhibition of end resection, and suppression of HR. Thus, the deposition of unmodified histone H4 marks sister chromatids as appropriate templates for accurate HR, while H2A K15ub targets BARD-BRCA1 to damaged sites within replicated chromatin.

The antagonism between BRCA1 and 53BP1 is underscored by seminal studies showing that loss of 53BP1 reverses the cell and organismal lethality caused by *BRCA1* mutations and results in the resistance of BRCA1-deficient cells to poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) (18, 19). Loss of 53BP1 restores end resection and HR in BRCA1-deficient cells, thereby suppressing NHEJ-dependent formation of lethal radial chromosomes during S-phase. The finding that end resection is proficient in cells lacking 53BP1 and BRCA1 suggests that BRCA1 promotes end resection largely indirectly through modulation of 53BP1 binding to replicated chromatin, although an additional direct role of BRCA1 cannot be ruled out. BRCA1 physically interacts with CtIP phosphorylated at S327 by CDK, and although this interaction is not required for end resection, HR, or cell viability, it may affect resection speed (45, 112, 125, 131).

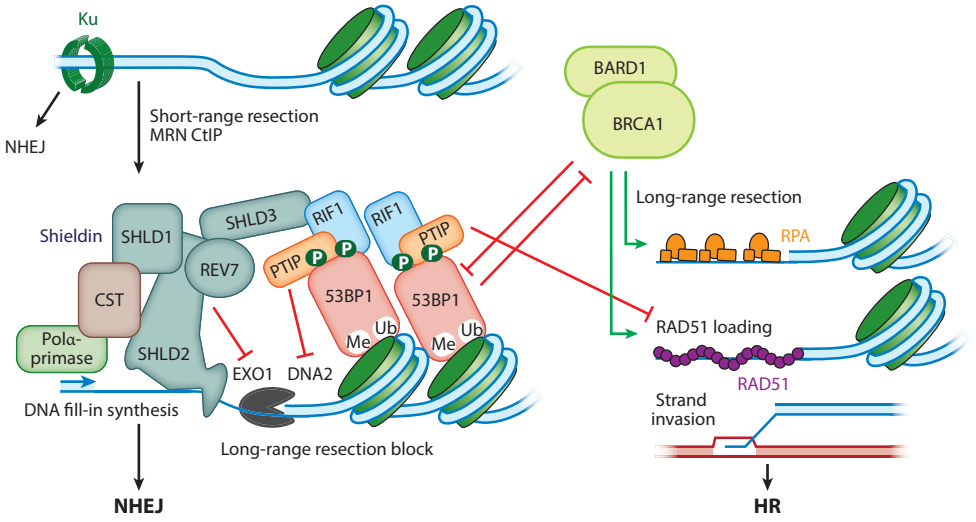

Figure 2

The role of the 53BP1-RIF1-Shieldin complex in DNA end protection. 53BP1 binds to nucleosomes modified by H4 K20 methylation and H2A K15 ubiquitylation, where it is phosphorylated by ATM and/or ATR and recruits the downstream effectors RIF1 and PTIP. RIF1 interacts with the Shieldin complex (REV7, SHLD1, SHLD2, and SHLD3), which in turn recruits the CST complex and Polα-primase to initiate fill-in synthesis, counteracting resection by EXO1 and DNA2. Shieldin and PTIP may also function independently of CST to inhibit long-range resection. The BRCA1-BARD1 complex binds to unmethylated nucleosomes in newly replicated DNA to promote end resection and RAD51 loading in S/G2-phase cells. See main text for details. Abbreviations: CST, CTC1-STN1-TEN1; HR, homologous recombination; Ku, Ku70-Ku80 heterodimer; Me, methylation; NHEJ, nonhomologous end joining; RPA, replication protein A; Ub, ubiquitylation.

In mammalian cells, the repressive effect of 53BP1 on end resection requires additional factors, many of which were identified by physical interactome analyses or in CRISPR-based screens for mutations that increase the resistance of BRCA1-deficient cells to PARPi or suppress genomic instability caused by dysfunctional telomeres. These studies identified PTIP and RIF1 as ATM-phosphorylation-dependent interactors of 53BP1 (20, 31, 53, 184) and SHLD1, SHLD2, SHLD3, and REV7 as components of the Shieldin complex (16, 52, 57, 60, 65, 119, 170). Loss of RIF1 or Shieldin causes phenotypes similar to 53BP1 deficiency, including increased end resection and restoration of RAD51 foci formation to BRCA1-deficient cells and loss of NHEJ repair in specific contexts. The finding that the protection of DNA ends is lost when RIF1 or Shieldin components are depleted indicates that the 53BP1-nucleosome interaction is insufficient to repress end resection in mammals. This raises the question, What is the molecular mechanism for end protection? Some insight into how Shieldin attenuates end resection came from biochemical studies showing that SHLD2 binds to ssDNA via three oligonucleotide/oligosaccharide-binding (OB) folds in the N terminus of the protein. These are required for end protection in cells (57). Thus, Shieldin could be anchored to damaged chromatin via RIF1 and 53BP1 and bind to the short ssDNA overhangs generated by MRN-CtIP-catalyzed resection, thereby blocking access to the long-range nucleases (**Figure 2**).

The other nonexclusive model for 53BP1-RIF1-Shieldin-mediated end protection arose from the observation that resected DSBs may be subject to fill-in synthesis by DNA Polα-primase as a mechanism to counteract end resection, similar to the mechanism used at telomeres to regulate

G-overhang length (104). The heterotrimeric CTC1-STN1-TEN1 (CST) complex has structural similarity to RPA and binds to the G-rich strand at telomeric overhangs where it recruits Polα-primase to fill in ends over-resected by EXO1. The CST complex makes multiple contacts with the Shieldin complex and recruits Polα-primase to DSBs (105) (**Figure 2**). Importantly, depletion of CST suppresses formation of radial chromosomes in PARPi-treated BRCA1-deficient cells, similar to loss of 53BP1. Recent studies have verified this model by showing CST- and Polα-primase-dependent bromodeoxyuridine incorporation at DSBs in G2-arrested cells (Z. Mirman, N. Sasi & T. de Lange, personal communication). Furthermore, artificially tethering CST to DNA damage-modified nucleosomes can bypass the requirement for Shieldin. Fill-in synthesis is likely to leave short 3′ overhangs, corresponding to the size of the RNA primers that would be removed by ribonuclease H (RNase H) plus the footprint of the CST complex. Such short overhangs should be insufficient for HR, thus favoring repair by NHEJ. These new findings support the idea that 53BP1-RIF1-Shieldin counteracts end resection by fill-in synthesis of resection tracts; however, a separate role in inhibiting the long-range nucleases cannot be excluded.

Competition for H4 K20 binding by BRCA1-BARD1 is one mechanism to attenuate the 53BP1-RIF1-Shieldin axis. Another is through active disassembly of the Shieldin complex. REV7 is a HORMA domain protein that exists in active (closed) and inactive (open) conformations that are regulated by the TRIP13 AAA^+ ATPase (41). TRIP13 converts REV7 to the open form, resulting in dissociation from SHLD3, which releases REV7-SHLD2-SHLD1 from chromatin. Consistent with this observation, overexpression of TRIP13 confers PARPi resistance to BRCA1-deficient tumors, while TRIP13 depletion decreases the efficiency of HR (41).

PTIP appears to function independently of RIF1 and Shieldin to guard against extensive resection (20). While loss of PTIP reduces telomere fusions, CSR is unaffected, suggesting that PTIP promotes NHEJ only in specific contexts. Interestingly, resection in BRCA1-deficient $53BP1^{S25A}$ cells, which fail to recruit PTIP to DSBs and exhibit elevated RIF1 focal accumulation, is mostly dependent on DNA2, whereas RIF1-Shieldin blocks EXO1-dependent resection (21). In addition, studies using the $53BP1^{S25A}$ separation-of-function allele identified a novel function for RIF1-Shieldin to suppress RAD51 loading at DSBs, which could contribute to the hyper-resection phenotype observed (21).

Attenuation of End Resection by Rad9 and Rif Proteins in Yeast

Rad9 and Crb2 are homologs of 53BP1 that mediate the DNA damage checkpoint response in budding and fission yeast, respectively (164). *S. cerevisiae* Rad9 interacts with nucleosomes modified by H3 K79 methylation and DNA-damage-inducible H2A S129 phosphorylation (equivalent to γH2AX). Once bound to chromatin in the vicinity of DSBs, Rad9 is phosphorylated by Tel1 and/or Mec1, thereby promoting multimerization and interaction with the effector kinase Rad53/CHK2 (164). Like 53BP1, Rad9/Crb2 attenuates DNA end resection, and this function requires chromatin binding and subsequent phosphorylation by the apical kinases (36, 56, 86, 87). Rad9/Crb2 acts mainly to suppress long-range resection by the Sgs1-Dna2 pathway (Rqh1-Dna2 in *S. pombe*) with only minor effects on Exo1-catalyzed resection (87, 115). The Rad9-nucleosome interaction is thought to cause a barrier to end resection that can be partially alleviated by Fun30. Additionally, Fun30 may promote long-range resection by chromatin remodeling independently of Rad9 (8). Rev7 is conserved in yeast, but its role in suppression of end resection is controversial. In one study, loss of *S. pombe* Rev7 was reported to increase end resection and to be epistatic to Crb2 deficiency; however, another group found no increase in end resection, and no physical interaction between Crb2 and Rev7 has been reported (87, 173). Orthologs of the SHLD proteins have not been identified in yeast. Although Cdc13 (the budding yeast ortholog of CST1) has been

detected at persistent DSBs by chromatin immunoprecipitation (ChIP), this recruitment seems to be associated with rare de novo telomere addition instead of NHEJ repair (38).

The budding yeast Rif1 and Rif2 proteins were identified by their interaction with the telomere-binding protein Rap1, and both contribute to telomere homeostasis. Rif1 localizes to telomeres through its interaction with Rap1 and its intrinsic DNA-binding activity, whereas its recruitment to DSBs is independent of Rap1 (95). In both contexts Rif1 attenuates end resection; however, the functional interaction with Rad9 is currently unclear (95). In one study, Rif1 was shown to promote end resection in G1-arrested cells by antagonizing Rad9 binding, and, in contrast to *rad9*Δ, *rif1*Δ does not suppress the DNA damage sensitivity of *sae2*Δ cells (94). Rif2 exerts a greater inhibition of resection at telomeric sequences than Rif1 exerts and does so by counteracting MRX and Exo1 (17). Rif2 acts directly on Rad50 to promote nonproductive ATP hydrolysis and, by discharging the ATP-bound state of Rad50, inhibits end resection and Tel1 activation (27, 66, 80a, 93, 133a).

Regulation of End Resection by DNA Damage Signaling

Factors involved in DNA damage recognition and signaling play both positive and negative roles in end resection, which is consistent with the view that resection initiates in response to DNA damage but is held in check to prevent excessive exposure of ssDNA. As described above, the ATM/Tel1 kinase is important for regulating the initiation of end resection in meiotic and somatic cells, and phosphorylation of CtIP/Sae2 and other MRN-interacting factors by ATM is also important for this function (6, 9, 102, 121, 161, 172). However, RPA phosphorylation, which is catalyzed by ATM, ATR, CDK, and DNA-PK, attenuates end resection primarily via inhibition of BLM helicase (147). *S. cerevisiae* Rad9, which requires Tel1- or Mec1-catalyzed phosphorylation for stable chromatin binding, attenuates extensive resection by inhibition of Sgs1-Dna2-catalyzed end resection and indirectly by being necessary for Rad53 inhibitory phosphorylation of Exo1 (107, 115, 176). Sae2 functions independently of its role in resection initiation with Mre11 nuclease to dampen Rad9-dependent checkpoint signaling (176). Indeed, loss of Rad9 or Rad53 kinase activity partially restores DNA damage resistance to *sae2*Δ but not to *mre11-nd* cells (56, 63, 176).

A recent study reported a novel function for the heterotrimeric 9-1-1 DNA damage clamp in suppression of MRX-dependent end resection in yeast (61). Components of the 9-1-1 clamp and clamp loader complex were identified in a screen for mutations that suppress the DNA damage sensitivity of cells lacking the long-range resection nucleases. Resection tracts in *exo1*Δ *sgs1*Δ cells were increased from approximately 350 nt to more than 1.7 kb in the absence of Ddc1 or Rad24 (61). Given the ability of the 9-1-1 clamp to bind to the recessed 5′ end at ssDNA/dsDNA junctions and to slide on dsDNA, it is possible that it restricts the access of MRX to the flanking dsDNA, preventing subsequent cleavages by MRX-Sae2. Additionally, the 9-1-1 clamp restricts long-range resection by stabilizing Rad9 binding to chromatin in the vicinity of DSBs, attenuating the Sgs1-Dna2 pathway (115). In contrast to the inhibitory effect of the 9-1-1 complex on MRX activity, 9-1-1 promotes resection by Exo1 in yeast, although the underlying mechanisms have yet to be elucidated (61, 115).

Additional Regulation of Long-Range Resection

Yeast Rad52 and human HELB have been shown to limit long-range resection independently of Rad9/Crb2/53BP1 (156, 173). Rad52 is essential for all homology-directed repair processes in budding and fission yeasts, where it functions to promote annealing of RPA-coated ssDNA and to mediate assembly of Rad51 nucleoprotein filaments (152). Rad52 limits resection by Sgs1/Rqh1,

independent of its role in promoting strand invasion, by directly inhibiting helicase translocation on ssDNA (173). Long-range resection in *S. pombe* is mostly driven by Exo1 (84, 87), and, consequently, the inhibitory effect of Rad52 is more prominent in *S. pombe* than in *S. cerevisiae* (173). The HELB ATP-dependent translocase was identified as an RPA-interacting factor that limits extensive resection by antagonizing EXO1 and BLM-DNA2 (156). Nuclear localization of HELB is regulated during the cell cycle such that HELB is nuclear during the G1 phase when end resection is restricted and is exported from the nucleus during the S and G2 phases when cells are primed for HR repair (156).

PATHOLOGICAL OUTCOMES OF DYSREGULATED END RESECTION AT DNA DOUBLE-STRAND BREAKS AND STALLED REPLICATION FORKS

End resection is essential for HR-mediated repair of DSBs in all organisms studied; however, unregulated resection threatens genome integrity. ssDNA is susceptible to chemical alterations or to degradation by nucleases, resulting in clustered mutations or loss of 3′ overhangs, respectively (35, 135). In addition, resection tracts are substrates for the noncanonical homology-directed repair mechanisms, single-strand annealing (SSA), and microhomology-mediated end joining (MMEJ), particularly in the absence of RAD51 and associated proteins (15, 142). SSA and MMEJ rely on the exposure of homologous sequences flanking a DSB by end resection and are mutagenic mechanisms because they can form deletions. MMEJ is CtIP-dependent and typically involves a homology of <15 nt (12, 158), whereas the long-range resection nucleases are required to expose longer homologous sequences for repair by SSA (99, 183). Coordinating end resection with homologous pairing is important to ensure that resection tracts are channeled to the correct repair mechanism, and in this regard, BRCA1 plays a particularly important role to suppress aberrant joining of replication-associated DSBs. As described below, recent studies have revealed critical functions for BRCA1 and BRCA2 at stalled replication forks to ensure accurate replication restart by HR and to protect nascent strands from unscheduled degradation.

Degradation and Protection of Stalled and Reversed Replication Forks

Replication forks may be perturbed by damaged templates or repetitive sequences, collisions with transcription machineries, imbalance or lack of nucleotides, or, more indirectly, overexpression of oncogenes. In response to these challenges, replication forks may pause or stall, leading to the accumulation of ssDNA gaps behind the forks (67). Seminal studies have shown that human RAD51, BRCA1, BRCA2, and a growing number of additional factors have recombination-independent functions to protect stalled replication forks to prevent nascent DNA degradation (67, 137, 138). This section summarizes data on DNA end resection nucleases, including MRE11, DNA2, and EXO1, which were found to act pathologically, in most cases, in the degradation of nascent DNA upon replication stress in various mutant genetic backgrounds (69, 70, 88, 97, 137, 138, 171). Pathological nascent DNA degradation was also observed in yeast. In budding and fission yeast, forks stalled at protein-induced barriers may undergo unrestricted and unscheduled degradation by Exo1, which can be counteracted by Rad51 (2, 85). Sgs1-Dna2 were also found to pathologically degrade DNA upon replication fork stalling with hydroxyurea in the absence of Rif1 (106). As most insights into replication fork metabolism are based on studying human cells, we focus on the human system for the remainder of this section.

In contrast to DNA end resection, the regulation and underlying mechanisms of nuclease action on replication forks are much less understood. It appears that paradigms learned from DSB resection are not directly applicable to studies of DNA metabolism at stalled replication forks.

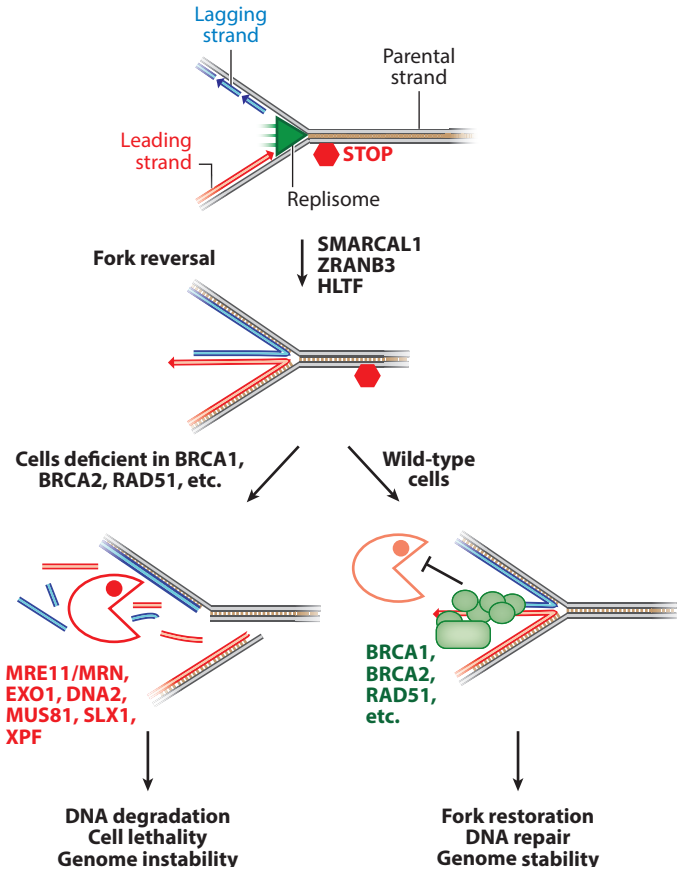

Figure 3

Protection of stalled replication fork from unscheduled degradation. Various stresses can cause replication forks to stall and undergo remodeling by SNF family translocases. The protection of remodeled replication forks requires RAD51, BRCA1, BRCA2, and many other fork protection factors. In the absence of RAD51 and associated proteins, HR-dependent fork restart is prevented and nascent strands are degraded by nucleases, resulting in genome instability and cell lethality. Abbreviation: HR, homologous recombination.

Nascent DNA degradation likely potentiates the efficacy of chemotherapy of BRCA-deficient tumors, and restoration of fork protection is one of the mechanisms of chemoresistance (129). Therefore, understanding the principles of DNA metabolism at stalled forks not only is an attractive area of basic research, but has direct implications for cancer therapy.

Which structures represent the entry points for nucleolytic degradation upon replication fork stalling? Initial reports pointed toward ssDNA gaps behind stalled forks (67, 181). Later, it was observed that nascent DNA degradation, e.g., in BRCA-deficient cells, is largely dependent on factors implicated in replication fork reversal, including the motor proteins SMARCAL1, ZRANB3, and HLTF. Therefore, reversed forks are now thought to be important intermediates that trigger DNA degradation in the absence of fork protection (83, 88, 97, 137, 153) (**Figure 3**). The annealed nascent strands of reversed forks resemble a DSB and thus can recruit the resection enzymes. Although replication fork reversal can lead to nascent DNA degradation and genome instability, fork reversal may be a protective mechanism, depending on the cellular context. In particular, in

wild-type cells with intact DNA protection, fork reversal can temporarily stabilize the replisome, giving cells time to deal with DNA damage and prevent DNA breakage (130). Accordingly, depletion of SMARCAL1 and ZRANB3 gives rise to genome instability in otherwise wild-type cells. However, in cells impaired in fork protection, such as in a BRCA-deficient background, inactivation of fork reversal through the depletion of SMARCAL1, ZRANB3, or HLTF instead prevents DNA breakage and chromosomal instability (7, 39, 40, 166, 177, 178). Depending on the context and cellular background, structures other than reversed forks may be subject to unscheduled nucleolytic degradation. For example, it was observed that intermediates of DNA interstrand crosslink repair need to be similarly protected by RAD51 and BRCA2 (133, 160).

Which nucleases pathologically degrade nascent DNA? In BRCA-deficient cells, DNA degradation at challenged forks can be prevented by treatment with the MRE11 nuclease inhibitor mirin or the depletion of MRE11 (137). Consequently, the 3′→5′ exonuclease of MRE11 has been implicated in nascent DNA degradation. The activity of the MRE11 nuclease in vitro is rather weak (123), and, similarly, the extent of MRE11-dependent resection in DSB processing is limited (64, 99, 183). Therefore, it appears unlikely that the MRE11 nuclease, acting alone or within the MRN complex in conjunction with CtIP, could account for the observed DNA degradation, which extends for multiple kilobases (88, 137). In accord, depletion of EXO1 prevents DNA degradation, suggesting that EXO1 may act downstream of MRE11, similar to its role in DSB processing (88). Activation of MRE11 nuclease by ATM-dependent phosphorylation in response to metabolic assaults might also contribute to the extensive nascent strand degradation observed in hydroxyurea-treated BRCA-deficient cells (146). DNA2 does not notably contribute to nascent DNA degradation in BRCA-deficient cells. In contrast, DNA2, but not MRE11, was found responsible for pathological DNA degradation in BRCA-proficient cells lacking BOD1L, which links fork protection with histone H3K4 methylation by SETD1A (69, 70). DNA2, together with WRN, was found to mediate limited reversed fork DNA degradation leading to fork restart in wild-type cells, showing that limited DNA degradation may have a physiological function (155).

Interestingly, CtIP was also found to mediate fork protection epistatically with BOD1L (69, 127), showing that CtIP may have roles in both DNA protection and DNA degradation (44, 88). DNA2, WRN, and to a lesser degree MRE11 were found to degrade reversed replication forks in the absence of RIF1, which acts in conjunction with protein phosphatase 1 (PP1) (59, 76a, 109). However, the exonuclease activity of WRN, as well as the WRN-interacting protein (WRNIP1), was found instead to protect nascent DNA against DNA degradation by MRE11 and EXO1 when RIF1 was present (76, 126). DNA2 and WRN are also responsible for pathological DNA degradation of unprotected intermediates of DNA interstrand crosslink repair (160). Interestingly, BRCA2 protects against DNA2-WRN degradation in interstrand crosslink repair but not at reversed forks, where it protects against MRE11 and EXO1 (88, 133, 137, 160). Similar antagonistic functions in DNA resection and protection of reversed forks were also attributed to BRCA1 and MRNIP (13, 19, 126, 127).

Another key difference between DSB resection and nascent DNA degradation at replication forks is the polarity of the nucleolytic processing. In DSB resection, only the 5′-terminated strand is degraded, while the 3′ end is largely protected. Nascent DNA degradation is usually monitored in mammalian cells by a DNA fiber assay that only detects events where both strands are degraded, indicating that the 3′-terminated strand is also lost at replication forks (137). It is unclear whether the canonical DNA end resection nucleases can promote degradation of both DNA strands at reversed forks. The 3′→5′ polarity of the MRE11 exonuclease makes it a potential candidate for the 3′ strand degradation, but its limited activity appears unlikely to explain the extended DNA loss observed in the fiber assays (137). Rather, enzymes other than MRE11, EXO1, or DNA2 are likely responsible for the loss of the 3′ strand. Potential candidates for this function

are structure-selective nucleases such as MUS81-EME1, SLX1-SLX4, and ERCC1-XPF and/or their higher-order complexes (169). These enzymes are capable of cleaving 3′ flaps, replication forks, or even four-way junctions at the branch points. Although they do not function in canonical DSB resection, they have been implicated in fork processing by several studies, with the relative involvement of each structure-selective nuclease depending on genetic background. MUS81 appears to be active in both BRCA-deficient and wild-type cells and has been implicated in pathological DNA degradation as well as in fork restart that promotes genetic stability (1, 33, 88, 122, 134). In contrast, SLX1-SLX4 and XPF-ERCC1 are activated in the absence of WRNIP1 (126). The interplay of these nucleases with MRE11/MRN, EXO1, and DNA2 remains to be defined.

Taken together, it is apparent that there are several pathways of fork protection and fork degradation, but at present it is not possible to propose a unified mechanistic model to consolidate the observations from the various laboratories. The involvement of different nucleases often varies, depending on genetic background and the kind of genotoxic treatment. Paradoxically, some factors, including CtIP, BRCA1, MRNIP, and WRN, may have opposing roles in DNA degradation and DNA protection. DNA metabolism at DSBs and stalled replication forks may be additionally governed by chromatin, through posttranslational modifications, or by additional regulatory factors (82, 139). Investigating these relationships will represent a fascinating direction of future research.

PERSPECTIVE AND FUTURE DIRECTIONS

Knowledge of how MRN/X initiates resection has advanced considerably over the last decade, but several mechanistic questions remain unanswered. MRN/X cleaves next to end-bound proteins, and even nucleosomes, raising the question of how the DNA end is sensed by MRN/X to prevent nicking at chromosome internal sites. How CtIP/Sae2 interacts with RAD50 to promote cleavage of 5′-terminated strands is also not fully understood. Until recently, resection initiation had been considered to commit cells to HR, thus preventing repair by NHEJ. The observation of CST and Polα-primase-dependent fill-in synthesis in mammalian cells challenges this view and raises questions about the minimal length of the 3′ overhang required to promote HR and inhibit NHEJ repair. Resection at stalled replication forks has emerged as a major research area in recent years. Whether this is a physiological response to fork stalling to promote replication restart by HR or a pathological response to nucleotide depletion or loss of fork protection factors is currently unclear. In addition, how resection functions in the chromatin context—in particular, its interaction with other processes such as transcription—and how it proceeds through nucleosomes are areas of active investigation. We anticipate these questions will inspire future research in the field.

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LITERATURE CITED

1. Aiello FA, Palma A, Malacaria E, Zheng L, Campbell JL, et al. 2019. RAD51 and mitotic function of mus81 are essential for recovery from low-dose of camptothecin in the absence of the WRN exonuclease. *Nucleic Acids Res.* 47:6796–810
2. 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
3. Anand R, Jasrotia A, Bundschuh D, Howard SM, Ranjha L, et al. 2019. NBS1 promotes the endonuclease activity of the MRE11-RAD50 complex by sensing CtIP phosphorylation. *EMBO J.* 38:e101005
4. 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
5. 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
6. Bai Y, Wang W, Li S, Zhan J, Li H, et al. 2019. C1QBP promotes homologous recombination by stabilizing MRE11 and controlling the assembly and activation of MRE11/RAD50/NBS1 complex. *Mol. Cell* 75:1299–314.e6
7. Bansbach CE, Betous R, Lovejoy CA, Glick GG, Cortez D. 2009. The annealing helicase SMARCAL1 maintains genome integrity at stalled replication forks. *Genes Dev.* 23:2405–14
8. Bantele SCS, Pfander B. 2019. Nucleosome remodeling by Fun30^{SMARCAD1} in the DNA damage response. *Front. Mol. Biosci.* 6:78
9. Baroni E, Viscardi V, Cartagena-Lirola H, Lucchini G, Longhese MP. 2004. The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. *Mol. Cell. Biol.* 24:4151–65
10. Becker JR, Bonnet C, Clifford G, Groth A, Wilson MD, Chapman JR. 2021. BARD1 links histone H2A Lysine-15 ubiquitination to initiation of BRCA1-dependent homologous recombination. *Nature* 596:433–37
11. Becker JR, Cuella-Martin R, Barazas M, Liu R, Oliveira C, et al. 2018. The ASCIZ-DYNLL1 axis promotes 53BP1-dependent non-homologous end joining and PARP inhibitor sensitivity. *Nat. Commun.* 9:5406
12. Bennardo N, Cheng A, Huang N, Stark JM. 2008. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. *PLOS Genet.* 4:e1000110
13. Bennett LG, Wilkie AM, Antonopoulou E, Ceppi I, Sanchez A, et al. 2020. MRNIP is a replication fork protection factor. *Sci. Adv.* 6:eaba5974
14. Betermier M, Borde V, de Villartay JP. 2020. Coupling DNA damage and repair: an essential safeguard during programmed DNA double-strand breaks? *Trends Cell Biol.* 30:87–96
15. Bhargava R, Onyango DO, Stark JM. 2016. Regulation of single-strand annealing and its role in genome maintenance. *Trends Genet.* 32:566–75
16. Boersma V, Moatti N, Segura-Bayona S, Peuscher MH, van der Torre J, et al. 2015. MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5′ end resection. *Nature* 521:537–40
17. Bonetti D, Clerici M, Manfrini N, Lucchini G, Longhese MP. 2010. The MRX complex plays multiple functions in resection of Yku- and Rif2-protected DNA ends. *PLOS ONE* 5:e14142
18. Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, et al. 2010. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat. Struct. Mol. Biol.* 17:688–95
19. Bunting SF, Callén E, Wong N, Chen HT, Polato F, et al. 2010. 53BP1 inhibits homologous recombination in *Brca1*-deficient cells by blocking resection of DNA breaks. *Cell* 141:243–54
20. Callen E, Di Virgilio M, Kruhlak MJ, Nieto-Soler M, Wong N, et al. 2013. 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell* 153:1266–80
21. Callen E, Zong D, Wu W, Wong N, Stanlie A, et al. 2020. 53BP1 enforces distinct pre- and post-resection blocks on homologous recombination. *Mol. Cell* 77:26–38.e7
22. Cannavo E, Cejka P. 2014. Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. *Nature* 514:122–25

23. 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
24. Cannavo E, Johnson D, Andres SN, Kissling VM, Reinert JK, et al. 2018. Regulatory control of DNA end resection by Sae2 phosphorylation. *Nat. Commun.* 9:4016
25. Cannavo E, Reginato G, Cejka P. 2019. Stepwise 5′ DNA end-specific resection of DNA breaks by the Mre11-Rad50-Xrs2 and Sae2 nuclease ensemble. *PNAS* 116:5505–13
26. Cannon B, Kuhnlein J, Yang SH, Cheng A, Schindler D, et al. 2013. Visualization of local DNA unwinding by Mre11/Rad50/Nbs1 using single-molecule FRET. *PNAS* 110:18868–73
27. Cassani C, Gobbini E, Wang W, Niu H, Clerici M, et al. 2016. Tel1 and Rif2 regulate MRX functions in end-tethering and repair of DNA double-strand breaks. *PLOS Biol.* 14:e1002387
28. 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
29. Ceppi I, Howard SM, Kasaciunaite K, Pinto C, Anand R, et al. 2020. CtIP promotes the motor activity of DNA2 to accelerate long-range DNA end resection. *PNAS* 117:8859–69
30. 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
31. Chapman JR, Barral P, Vannier JB, Borel V, Steger M, et al. 2013. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol. Cell* 49:858–71
32. Chapman JR, Sossick AJ, Boulton SJ, Jackson SP. 2012. BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. *J. Cell Sci.* 125:3529–34
33. Chappidi N, Nascakova Z, Boleslavska B, Zellweger R, Isik E, et al. 2020. Fork cleavage-religation cycle and active transcription mediate replication restart after fork stalling at co-transcriptional R-loops. *Mol. Cell* 77:528–41.e8
34. Cheblal A, Challa K, Seeber A, Shimada K, Yoshida H, et al. 2020. DNA damage-induced nucleosome depletion enhances homology search independently of local break movement. *Mol. Cell* 80:311–26.e4
35. Chen H, Lisby M, Symington LS. 2013. RPA coordinates DNA end resection and prevents formation of DNA hairpins. *Mol. Cell* 50:589–600
36. Chen X, Cui D, Papusha A, Zhang X, Chu C-D, et al. 2012. The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. *Nature* 489:576–80
37. Chen X, Niu H, Chung W-H, Zhu Z, Papusha A, et al. 2011. Cell cycle regulation of DNA double-strand break end resection by Cdk1-dependent Dna2 phosphorylation. *Nat. Struct. Mol. Biol.* 18:1015–19
38. Chung WH, Zhu Z, Papusha A, Malkova A, Ira G. 2010. Defective resection at DNA double-strand breaks leads to de novo telomere formation and enhances gene targeting. *PLOS Genet.* 6:e1000948
39. Ciccia A, Bredemeyer AL, Sowa ME, Terret M-E, Jallepalli PV, et al. 2009. The SIOD disorder protein SMARCAL1 is an RPA-interacting protein involved in replication fork restart. *Genes Dev.* 23:2415–25
40. Ciccia A, Nimonkar AV, Hu Y, Hajdu I, Achar YJ, et al. 2012. Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress. *Mol. Cell* 47:396–409
41. Clairmont CS, Sarangi P, Ponnienselvan K, Galli LD, Csete I, et al. 2020. TRIP13 regulates DNA repair pathway choice through REV7 conformational change. *Nat. Cell Biol.* 22:87–96
42. Colnaghi R, Carpenter G, Volker M, O'Driscoll M. 2011. The consequences of structural genomic alterations in humans: genomic disorders, genomic instability and cancer. *Semin. Cell Dev. Biol.* 22:875–85
43. Connelly JC, de Leau ES, Leach DR. 2003. Nucleolytic processing of a protein-bound DNA end by the *E. coli* SbcCD (MR) complex. *DNA Repair* 2:795–807
44. Coquel F, Silva MJ, Techer H, Zadorozhny K, Sharma S, et al. 2018. SAMHD1 acts at stalled replication forks to prevent interferon induction. *Nature* 557:57–61
45. Cruz-García A, López-Saavedra A, Huertas P. 2014. BRCA1 accelerates CtIP-mediated DNA-end resection. *Cell Rep.* 9:451–59
46. Daddacha W, Koyen AE, Bastien AJ, Head PE, Dhere VR, et al. 2017. SAMHD1 promotes DNA end resection to facilitate DNA repair by homologous recombination. *Cell Rep.* 20:1921–35

47. Daley JM, Tomimatsu N, Hooks G, Wang W, Miller AS, et al. 2020. Specificity of end resection pathways for double-strand break regions containing ribonucleotides and base lesions. *Nat. Commun.* 11:3088
48. Desai-Mehta A, Cerosaletti KM, Concannon P. 2001. Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization. *Mol. Cell. Biol.* 21:2184–91
49. Deshpande RA, Lee J-H, Arora S, Paull TT. 2016. Nbs1 converts the human Mre11/Rad50 nuclease complex into an endo/exonuclease machine specific for protein-DNA adducts. *Mol. Cell* 64:593–606
50. Deshpande RA, Myler LR, Soniat MM, Makharashvili N, Lee L, et al. 2020. DNA-dependent protein kinase promotes DNA end processing by MRN and CtIP. *Sci. Adv.* 6:eaay0922
51. Deshpande RA, Williams GJ, Limbo O, Williams RS, Kuhnlein J, et al. 2014. ATP-driven Rad50 conformations regulate DNA tethering, end resection, and ATM checkpoint signaling. *EMBO J.* 33:482–500
52. Dev H, Chiang T-WW, Lescale C, de Krijger I, Martin AG, et al. 2018. Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. *Nat. Cell Biol.* 20:954–65
53. Di Virgilio M, Callen E, Yamane A, Zhang W, Jankovic M, et al. 2013. Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 339:711–15
54. Dion V, Kalck V, Horigome C, Towbin BD, Gasser SM. 2012. Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat. Cell Biol.* 14:502–9
55. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, et al. 2013. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol. Cell* 49:872–83
56. Ferrari M, Dibitetto D, De Gregorio G, Eapen VV, Rawal CC, et al. 2015. Functional interplay between the 53BP1-ortholog Rad9 and the Mre11 complex regulates resection, end-tethering and repair of a double-strand break. *PLOS Genet.* 11:e1004928
57. Gao S, Feng S, Ning S, Liu J, Zhao H, et al. 2018. An OB-fold complex controls the repair pathways for DNA double-strand breaks. *Nat. Commun.* 9:3925
58. Garcia V, Phelps SEL, Gray S, Neale MJ. 2011. Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. *Nature* 479:241–44
59. Garzón J, Ursich S, Lopes M, Hiraga S-I, Donaldson AD. 2019. Human RIF1-protein phosphatase 1 prevents degradation and breakage of nascent DNA on replication stalling. *Cell Rep.* 27:2558–66.e4
60. Ghezraoui H, Oliveira C, Becker JR, Bilham K, Moralli D, et al. 2018. 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. *Nature* 560:122–27
61. Gobbini E, Casari E, Colombo CV, Bonetti D, Longhese MP. 2020. The 9-1-1 complex controls Mre11 nuclease and checkpoint activation during short-range resection of DNA double-strand breaks. *Cell Rep.* 33:108287
62. Gobbini E, Cassani C, Vertemara J, Wang W, Mambretti F, et al. 2018. The MRX complex regulates Exo1 resection activity by altering DNA end structure. *EMBO J.* 37:e98588
63. Gobbini E, Villa M, Gnugnoli M, Menin L, Clerici M, Longhese MP. 2015. Sae2 function at DNA double-strand breaks is bypassed by dampening Tel1 or Rad53 activity. *PLOS Genet.* 11:e1005685
64. 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
65. Gupta R, Somyajit K, Narita T, Maskey E, Stanlie A, et al. 2018. DNA repair network analysis reveals shieldin as a key regulator of NHEJ and PARP inhibitor sensitivity. *Cell* 173:972–88.e23
66. Hailemariam S, De Bona P, Galletto R, Hohl M, Petrini JH, Burgers PM. 2019. The telomere-binding protein Rif2 and ATP-bound Rad50 have opposing roles in the activation of yeast $Tel1^{ATM}$ kinase. *J. Biol. Chem.* 294:18846–52
67. 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
68. He YJ, Meghani K, Caron M-C, Yang C, Ronato DA, et al. 2018. DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells. *Nature* 563:522–26
69. 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

70. Higgs MR, Sato K, Reynolds JJ, Begum S, Bayley R, et al. 2018. Histone methylation by SETD1A protects nascent DNA through the nucleosome chaperone activity of FANCD2. *Mol. Cell* 71:25–41.e6
71. Hohl M, Kochańczyk T, Tous C, Aguilera A, Krężel A, Petrini JH. 2015. Interdependence of the Rad50 hook and globular domain functions. *Mol. Cell* 57:479–91
72. Hopfner K-P, Craig L, Moncalian G, Zinkel RA, Usui T, et al. 2002. The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature* 418:562–66
73. Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA. 2001. Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* 105:473–85
74. Huertas P, Cortes-Ledesma F, Sartori AA, Aguilera A, Jackson SP. 2008. CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature* 455:689–92
75. Huertas P, Jackson SP. 2009. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. *J. Biol. Chem.* 284:9558–65
76. Iannascoli C, Palermo V, Murfuni I, Franchitto A, Pichierri P. 2015. The WRN exonuclease domain protects nascent strands from pathological MRE11/EXO1-dependent degradation. *Nucleic Acids Res.* 43:9788–803
76a. Isobe S-Y, Hiraga S-I, Nagao K, Sasanuma H, Donaldson AD, Obuse C. 2021. Protein phosphatase 1 acts as a RIF1 effector to suppress DSB resection prior to Shieldin action. *Cell Rep.* 36:109383
77. Jachimowicz RD, Beleggia F, Isensee J, Velpula BB, Goergens J, et al. 2019. UBQLN4 represses homologous recombination and is overexpressed in aggressive tumors. *Cell* 176:505–19.e22
78. Kao H-I, Campbell JL, Bambara RA. 2004. Dna2p helicase/nuclease is a tracking protein, like FEN1, for flap cleavage during Okazaki fragment maturation. *J. Biol. Chem.* 279:50840–49
79. Käshammer L, Saathoff J-H, Lammens K, Gut F, Bartho J, et al. 2019. Mechanism of DNA end sensing and processing by the Mre11-Rad50 complex. *Mol. Cell* 76:382–94.e6
80. Keeney S, Kleckner N. 1995. Covalent protein-DNA complexes at the 5′ strand termini of meiosis-specific double-strand breaks in yeast. *PNAS* 92:11274–78
80a. Khayat F, Cannavo E, Alshmery M, Foster WR, Chahwan C, et al. 2021. Inhibition of MRN activity by a telomere protein motif. *Nat. Commun.* 12:3856
81. Kim JH, Grosbart M, Anand R, Wyman C, Cejka P, Petrini JHJ. 2017. The Mre11-Nbs1 interface is essential for viability and tumor suppression. *Cell Rep.* 18:496–507
82. Kim JJ, Lee SY, Choi J-H, Woo HG, Xhemalce B, Miller KM. 2020. PCAF-mediated histone acetylation promotes replication fork degradation by MRE11 and EXO1 in BRCA-deficient cells. *Mol. Cell* 80:327–44.e8
83. Kolinjivadi AM, Sannino V, De Antoni A, Zadorozhny K, Kilkenny 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
84. 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
85. Larsen NB, Liberti SE, Vogel I, Jorgensen SW, Hickson ID, Mankouri HW. 2017. Stalled replication forks generate a distinct mutational signature in yeast. *PNAS* 114:9665–70
86. Lazzaro F, Sapountzi V, Granata M, Pellicioli A, Vaze M, et al. 2008. Histone methyltransferase Dot1 and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. *EMBO J.* 27:1502–12
87. Leland BA, Chen AC, Zhao AY, Wharton RC, King MC. 2018. Rev7 and 53BP1/Crb2 prevent RecQ helicase-dependent hyper-resection of DNA double-strand breaks. *eLife* 7:e33402
88. 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
89. Levikova M, Klaue D, Seidel R, Cejka P. 2013. Nuclease activity of *Saccharomyces cerevisiae* Dna2 inhibits its potent DNA helicase activity. *PNAS* 110:E1992–2001
90. Levikova M, Pinto C, Cejka P. 2017. The motor activity of DNA2 functions as an ssDNA translocase to promote DNA end resection. *Genes Dev.* 31:493–502

91. Llorente B, Symington LS. 2004. The Mre11 nuclease is not required for 5′ to 3′ resection at multiple HO-induced double-strand breaks. *Mol. Cell. Biol.* 24:9682–94
92. Lobachev KS, Gordenin DA, Resnick MA. 2002. The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. *Cell* 108:183–93
93. Marsella A, Gobbini E, Cassani C, Tisi R, Cannavo E, et al. 2021. Sae2 and Rif2 regulate MRX endonuclease activity at DNA double-strand breaks in opposite manners. *Cell Rep*. 34:108906
94. Martina M, Bonetti D, Villa M, Lucchini G, Longhese MP. 2014. *Saccharomyces cerevisiae* Rif1 cooperates with MRX-Sae2 in promoting DNA-end resection. *EMBO Rep*. 15:695–704
95. Mattarocci S, Reinert JK, Bunker RD, Fontana GA, Shi T, et al. 2017. Rif1 maintains telomeres and mediates DNA repair by encasing DNA ends. *Nat. Struct. Mol. Biol.* 24:588–95
96. Mendez-Dorantes C, Bhargava R, Stark JM. 2018. Repeat-mediated deletions can be induced by a chromosomal break far from a repeat, but multiple pathways suppress such rearrangements. *Genes Dev*. 32:524–36
97. 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
98. Miller AS, Daley JM, Pham NT, Niu H, Xue X, et al. 2017. A novel role of the Dna2 translocase function in DNA break resection. *Genes Dev*. 31:503–10
99. Mimitou EP, Symington LS. 2008. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455:770–74
100. Mimitou EP, Symington LS. 2009. DNA end resection: Many nucleases make light work. *DNA Repair* 8:983–95
101. Mimitou EP, Symington LS. 2010. Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. *EMBO J.* 29:3358–69
102. Mimitou EP, Yamada S, Keeney S. 2017. A global view of meiotic double-strand break end resection. *Science* 355:40–45
103. Mine-Hattab J, Rothstein R. 2012. Increased chromosome mobility facilitates homology search during recombination. *Nat. Cell Biol.* 14:510–17
104. Mirman Z, de Lange T. 2020. 53BP1: a DSB escort. *Genes Dev*. 34:7–23
105. Mirman Z, Lottersberger F, Takai H, Kibe T, Gong Y, et al. 2018. 53BP1-RIF1-shieldin counteracts DSB resection through CST- and Polα-dependent fill-in. *Nature* 560:112–16
106. Monerawela C, Hiraga S-I, Donaldson AD. 2020. Checkpoint phosphorylation sites on budding yeast Rif1 protect nascent DNA from degradation by Sgs1-Dna2. bioRxiv 2020.06.25.170571. **https://doi.org/10.1101/2020.06.25.170571**
107. Morin I, Ngo HP, Greenall A, Zubko MK, Morrice N, Lydall D. 2008. Checkpoint-dependent phosphorylation of Exo1 modulates the DNA damage response. *EMBO J.* 27:2400–10
108. Moynahan ME, Jasin M. 2010. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nat. Rev. Mol. Cell Biol.* 11:196–207
109. Mukherjee C, Tripathi V, Manolika EM, Heijink AM, Ricci G, et al. 2019. RIF1 promotes replication fork protection and efficient restart to maintain genome stability. *Nat. Commun.* 10:3287
110. Myler LR, Gallardo IF, Soniat MM, Deshpande RA, Gonzalez XB, et al. 2017. Single-molecule imaging reveals how Mre11-Rad50-Nbs1 initiates DNA break repair. *Mol. Cell* 67:891–98.e4
111. Myler LR, Gallardo IF, Zhou Y, Gong F, Yang SH, et al. 2016. Single-molecule imaging reveals the mechanism of Exo1 regulation by single-stranded DNA binding proteins. *PNAS* 113:E1170–79
112. Nakamura K, Kogame T, Oshiumi H, Shinohara A, Sumitomo Y, et al. 2010. Collaborative action of Brca1 and CtIP in elimination of covalent modifications from double-strand breaks to facilitate subsequent break repair. *PLOS Genet*. 6:e1000828
113. Nakamura K, Saredi G, Becker JR, Foster BM, Nguyen NV, et al. 2019. H4K20me0 recognition by BRCA1-BARD1 directs homologous recombination to sister chromatids. *Nat. Cell Biol.* 21:311–18
114. Nath S, Nagaraju G. 2020. FANCJ helicase promotes DNA end resection by facilitating CtIP recruitment to DNA double-strand breaks. *PLOS Genet*. 16:e1008701
115. Ngo GH, Lydall D. 2015. The 9-1-1 checkpoint clamp coordinates resection at DNA double strand breaks. *Nucleic Acids Res*. 43:5017–32

116. 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
117. 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
118. Niu H, Chung WH, Zhu Z, Kwon Y, Zhao W, et al. 2010. Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature* 467:108–11
119. Noordermeer SM, Adam S, Setiaputra D, Barazas M, Pettitt SJ, et al. 2018. The shieldin complex mediates 53BP1-dependent DNA repair. *Nature* 560:117–21
120. Oh J, Al-Zain A, Cannavo E, Cejka P, Symington LS. 2016. Xrs2 dependent and independent functions of the Mre11-Rad50 complex. *Mol. Cell* 64:405–15
121. Paiano J, Wu W, Yamada S, Sciascia N, Callen E, et al. 2020. ATM and PRDM9 regulate SPO11-bound recombination intermediates during meiosis. *Nat. Commun.* 11:857
122. Pardo B, Moriel-Carretero M, Vicat T, Aguilera A, Pasero P. 2020. Homologous recombination and Mus81 promote replication completion in response to replication fork blockage. *EMBO Rep*. 21:e49367
123. Paull TT, Gellert M. 1998. The 3′ to 5′ exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. *Mol. Cell* 1:969–79
124. Pinto C, Kasaciunaite K, Seidel R, Cejka P. 2016. Human DNA2 possesses a cryptic DNA unwinding activity that functionally integrates with BLM or WRN helicases. *eLife* 5:e18574
125. Polato F, Callen E, Wong N, Faryabi R, Bunting S, et al. 2014. CtIP-mediated resection is essential for viability and can operate independently of BRCA1. *J. Exp. Med.* 211:1027–36
126. Porebski B, Wild S, Kummer S, Scaglione S, Gaillard P-HL, Gari K. 2019. WRNIP1 protects reversed DNA replication forks from SLX4-dependent nucleolytic cleavage. *iScience* 21:31–41
127. Przetocka S, Porro A, Bolck HA, Walker C, Lezaja A, et al. 2018. CtIP-mediated fork protection synergizes with BRCA1 to suppress genomic instability upon DNA replication stress. *Mol. Cell* 72:568–82.e6
128. Ranjha L, Howard SM, Cejka P. 2018. Main steps in DNA double-strand break repair: an introduction to homologous recombination and related processes. *Chromosoma* 127:187–214
129. 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
130. 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
131. Reczek CR, Szabolcs M, Stark JM, Ludwig T, Baer R. 2013. The interaction between CtIP and BRCA1 is not essential for resection-mediated DNA repair or tumor suppression. *J. Cell Biol.* 201:693–707
132. Reginato G, Cannavo E, Cejka P. 2017. Physiological protein blocks direct the Mre11-Rad50-Xrs2 and Sae2 nuclease complex to initiate DNA end resection. *Genes Dev*. 31:2325–30
133. Rickman KA, Noonan RJ, Lach FP, Sridhar S, Wang AT, et al. 2020. Distinct roles of BRCA2 in replication fork protection in response to hydroxyurea and DNA interstrand cross-links. *Genes Dev*. 34:832–46
133a. Roisné-Hamelin F, Pobiega S, Jézéquel K, Miron S, Dépagne J, et al. 2021. Mechanism of MRX inhibition by Rif2 at telomeres. *Nat. Commun*. 12:2763
134. Rondinelli B, Gogola E, Yücel H, Duarte AA, van de Ven M, et al. 2017. EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. *Nat. Cell Biol.* 19:1371–78
135. Saini N, Gordenin DA. 2020. Hypermutation in single-stranded DNA. *DNA Repair* 91–92:102868
136. Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, et al. 2007. Human CtIP promotes DNA end resection. *Nature* 450:509–14
137. 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. Correction. 2011. *Cell* 145:993
138. 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
139. Schmid JA, Berti M, Walser F, Raso MC, Schmid F, et al. 2018. Histone ubiquitination by the DNA damage response is required for efficient DNA replication in unperturbed S phase. *Mol. Cell* 71:897–910.e8

140. Schrank BR, Aparicio T, Li Y, Chang W, Chait BT, et al. 2018. Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. *Nature* 559:61–66
141. Setiaputra D, Durocher D. 2019. Shieldin—the protector of DNA ends. *EMBO Rep*. 20:e47560
142. Sfeir A, Symington LS. 2015. Microhomology-mediated end joining: a back-up survival mechanism or dedicated pathway? *Trends Biochem. Sci.* 40:701–14
143. Shibata A, Moiani D, Arvai AS, Perry J, Harding SM, et al. 2014. DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. *Mol. Cell* 53:7–18
144. Shim EY, Chung WH, Nicolette ML, Zhang Y, Davis M, et al. 2010. *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. *EMBO J.* 29:3370–80
145. Shiotani B, Zou L. 2009. Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Mol. Cell* 33:547–58
146. Somyajit K, Spies J, Coscia F, Kirik U, Rask M-B, et al. 2021. Homology-directed repair protects the replicating genome from metabolic assaults. *Dev. Cell* 56:461–77.e7
147. Soniat MM, Myler LR, Kuo H-C, Paull TT, Finkelstein IJ. 2019. RPA phosphorylation inhibits DNA resection. *Mol. Cell* 75:145–53.e5
148. Sriramachandran AM, Petrosino G, Méndez-Lago M, Schäfer AJ, Batista-Nascimento LS, et al. 2020. Genome-wide nucleotide-resolution mapping of DNA replication patterns, single-strand breaks, and lesions by GLOE-Seq. *Mol. Cell* 78:975–85.e7
149. Staples CJ, Barone G, Myers KN, Ganesh A, Gibbs-Seymour I, et al. 2016. MRNIP/C5orf45 interacts with the MRN complex and contributes to the DNA damage response. *Cell Rep*. 16:2565–75
150. 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
151. Symington LS, Gautier J. 2011. Double-strand break end resection and repair pathway choice. *Annu. Rev. Genet.* 45:247–71
152. Symington LS, Rothstein R, Lisby M. 2014. Mechanisms and regulation of mitotic recombination in *Saccharomyces cerevisiae*. *Genetics* 198:795–835
153. Taglialatela A, Alvarez S, Leuzzi G, Sannino V, Ranjha L, et al. 2017. Restoration of replication fork stability in BRCA1- and BRCA2-deficient cells by inactivation of SNF2-family fork remodelers. *Mol. Cell* 68:414–30.e8
154. Tatebe H, Lim CT, Konno H, Shiozaki K, Shinohara A, et al. 2020. Rad50 zinc hook functions as a constitutive dimerization module interchangeable with SMC hinge. *Nat. Commun.* 11:370
155. 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
156. Tkáč J, Xu G, Adhikary H, Young JTF, Gallo D, et al. 2016. HELB is a feedback inhibitor of DNA end resection. *Mol. Cell* 61:405–18
157. Tran PT, Erdeniz N, Dudley S, Liskay RM. 2002. Characterization of nuclease-dependent functions of Exo1p in *Saccharomyces cerevisiae*. *DNA Repair* 1:895–912
158. Truong LN, Li Y, Shi LZ, Hwang PY-H, He J, et al. 2013. Microhomology-mediated end joining and homologous recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *PNAS* 110:7720–25
159. Tsukamoto Y, Mitsuoka C, Terasawa M, Ogawa H, Ogawa T. 2005. Xrs2p regulates Mre11p translocation to the nucleus and plays a role in telomere elongation and meiotic recombination. *Mol. Biol. Cell* 16:597–608
160. 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
161. Wang H, Shi LZ, Wong CC, Han X, Hwang PY, et al. 2013. The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. *PLOS Genet*. 9:e1003277
162. Wang W, Daley JM, Kwon Y, Krasner DS, Sung P. 2017. Plasticity of the Mre11-Rad50-Xrs2-Sae2 nuclease ensemble in the processing of DNA-bound obstacles. *Genes Dev*. 31:2331–36

163. Wang W, Daley JM, Kwon Y, Xue X, Krasner DS, et al. 2018. A DNA nick at Ku-blocked double-strand break ends serves as an entry site for exonuclease 1 (Exo1) or Sgs1-Dna2 in long-range DNA end resection. *J. Biol. Chem.* 293:17061–69
164. Waterman DP, Haber JE, Smolka MB. 2020. Checkpoint responses to DNA double-strand breaks. *Annu. Rev. Biochem.* 89:103–33
165. Westmoreland JW, Resnick MA. 2016. Recombinational repair of radiation-induced double-strand breaks occurs in the absence of extensive resection. *Nucleic Acids Res.* 44:695–704
166. Weston R, Peeters H, Ahel D. 2012. ZRANB3 is a structure-specific ATP-dependent endonuclease involved in replication stress response. *Genes Dev.* 26:1558–72
167. Williams RS, Dodson GE, Limbo O, Yamada Y, Williams JS, et al. 2009. Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. *Cell* 139:87–99
168. Williams RS, Moncalian G, Williams JS, Yamada Y, Limbo O, et al. 2008. Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell* 135:97–109
169. Wyatt HDM, Laister RC, Martin SR, Arrowsmith CH, West SC. 2017. The SMX DNA repair trinuclease. *Mol. Cell* 65:848–60.e11
170. Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, et al. 2015. REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 521:541–44
171. Xu S, Wu X, Wu L, Castillo A, Liu J, et al. 2017. Abro1 maintains genome stability and limits replication stress by protecting replication fork stability. *Genes Dev.* 31:1469–82
172. Yamada S, Hinch AG, Kamido H, Zhang Y, Edelmann W, Keeney S. 2020. Molecular structures and mechanisms of DNA break processing in mouse meiosis. *Genes Dev.* 34:806–18
173. Yan Z, Xue C, Kumar S, Crickard JB, Yu Y, et al. 2019. Rad52 restrains resection at DNA double-strand break ends in yeast. *Mol. Cell* 76:699–711.e6
174. You Z, Shi LZ, Zhu Q, Wu P, Zhang YW, et al. 2009. CtIP links DNA double-strand break sensing to resection. *Mol. Cell* 36:954–69
175. Yu TY, Garcia VE, Symington LS. 2019. CDK and Mec1/Tel1-catalyzed phosphorylation of Sae2 regulate different responses to DNA damage. *Nucleic Acids Res.* 47:11238–49
176. Yu TY, Kimble MT, Symington LS. 2018. Sae2 antagonizes Rad9 accumulation at DNA double-strand breaks to attenuate checkpoint signaling and facilitate end resection. *PNAS* 115:E11961–69
177. Yuan J, Ghosal G, Chen J. 2009. The annealing helicase HARP protects stalled replication forks. *Genes Dev.* 23:2394–99
178. 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
179. Zakharyevich K, Ma Y, Tang S, Hwang PY, Boiteux S, Hunter N. 2010. Temporally and biochemically distinct activities of Exo1 during meiosis: double-strand break resection and resolution of double Holliday junctions. *Mol. Cell* 40:1001–15
180. Zdravković A, Daley JM, Dutta A, Niwa T, Murayama Y, et al. 2021. A conserved Ctp1/CtIP C-terminal peptide stimulates Mre11 endonuclease activity. *PNAS.* 118:e2016287118
181. 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
182. Zhou C, Pourmal S, Pavletich NP. 2015. Dna2 nuclease-helicase structure, mechanism and regulation by Rpa. *eLife* 4:e09832
183. 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
184. Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 2013. 53BP1 regulates DSB repair using Rif1 to control 5′ end resection. *Science* 339:700–4

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