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End Resection at Double-Strand Breaks: Mechanism and Regulation

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RecA/Rad51 catalyzed pairing of homologous DNA strands, initiated by polymerization of the recombinase on single-stranded DNA (ssDNA), is a universal feature of homologous recombination (HR). Generation of ssDNA from a double-strand break (DSB) requires nucleolytic degradation of the 5′-terminated strands to generate 3′-ssDNA tails, a process referred to as 5′–3′ end resection. The RecBCD helicase–nuclease complex is the main end-processing machine in Gram-negative bacteria. Mre11-Rad50 and Mre11-Rad50-Xrs2/Nbs1 can play a direct role in end resection in archaea and eukaryota, respectively, by removing end-blocking lesions and act indirectly by recruiting the helicases and nucleases responsible for extensive resection. In eukaryotic cells, the initiation of end resection has emerged as a critical regulatory step to differentiate between homology-dependent and end-joining repair of DSBs.

DSBs can arise accidentally during normal cell metabolism or after exposure of cells to DNA-damaging agents, and also serve as intermediates in a number of programmed recombination events in eukaryotic cells (Mehta and Haber 2014). The repair of DSBs is critical for maintenance of genome integrity, and misrepair, or failure to repair, is associated with chromosome rearrangements, chromosome loss, or even cell death. Both prokaryotic and eukaryotic cells have evolved elaborate mechanisms for the recognition and repair of DSBs. The two predominant repair mechanisms are HR and nonhomologous end joining (NHEJ). HR relies on the presence of an intact homologous duplex to template repair of the broken strands, whereas NHEJ repairs DSBs by direct ligation of the DNA ends. For DSBs to be repaired by HR, the ends must first be degraded to generate long 3′-ssDNA tails, a process referred to as 5′–3′ end resection. The 3′-ssDNA tails are then bound by a member of the RecA/Rad51 family of proteins to initiate homologous pairing and serve as primers for DNA synthesis following strand invasion. Strand invasion intermediates are further processed by helicases and/or nucleases (Bizard and Hickson 2014; Wyatt and West 2014), and ultimately by gap-filling DNA synthesis and ligation, to generate mature recombinant products. The DNA end-resection step of HR is conserved in all domains of life, but the mechanisms used for generating ssDNA are distinct. Here, we review the basic machinery for DNA end resection in bacteria, archaea, and eukaryota and the regulation of end resection in eukaryotic cells.

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END RESECTION IN BACTERIA

The heterotrimeric RecBCD nuclease is the major end-processing machine in *Escherichia coli* and is conserved across the majority of Gram-negative bacteria (Dillingham and Kowalczykowski 2008). RecBCD is a complex enzyme that couples ATP-dependent unwinding to DNA degradation (Smith 2001; Dillingham and Kowalczykowski 2008). The potent exonuclease activity of RecBCD can degrade thousands of bases per second. This destructive activity of RecBCD plays an important role in protecting bacteria from invading bacteriophages with linear genomes. Nuclease activity resides in the carboxy-terminal region of the RecB subunit and is regulated by RecC and by interaction with a specific sequence called Chi (5′-GCTGGTGG-3′) (Wang et al. 2000). Chi sites suppress the nuclease activity of RecBCD and stimulate recombination locally (Lam et al. 1974; Dixon and Kowalczykowski 1993). The 8-bp nonpalindromic Chi sites are overrepresented in the *E. coli* genome and are oriented toward the replication origin such that loading of RecBCD at a collapsed replication fork would lead to suppression of DNA degradation upon Chi recognition by RecBCD and activation of HR (Blattner et al. 1997).

Our current view of how RecBCD promotes recombination derives from a combination of bulk-phase biochemistry, single-DNA molecule imaging, electron microscopy (EM), and structural studies. RecBCD binds with high affinity to blunt or nearly blunt-ended linear duplex DNA (Taylor and Smith 1985). Unwinding is driven by the RecB and RecD subunits, which are helicases with opposite polarities and thus translocate the complex on both strands of duplex DNA in the same direction (Dillingham et al. 2003; Taylor and Smith 2003). The robust translocase activity of the RecBCD complex is able to displace tightly bound proteins from duplex DNA (Finkelstein et al. 2010). Under conditions in which the nuclease activity of the complex is minimized, the enzyme unwinds duplex DNA to produce one long 5′-ssDNA tail and an ssDNA loop associated with a short 3′-ssDNA tail owing to the two helicases operating at different speeds (Taylor and Smith 2003). RecD is the fast, or lead, motor on the 5′-terminated strand, whereas RecB translocates more slowly on the 3′-terminated strand until the complex encounters a Chi site (Fig. 1). Upon Chi recognition, the enzyme pauses, the RecD subunit is inactivated, and continued unwinding is driven by the RecB helicase, resulting in a slower translocation rate (Spies et al. 2003). Before encountering Chi, the 3′ end is more extensively cleaved by the RecB endonuclease than the 5-terminated strand, but after Chi recognition, degradation of the 3′ end is suppressed, and cleavage of the 5′-terminated strand is stimulated, generating a 3′-ssDNA tail (Anderson and Kowalczykowski 1997a). In addition, RecB facilitates loading of RecA onto the 3′-terminated strand after Chi recognition (Anderson and Kowalczykowski 1997b). How does Chi regulate the nuclease activities of the RecBCD complex? Structural studies indicate that a "pin" in RecC separates the strands of duplex DNA entering the complex driven by the RecB and RecD translocases (Singleton et al. 2004). As the separated strands pass through the RecBCD complex, the RecC subunit recognizes Chi, resulting in a conformational change that opens a molecular latch allowing the 3′-terminated strand to bypass the RecB nuclease domain and exit the complex (Handa et al. 2012; Yang et al. 2012).

The RecBC enzyme behaves similarly to Chi-modified RecBCD. RecBC unwinds double-stranded DNA (dsDNA) more slowly than RecBCD and constitutively loads RecA onto the 3′ end of the unwound strands. Consistent with the in vitro studies, *recD* mutants are recombination proficient and recombination is stimulated at ends instead of in the vicinity of Chi sites (Thaler et al. 1989; Churchill et al. 1999). By contrast, *recB* and *recC* mutants show high sensitivity to X rays and low frequency of recombination as measured by conjugation or transduction (Persky and Lovett 2008). However, these defects can be suppressed by inactivation of the 3′ exonucleases ExoI and SbcCD, suggesting that an alternative mechanism is able to generate 3′-ssDNA tailed intermediates in the absence of RecBCD, but the ends are unstable because of 3′ nuclease activity. Recombination in the *recBC*-suppressed strains is caused by the

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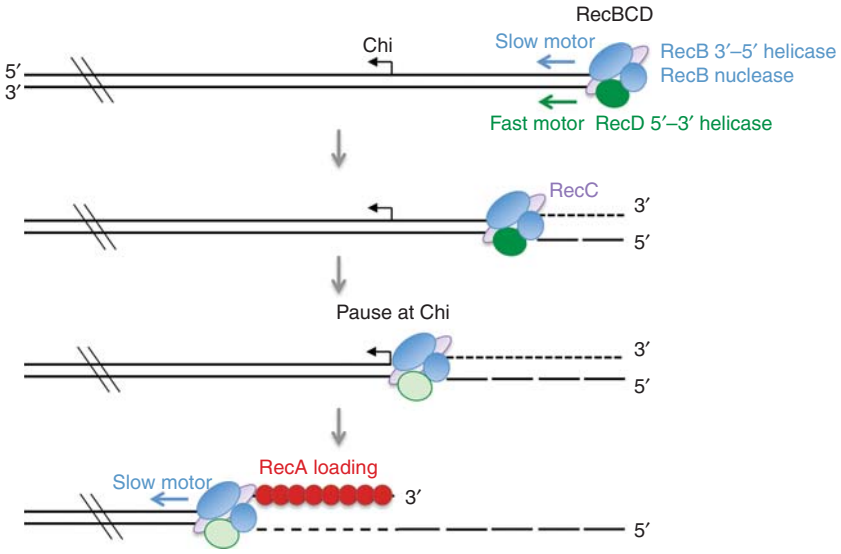

Figure 1. End processing by the RecBCD complex. RecBCD loads at ends and translocates on both strands using the RecD and RecB helicase subunits. RecB degrades both DNA strands exiting the complex, but with more incisions on the 3′ strand than the 5′-terminated strand. RecBCD pauses at a Chi site, and the RecD subunit is modified; continued translocation is driven by the RecB helicase. After Chi recognition, RecB directs loading of RecA onto the 3′ end and degrades only the 5′ strand.

RecF pathway of recombination, which normally functions during ssDNA gap repair (Persky and Lovett 2008). Resection by the RecF pathway requires the 5′–3′ exonuclease, RecJ, and is stimulated by the RecQ 3′–5′ helicase and the ssDNA-binding protein, SSB (Han et al. 2006; Handa et al. 2009). RecJ requires an ssDNA tail of >6 nucleotides for binding and degrades to the ssDNA–dsDNA junction, releasing mononucleotide products (Han et al. 2006). Although originally characterized biochemically as an ssDNA-specific exonuclease, RecJ shows limited degradation of dsDNA (Lovett and Kolodner 1989; Handa et al. 2009). In a reconstituted reaction with other RecF pathway proteins, RecJ was shown to generate sufficient ssDNA to promote RecA-catalyzed strand invasion, although the extent of degradation and joint molecule formation was less than observed in the presence of RecQ (Handa et al. 2009).

The normal function of the RecF pathway is to promote recombination at ssDNA gaps formed during replication—for example, when a UV-induced pyrimidine dimer prevents primer extension by DNA polymerase III (see Syeda et al. 2014). *recJ* and *recQ* mutants show UV sensitivity and may be required to expand ssDNA gaps to facilitate RecA binding (Persky and Lovett 2008). RecJ can also cooperate with RecB and RecC in the absence of RecD (Lovett et al. 1988; Dermic 2006). The high frequency of conjugal recombination observed in *recD* mutants is reduced by mutation of *recJ*, but not by *recQ*. The residual recombination observed in the *recD recJ* mutant requires ExoVII, which degrades ssDNA from 5′ or 3′ ends, but the *recD xseA* mutant is recombination proficient, indicating that RecJ is the main activity with ExoVII serving as a backup function (Dermic 2006).

Ironically, there appears to be no role for SbcCD in end resection in bacteria, in contrast to archaea and eukaryota, where the SbcC and SbcD orthologs, Rad50 and Mre11, respectively, play an important role in coordinating DNA end processing (see below). Instead, the main function of SbcCD is to resolve hairpin structures formed by annealing between closely spaced inverted repeats, a role that is conserved in yeast (Lobachev et al. 2002; Rattray et al. 2005; Eykelenboom et al. 2008).

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END RESECTION IN ARCHAEA

Homologs of the helicases and nucleases responsible for end resection in bacteria have not been identified in any of the archaeal genomes examined to date; RecQ-like helicases have been found but have no characterized role in end resection (Guy and Bolt 2005; Fujikane et al. 2006; Oyama et al. 2009). Mre11 and Rad50 are present in archaea, and biochemical studies suggest a role in end processing. Most of the structural analyses have been performed with the archaeal proteins; however, the functional analysis of catalytic and architectural motifs has mainly been performed in yeast (see below). Mre11 shows Mn^{2+}-dependent 3′–5′ exonuclease activity in vitro and an endonuclease activity that acts at the dsDNA–ssDNA transition of secondary structures within ssDNA (Hopfner et al. 2000a; Trujillo and Sung 2001). The genes encoding Mre11 and Rad50 are within the same operon as the HerA helicase and the NurA nuclease in thermophilic archaea, suggesting they might functionally cooperate to promote end resection (Hopkins and Paull 2008). HerA is a member of the FtsK superfamily of hexameric translocases and helicases, and NurA forms a dimer with RNaseH-like domains (Blackwood et al. 2012). HerA and NurA physically interact, and the catalytic activities are mutually interdependent (Hopkins and Paull 2008; Blackwood et al. 2012). In assays with limiting amounts of HerA and NurA, addition of Mre11 and Rad50 strongly stimulates ATP-dependent DNA degradation, requiring the helicase and nuclease activities of HerA and NurA, respectively (Hopkins and Paull 2008). Interestingly, the Mre11–Rad50 (MR) complex alone is able to remove 15–55 nt from the 5′ ends of long linear substrates in a reaction dependent on the Mre11 nuclease and Mg^{2+}. The initial processing by MR stimulates degradation by NurA but is not essential for end resection in the reconstituted reaction (Hopkins and Paull 2008).

END RESECTION IN EUKARYOTES

Much of our knowledge of the factors involved in eukaryotic end resection has come from genetic analysis in *Saccharomyces cerevisiae,* where DNA end processing can be followed physically at sites of endonuclease-generated DSBs in vegetatively dividing (mitotic) cells or Spo11-induced DSBs in meiosis. These studies identified the Mre11-Rad50-Xrs2 (MRX) complex, Sae2, Exo1, Replication Protein A (RPA), Sgs1, and Dna2 as key factors for 5′–3′ end resection, and their activities are conserved in other eukaryotes investigated (human NBS1, CtIP, and BLM are the functional orthologs of Xrs2, Sae2, and Sgs1, respectively) (Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008; Nimonkar et al. 2011; Peterson et al. 2011; Karanja et al. 2012; Chen et al. 2013). A widely accepted view is for MRX/N and Sae2/CtIP to initiate end resection by endonucleolytic cleavage of the 5′ ends internal to break ends releasing oligonucleotides. The short 3′-ssDNA tails formed are then subject to extensive resection executed via two parallel pathways. One is dependent on the 5′–3′ exonuclease, Exo1, whereas the other depends on the concerted action of the Sgs1/BLM-Top3-Rmi1 complex with the Dna2 endonuclease, hereafter referred to as STR-Dna2. The extensively resected ssDNA tracts formed vary in length from a few hundred nucleotides to tens of kilobases, depending on the availability and location of the homologous template, and correlate with the kinetics of repair (Chung et al. 2010).

Biochemical and Structural Characterization of Resection Nucleases

MRX/N

The *MRE11*, *RAD50,* and *XRS2* genes were originally identified by their essential roles for ionizing radiation (IR) resistance and meiotic recombination, and null mutations confer similar phenotypes (Mimitou and Symington 2009). Mre11, Rad50, and Xrs2/Nbs1 interact and copurify as a complex (Trujillo et al. 1998; Usui et al. 1998; Paull and Gellert 1999). Mre11 has five conserved phosphoesterase motifs in the amino-terminal half of the protein that are required for Mn^{2+}-dependent 3′–5′ dsDNA exonuclease and ssDNA endonuclease activities in

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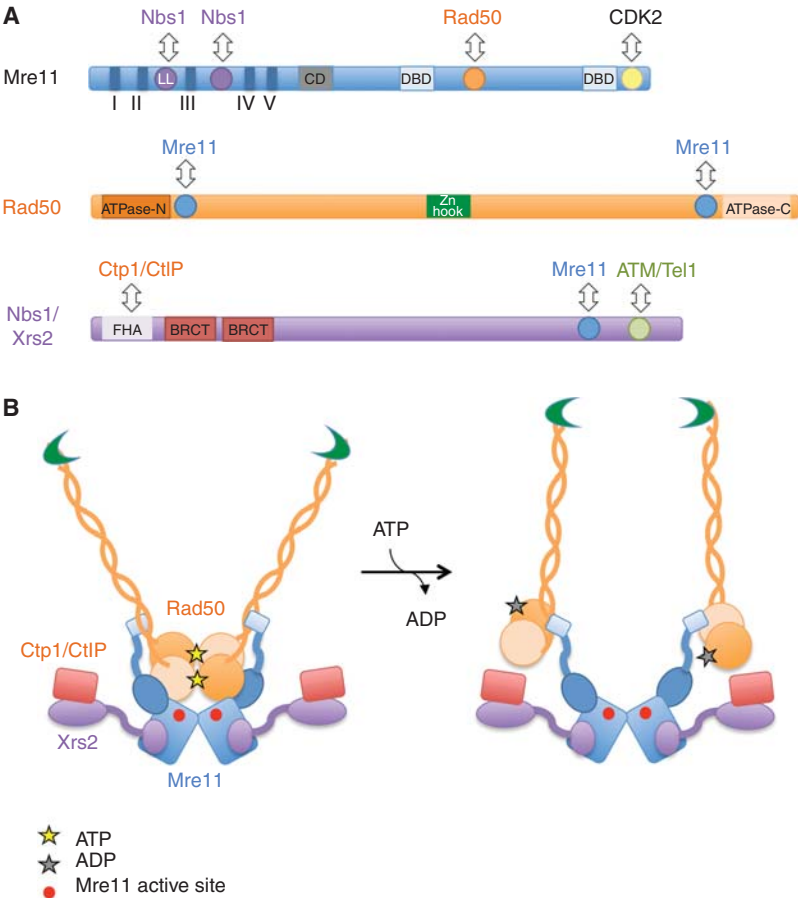

Figure 2. Structural organization of the MRX/N complex. (*A*) Schematic showing domains of the Mre11, Rad50, and Nbs1/Xrs2 proteins. Interaction domains are shown as color-coded circles, and other functional domains are indicated by rectangles. LL, latching loop; CD, capping domain; DBD, DNA-binding domain; FHA, forkhead associated; BRCT, BRCA1 carboxy-terminal domain. (*B*) Panel created from data in Lim et al. (2011) to show how ATP hydrolysis by Rad50 causes a conformational change exposing the Mre11 active site. Note that there are no structures of the entire MRX/N complex, or the complex with Sae2/Ctp1/CtIP, and the cartoon depicts the known interactions based on partial complexes.

vitro (Fig. 2A) (Bressan et al. 1998; Furuse et al. 1998; Usui et al. 1998; Moreau et al. 1999; Trujillo and Sung 2001). Substitution of conserved Asp or His residues within the nuclease motifs (e.g., D16, D56, H125, or H213 of ScMre11) with Asn or Ala abolishes exo- and endonuclease activities in vitro (Furuse et al. 1998; Usui et al. 1998; Moreau et al. 1999); hereafter, nuclease-deficient *mre11* alleles are referred to as *mre11-nd*. Mutation of His59 reduces the exonuclease activity to a greater extent than the endonuclease activity and has been used to evaluate the role of the exonuclease activity in vivo (Williams et al. 2008; Garcia et al. 2011). Two α helices of Mre11 located carboxy terminal to the nuclease core domain are responsible for interaction with the Rad50 coiled-coil base (Lammens et al. 2011; Lim et al. 2011; Williams et al. 2011). *Schizosaccharomyces pombe* Mre11 interacts with Nbs1 via a eukaryotic-specific insertion between phosphoesterases motifs II and III, referred to as the latching loop, and through additional residues in the amino-terminal region (Schiller et al. 2012). Mutations within the latching loop

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that are found in individuals with ataxia-telangiectasia-like disorder (ATLD) or Nijmegen breakage syndrome (NBS)-like disorder disrupt the interaction with Nbs1. In the absence of Xrs2/Nbs1, or point mutations that abolish the interaction with Nbs1, Mre11 fails to localize to the nucleus. Interestingly, addition of an nuclear localization signal (NLS) to Mre11 can partially suppress the DNA damage sensitivity of the *xrs2*Δ mutant, indicating that one of the main functions for Xrs2 is Mre11 localization to the nucleus (Tsukamoto et al. 2005). The carboxy-terminal 54 residues of murine Mre11 interact with cyclin-dependent kinase 2 (CDK2) to facilitate CtIP phosphorylation and stability (Buis et al. 2012).

Rad50 has a similar domain organization to the structural maintenance of chromosomes family of proteins, which are characterized by Walker A and B ATP-binding cassettes located at the amino- and carboxy-terminal regions of the primary sequence that come together by collapse of the intervening sequence to form a long antiparallel coiled-coil (Fig. 2) (Hopfner et al. 2000b). Two Rad50 ATP-binding cassettes interact with an Mre11 dimer to form a "head" domain with DNA-binding and ATP-regulated nuclease activity (Fig. 2B). The Rad50 coiled-coil domains emanate from the head and can interact with other MR complexes by Zn^{2+}-mediated dimerization of the hook domains at the apexes of the coiled-coils intramolecularly, or intermolecularly to tether linear DNA molecules (Anderson et al. 2001; Chen et al. 2001; de Jager et al. 2001; Hopfner et al. 2002; Wiltzius et al. 2005). Mre11 stabilizes dimerization of Rad50 and stimulates Rad50 ATP hydrolysis. The ATP-bound form of Rad50 negatively regulates the Mre11 nuclease activity by masking the active site of Mre11 (Lim et al. 2011). ATP hydrolysis triggers substantial conformational changes of both Rad50 and Mre11 within the MR complex, resulting in exposure of the Mre11 nuclease site and activation of DNA degradation (Lim et al. 2011; Mockel et al. 2012). Mutation of conserved residues in the Walker A-type ATPase domain confer a *rad50* null phenotype, whereas a class of mutations located close to the ATPase domain called *rad50S* behave similarly to *mre11-nd* alleles (Alani et al. 1990). Exactly how the *rad50S* mutations affect the in vitro functions of the Mre11 complex has not been determined.

Although Mre11 and Rad50 are conserved in bacteria, bacteriophage T4, and archaea, Xrs2 and Nbs1 are unique to eukaryotes and are more diverged. The amino-terminal region of Xrs2/Nbs1 has phosphoprotein-binding motifs that are separated from the Mre11 and Tel1/ATM interaction regions in the carboxyl terminus by a flexible linker (Lloyd et al. 2009; Williams et al. 2009). Xrs2 has only the conserved FHA domain, whereas Nbs1 has two BRCT domains adjacent to the FHA domain. Diphosphorylated pSDpTD motifs are Nbs1 FHA domain-binding targets and direct the interaction between Ctp1 (*S. pombe* Sae2 ortholog) and Nbs1; this interaction is important for resistance to IR and the topoisomerase I poison, camptothecin (CPT) in fission yeast (Lloyd et al. 2009; Williams et al. 2009). A conserved region within the carboxy-terminal region of Xrs2/Nbs1 is responsible for Mre11 interaction (Tsukamoto et al. 2005).

Sae2/Ctp1/CtIP

Sae2 (also known as Com1) was originally identified by its requirement to process meiotic DSBs, and the phenotype conferred by *sae2*Δ is very similar to *mre11-nd* and *rad50S* mutations (McKee and Kleckner 1997; Prinz et al. 1997; Mimitou and Symington 2009). Mammalian CtIP is thought to be the ortholog of Sae2, but sequence homology is limited to a small region of the carboxyl terminus that includes sites for cyclin-dependent kinase (CDK) and Mec1/ATR and Tel1/ATM phosphorylation, and an oligomerization motif ($LKEX_4$ EV/L) close to the amino terminus (Sartori et al. 2007; Kim et al. 2008; Wang et al. 2012). Although Sae2 does not form a stable complex with MRX in solution, together they form a higher-order complex in association with DNA (Lengsfeld et al. 2007). The similarity of the *sae2*Δ and *mre11-nd* phenotypes initially led to speculation that Sae2 activates the Mre11 nuclease, and recombinant Sae2 does indeed stimulate the 3′–5′ Mre11 exonuclease activity. In addition, Sae2 alone functions as an endonucle-

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ase and cuts a variety of branched DNA structures with a preference for cleavage within an ssDNA region near a branch or hairpin-capped end, and the activity toward hairpin structures is stimulated by MRX (Lengsfeld et al. 2007).

Exo1

Exo1 is a member of the XPG family of nucleases, which includes Rad2/XPG, Rad27/FEN-1, and Yen1/GEN1 in eukaryotes (Szankasi and Smith 1995). These proteins have conserved nuclease motifs in the amino-terminal region but have distinct substrate preferences. Exo1 shows 5′–3′ dsDNA exonuclease and 5′ flap endonuclease activities in vitro and is able to degrade from a dsDNA end or an internal nick releasing mononucleotide products (Szankasi and Smith 1992; Tran et al. 2004). Exo1 acts preferentially on dsDNA substrates with recessed 5′ ends, analogous to the ends produced by MRX and Sae2 in vivo (Cannavo et al. 2013). RPA stimulates Exo1 nucleolytic processing by binding to the ssDNA produced by end resection and preventing formation of nonproductive Exo1-ssDNA complexes (Cannavo et al. 2013). MRX and Sae2 also stimulate Exo1-catalyzed degradation, which could occur by MRX-mediated unwinding of duplex ends to create the preferred substrate for Exo1 binding and flap endonuclease activity, or by clipping the 5′ strand to generate a recessed 5′ end for the exonuclease activity (Nicolette et al. 2010; Cannavo et al. 2013). Although no direct interaction between MRX or Sae2 and Exo1 has been reported, human CtIP and EXO1 do interact, and CtIP is required for recruitment of EXO1 to damaged sites in vivo (Eid et al. 2010). In yeast, MRX is required for Exo1 localization to DSBs, but Sae2 and Mre11 nuclease activity are not, suggesting the strand-separation function of MRX might be more important for recruitment than end clipping (Paull and Gellert 1999; Shim et al. 2010; Cannon et al. 2013). BLM is also able to stimulate EXO1 degradation by increasing the affinity of EXO1 for DNA ends, but this function is independent of the ATPase activity and is not conserved in yeast (Nimonkar et al. 2011; Cannavo et al. 2013).

Sgs1/BLM-Dna2-RPA

Sgs1, BLM, and WRN are members of the RecQ family of helicases that unwind DNA by ATP-dependent 3′–5′ translocation on the 3′-terminated strand (Bernstein et al. 2010). Dna2, which is related to bacterial RecB proteins, shows helicase and ssDNA endonuclease activities in vitro (Bae et al. 1998; Budd et al. 2000). The nuclease activity of Dna2 is essential for end resection, but the helicase activity is dispensable, and Dna2 is dependent on Sgs1/BLM to generate the ssDNA substrate for degradation (Zhu et al. 2008; Cejka et al. 2010; Niu et al. 2010; Nimonkar et al. 2011). Sgs1-Dna2-catalyzed end resection is completely dependent on RPA (Cejka et al. 2010; Niu et al. 2010; Nimonkar et al. 2011; Chen et al. 2013). RPA directly interacts with Sgs1 and stimulates Sgs1 unwinding. The function of yRPA is only partially substituted by hRPA or *E. coli* SSB, suggesting a species-specific interaction is important and the role of RPA is not restricted to stabilizing the unwound strands. This is in contrast to the role of RPA in stimulation of Exo1 resection, which can be substituted by *E. coli* SSB (Cannavo et al. 2013). The Dna2 endonuclease can degrade either 3′- or 5′-terminated ssDNA; however, in the presence of RPA, the 3′ nuclease activity is attenuated and the 5′ endonuclease activity is stimulated, explaining the strand bias of end resection (Cejka et al. 2010; Niu et al. 2010). Dna2 fails to localize to DSBs in the absence of RPA, which could account for the strict RPA requirement for Dna2-catalyzed resection in vivo (Chen et al. 2013).

Top3 and Rmi1, which function with Sgs1 to dissolve double Holliday intermediates (Bizard and Hickson 2014), stimulate end resection by increasing the affinity of Sgs1 for DNA ends (Cejka et al. 2010). Unlike dissolution, the role of Top3 in end resection is independent of its catalytic activity (Niu et al. 2010). Although Top3 and Rmi1 are not essential for Sgs1-Dna2-RPA end resection in vitro, they are necessary in vivo (Zhu et al. 2008). Similarly, MRX stimulates end resection by Sgs1-Dna1-RPA by increasing Sgs1 helicase activity. The MRX stim-

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ulation can be bypassed by providing a dsDNA substrate with 5′ overhangs, suggesting MRX recruits Sgs1 to DNA ends or creates an unwound end that is the preferred substrate for Sgs1 binding (Niu et al. 2010). Sgs1 and Mre11 cofractionate after DNA damage, and MRX is required for Sgs1 and Dna2 recruitment to DSBs in vivo (Chiolo et al. 2005; Niu et al. 2010; Shim et al. 2010).

Resection of Meiotic DSBs

The Spo11 transesterase generates meiotic DSBs by forming a covalent linkage between a conserved tyrosine residue and the 5′ end of the cleaved strand (Keeney et al. 1997; Lam and Keeney 2014). A dimer of Spo11 acts to cut both DNA strands in concert. Spo11 is then removed endonucleolytically, releasing it with a short (12- to 40-nt) oligonucleotide attached (Neale et al. 2005). The *sae2*Δ/*ctp1*Δ, *rad50S*, and *mre11-nd* mutants of budding and fission yeast generate meiotic DSBs with Spo11 stably bound to the 5′ ends, suggesting the endonuclease activity of the MRX/N complex and/or Sae2/Ctp1 incises DNA internal to the DSB ends to liberate Spo11-oligonucleotides (Neale et al. 2005; Hartsuiker et al. 2009; Milman et al. 2009). Mutation of the Mre11 exonuclease activity (*mre11-H59S*) results in release of longer oligonucleotides attached to Spo11 (Garcia et al. 2011). In wild-type cells, the average length of 3′-ssDNA tails formed by end resection is ~800 nt but is reduced to ~270 nt in the *exo1*Δ mutant (Zakharyevich et al. 2010; Hodgson et al. 2011; Keelagher et al. 2011). These findings are consistent with a model whereby MRX and Sae2 incise the 5′ strand 250–300 nt from the Spo11-bound end and the Mre11 3′–5′ exonuclease degrades from the nick toward Spo11, whereas Exo1 degrades in the opposite direction, removing an additional ~500 nt (Fig. 3) (Zakharyevich et al. 2010; Garcia et al. 2011). DSB formation and processing are highly coordinated events during meiosis, and the intermediates with Spo11 attached to ends, or products of MRX-Sae2 processing are not observed in wild-type cells (Zakharyevich et al. 2010). STR-Dna2 does not contribute to resection in meiosis, except in the absence of the Dmc1 recombinase (Manfrini et al. 2010; Zakharyevich et al. 2010). Loss of Exo1 nuclease activity does not significantly impair meiotic recombination, indicating that the short ssDNA tails generated by MRX and Sae2 are sufficient for homologous pairing (Zakharyevich et al. 2010).

Resection of Endonuclease-Generated DSBs

MRX/N rapidly localizes to DSBs and precedes recruitment of RPA and Rad51 (Nelms et al. 1998; Lisby et al. 2004). MRX localizes very close to a DSB and does not spread from the break site, consistent with a role in resection initiation but not in extensive resection (Shroff et al. 2004). In the absence of MRX, DSBs generated by the HO or I-SceI endonucleases remain stable for several hours (Ivanov et al. 1994; Tsubouchi and Ogawa 1998). Resection can be initiated by Exo1, but is inhibited by Ku binding to DNA ends; the absence of Ku, or Exo1 overexpression, results in suppression of the *mre11*Δ resection initiation defect (Bressan et al. 1999; Lee et al. 2002; Tomita et al. 2003; Williams et al. 2008; Mimitou and Symington 2010; Shim et al. 2010). STR-Dna2 is unable to initiate end resection without MRX, even in the absence of Ku (Mimitou and Symington 2010). Loss of the Mre11 nuclease activity or Sae2 results in a much shorter delay in resection initiation than observed in the absence of the MRX complex, attributed to the role of MRX in recruiting Exo1, Sgs1, and Dna2 to DSBs (Fig. 3) (Llorente and Symington 2004; Mimitou and Symington 2008; Shim et al. 2010). However, the Mre11 nuclease and Sae2 are essential for processing DSBs that have covalent adducts at the 5′ ends, such as Spo11-induced DSBs (see above) or hairpin-capped ends; these phenotypes are shared by *rad50S* mutants (Mimitou and Symington 2009). It is possible that the mechanism of end resection envisioned during meiosis occurs during DSB processing in mitotic cells, with Exo1 and Mre11 degrading bidirectionally from a nick created internal to the ends. Having Exo1 initiate resection from a nick

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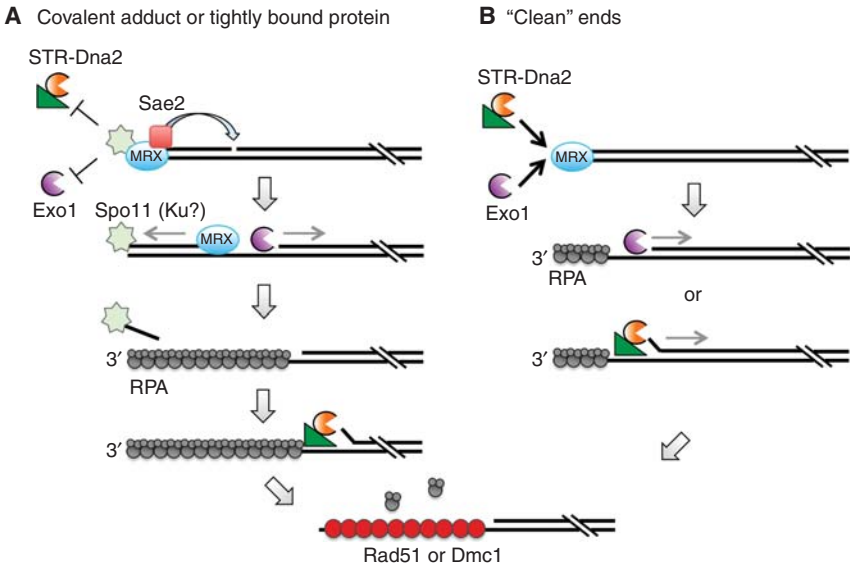

Figure 3. End processing in eukaryotes. (*A*) Resection of meiotic DSBs involves MRX- and Sae2-dependent incision of the 5′ strand ~270 nt internal to the Spo11-bound end. MRX and Exo1 degrade bidirectionally from the nick to generate an ssDNA tail of ~800 nt, and Spo11 is released from ends with a short (15- to 40-nt) oligonucleotide attached. The resulting ssDNA is bound by RPA, which is rapidly replaced by Rad51 and Dmc1 to promote strand invasion. In the absence of Dmc1, STR and Dna2 carry out more extensive end processing. This model could also apply to resection of DSBs blocked by a covalent adduct, or tightly bound protein such as Ku, in vegetatively dividing cells. (*B*) Resection of ends with no covalent modification (e.g., ends produced by the HO and I-SceI endonucleases) can initiate directly by STR-Dna2 or Exo1 via MRX recruitment. The two extensive resection mechanisms appear to function independently and redundantly at endonuclease-induced DSBs.

would overcome the problem of Ku inhibiting Exo1 at DNA ends.

In fission yeast and mammalian cells, the initial processing step by Mre11 nuclease and Sae2/Ctp1/CtIP appears to be more important for homology-dependent repair than in budding yeast (Limbo et al. 2007; Sartori et al. 2007; Buis et al. 2008; Langerak et al. 2011; Truong et al. 2013). The fission yeast *ctp1*Δ and *mre11-H134S* mutants show similar DNA damage sensitivity to the *mre11*Δ mutant, and recruitment of RPA adjacent to an HO-induced DSB is greatly reduced (Limbo et al. 2007; Williams et al. 2008). Interestingly, null mutation of *CtIP* or the $Mre11^{H129N/H129N}$ mutation (nuclease defective) causes mouse embryonic lethality, highlighting the importance of MRN-CtIP regulated processing in mammalian cells (Chen et al. 2005; Buis et al. 2008). Knockdown of CtIP in human cells, or use of a conditional $Mre11^{H129N/H129N}$ cell line, results in a dramatic reduction in resection as determined by formation of IR-induced RPA or Rad51 foci (Sartori et al. 2007; Buis et al. 2008).

In the absence of Exo1 resection initiation occurs with normal kinetics, but resection 1–5 kb from the DSB is reduced (Llorente and Symington 2004; Mimitou and Symington 2008). DNA ends are stable for ~6 h in the *mre11*Δ *exo1*Δ double mutant, but some end processing eventually occurs that must be caused by low residual STR-Dna2 activity (Tsubouchi and Ogawa 2000; Moreau et al. 2001). STR-Dna2 is mainly responsible for resection >5 kb from DSB ends and acts redundantly with Exo1 in early resection (Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008). The *mre11-nd sgs1*Δ double mutant shows synergistic sensitivity to IR and CPT, and delayed resection initiation as compared to the single mu-

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tants, suggesting STR-Dna2 is able to initiate end resection at clean ends in the absence of the Mre11 nuclease (Budd and Campbell 2009; Mimitou and Symington 2010; Shim et al. 2010). In the absence of Sgs1-Dna2 and Exo1, resection initiates by an endonucleolytic mechanism removing nucleotides from the 5′ end in increments of ~100 nt; however, the length of ssDNA tails rarely exceeds 700 nt (Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008). Depletion of RPA from cells prevents extensive resection, similar to the phenotype of the *exo1*Δ *sgs1*Δ double mutant; furthermore, the 3′ tails formed by MRX-Sae2 are unstable because of formation of secondary structures and degradation (Chen et al. 2013).

Cell Cycle, Ku, and DNA Damage Checkpoint Regulation of End Resection

HR is generally restricted to the S and G_2 phases of the cell cycle, when a sister chromatid is available as a repair template. This restriction is mainly caused by reduced end resection in G_1 compared with cycling or G_2-arrested cells (Aylon et al. 2004; Ira et al. 2004; Jazayeri et al. 2006; Barlow et al. 2008; Zierhut and Diffley 2008). Indeed, restoration of end resection in G_1 permits HR repair if a donor sequence is available (Zhang et al. 2009; Trovesi et al. 2011). Reduced resection in G_1 results from Ku binding to DNA ends, NHEJ, and low CDK (Cdc28) activity. Elimination of Ku or Dnl4 restores resection initiation to G_1-phase cells, but extensive resection is still partially defective (Clerici et al. 2008; Zierhut and Diffley 2008). Activation of Cdc28 in G_1 restores resection, whereas inhibition of Cdc28 in G_2 cells blocks resection (Aylon et al. 2004; Ira et al. 2004; Clerici et al. 2008).

Sae2 and Dna2 show S-phase-specific phosphorylation and are targets for Cdc28-mediated regulation of end resection (Baroni et al. 2004; Huertas et al. 2008; Chen et al. 2011). Mutation of Ser267 of Sae2 to a nonphosphorylatable residue, S267A, phenocopies *sae2*Δ, including hypersensitivity to camptothecin, defective sporulation, reduced hairpin-induced recombination, impaired DSB processing, and delayed Rad52 recruitment (Huertas et al. 2008). Similarly, substitution of the equivalent CDK site in human CtIP (Thr847) to alanine impairs resection in human cells (Huertas and Jackson 2009). Cell-cycle regulation of *S. pombe* Ctp1 is mainly transcriptional (Limbo et al. 2007). Mutation of CDK consensus site residues in the amino-terminal region of Dna2 abolishes Cdc28-dependent phosphorylation (Chen et al. 2011). Substitution of Thr4, Ser17, and Ser327 with alanine (*dna2-3A* mutant) reduces extensive resection but not to the same extent as *dna2*Δ or by inhibition of Cdc28. Dna2 has a bipartite nuclear localization sequence overlapping Ser17, and the *dna2-S17A* mutation reduces nuclear entry during S phase and Dna2 localization to an HO-induced DSB (Kosugi et al. 2009; Chen et al. 2011); however, extensive resection is unaffected by the *dna2-S17A* mutant, suggesting the pool of nuclear Dna2 is sufficient for end processing. Expression of Dna2 with phosphomimetic substitutions of Thr4, Ser17, and Ser327 does not override Cdc28 regulation of extensive resection, indicating that there must be other Cdc28 targets.

The DNA damage sensitivity of the *sae2*Δ/*ctp1*Δ mutant is suppressed by elimination of Ku, and the suppression requires both Exo1 and Sgs1, suggesting CDK activation of Sae2 removes Ku from DNA ends to allow access to Exo1 or STR-Dna2 to DSBs (Fig. 3) (Limbo et al. 2007; Mimitou and Symington 2010; Langerak et al. 2011). It is possible that Ku is removed from ends by MRX-Sae2 clipping, similar to Spo11, or that a dynamic equilibrium exists between MRX-Sae2 and Ku binding, and that once MRX-Sae2 initiate resection, the preferred substrate for Ku binding is no longer available. Sae2 is still required for meiosis and hairpin cleavage in the absence of Ku, indicating an essential role for Sae2 nuclease, or activation of the Mre11 endonuclease by Sae2, to process these ends (Rattray et al. 2005; Mimitou and Symington 2010). Interestingly, the meiotic defect of the *Caenorhabditis elegans com-1* (Sae2/CtIP ortholog) mutant is suppressed in the absence of Ku, suggesting that Com1/Sae2 may not play a direct role in the endonucleolytic re-

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moval of Spo11 in all organisms (Lemmens et al. 2013).

Mec1/ATR and/or Tel1/ATM phosphorylate many of the proteins involved in end resection after DNA damage. Cell-cycle and DNA damage–dependent phosphorylation of Sae2 require Mec1 and Tel1, and mutations altering the main phosphorylation sites cause DNA damage sensitivity (Baroni et al. 2004). A highly conserved ATM/ATR site in the carboxy-terminal region of CtIP (Thr859 of hCtIP or Thr818 of XCtIP) is phosphorylated in response to DSBs and is required for CtIP association with chromatin, DNA end resection, and HR repair (Peterson et al. 2013; Wang et al. 2013).

Regulation of End Resection by Chromatin Binding and Remodeling Proteins

Rad9

Rad9 is considered as an adaptor protein in the DNA damage checkpoint linking the upstream Mec1 kinase to the effector kinases, Rad53 and Chk1 (Harrison and Haber 2006). The Tudor domain of Rad9 interacts with methylated K79 of histone H3, and the BRCT domain binds to H2A sites phosphorylated by Mec1/ATR or Tel1/ATM following DNA damage (γH2A). In addition to its role in DNA damage checkpoint signaling, several studies have shown that Rad9 prevents the accumulation of ssDNA at uncapped telomeres and slows resection of endonuclease-induced DSBs (Zubko et al. 2004; Lazzaro et al. 2008; Doksani and de Lange 2014). End resection is increased in the absence of Dot1 (encodes the methyltransferase for histone H3 K79) and in the nonphosphorylatable *h2a-S129A* mutant, indicating that the end-protection function of Rad9 requires chromatin association (Lazzaro et al. 2008; Chen et al. 2012; Eapen et al. 2012). Resection of uncapped subtelomeric sequences is mostly Exo1-dependent in the presence or absence of Rad9, with only a small contribution from the Sgs1 pathway (Ngo and Lydall 2010). However, the increased resection of sequences further from uncapped ends that is seen in the *rad9Δ* mutant is mainly caused by Sgs1 activity (Ngo and Lydall 2010).

53BP1 and RIF1

53BP1 (p53 binding protein 1) shares a similar domain organization to Rad9 and plays a comparable end-protection role at telomeres and DSBs. Like Rad9, 53BP1 binds chromatin constitutively through the Tudor domain and forms γ-H2AX-dependent foci in response to IR. Simultaneous loss of the mammalian telomere-binding complex, Shelterin, and 53BP1 causes extensive resection of telomeres, which is partially dependent on CtIP, BLM, and EXO1 (Sfeir and de Lange 2012). The association of 53BP1 with DSBs in G_1 prevents ATM-dependent resection of AID- (activation-induced cytidine deaminase) or IR-induced DSBs. BRCA1 competes with 53BP1, binding to ends when cells are in the S and G_2 phases of the cell cycle to promote end resection and homology-directed repair (Escribano-Diaz et al. 2013). Interestingly, the HR defect and chromosomal instability associated with loss of BRCA1 are abrogated in the absence of 53BP1 by restoration of CtIP and ATM-dependent end resection (Bouwman et al. 2010; Bunting et al. 2010). The role of 53BP1 in preventing end resection requires RIF1 (Chapman et al. 2013; Di Virgilio et al. 2013; Feng et al. 2013; Zimmermann et al. 2013). Rif1 was originally identified as a Rap1-interacting protein and modulates telomere length in budding yeast (Hardy et al. 1992). RIF1 has no obvious telomere function in mammals, but was shown to interact with the amino-terminal domain of 53BP1 (Silverman et al. 2004). Accumulation of RIF1 at DSBs is ATM- and 53BP1-dependent and requires ATM/ATR target sites (S/TQ) within the 53BP1 amino terminus.

Chromatin-Remodeling Complexes

How the resection machinery navigates nucleosomal DNA and nonhistone protein–DNA complexes is not well understood. Nucleosomes assembled on a linear dsDNA template impede resection by Exo1 in vitro, but the inhibitory effect is less for Sgs1-Dna2, particularly if a nucleosome-free gap is adjacent to the DNA ends (Adkins et al. 2013). ATP-dependent chromatin remodeling complexes translocate on dsDNA

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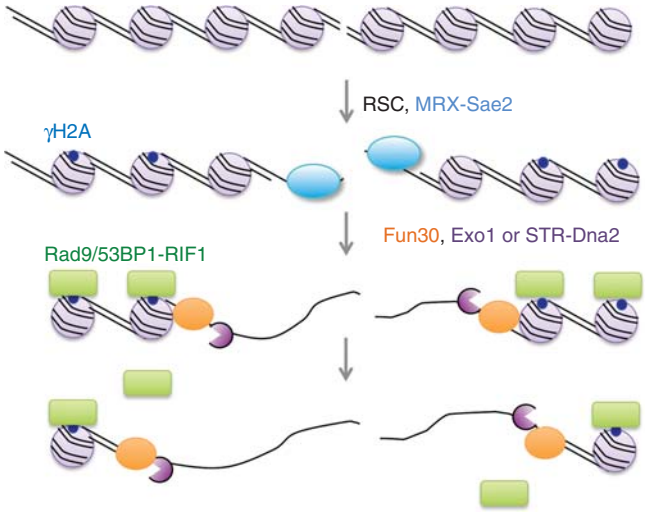

Figure 4. Chromatin and chromatin-bound proteins are barriers to end resection. The RSC complex is required for early end resection in collaboration with MRX, whereas more extensive end resection requires Fun30 acting with Exo1 and STR-Dna2. Chromatin-bound Rad9 or 53BP1-RIF1 complexes impose an additional barrier that requires Fun30.

disrupting histone-DNA contacts by nucleosome sliding, eviction, or histone exchange. In budding yeast, the RSC, SWI/SNF, INO80, SWR-C, and Fun30 remodeling enzymes are all recruited to HO-induced DSBs (Bennett et al. 2013). The RSC complex is required for early resection and promotes recruitment of the MRX complex to DSBs, whereas Fun30 is important for extensive resection (Fig. 4) (Shim et al. 2007; Chen et al. 2012; Costelloe et al. 2012; Eapen et al. 2012). A role for the INO80 complex in early resection is only apparent in the absence of Fun30 and the RSC complex (Chen et al. 2012). Although Fun30 facilitates both extensive resection mechanisms, the phenotype of *fun30*Δ is similar to *exo1*Δ and overexpression of Exo1 suppresses the DNA damage sensitivity of the *fun30*Δ mutant (Chen et al. 2012; Costelloe et al. 2012). Additionally, SMARCAD1, the human ortholog of Fun30, is required for RPA localization to laser-induced DNA damage, similar to the role of EXO1 (Costelloe et al. 2012; Tomimatsu et al. 2012). Although the recruitment of RSC, INO80, and Fun30 would be expected to precede resection, localization of these factors to DSBs is reduced in *exo1*Δ *sgs1*Δ cells; furthermore, recruitment of Sgs1, Dna2, and Exo1 is reduced in the *fun30*Δ mutant, indicating a complex interdependency. Interestingly, the extensive resection defect of the *fun30*Δ mutant is completely suppressed by elimination of Rad9, suggesting Fun30 helps to overcome the resection barrier formed by Rad9 (Chen et al. 2012; Eapen et al. 2012).

CONCLUDING REMARKS

Considerable progress has been made in identifying components of the end-resection machinery in eukaryotes, and the extensive resection pathways have been reconstituted in vitro. However, a detailed mechanistic understanding of resection initiation is lacking, in particular, how the Mre11 nuclease and Sae2/CtIP collaborate to initiate resection is poorly understood. NHEJ is a prominent repair pathway during the G_1 phase of the cell cycle in eukaryotes, and the initiation of end resection has emerged as a key regulatory step to differentiate between repair mechanisms. How the DNA damage checkpoint and CDKs coordinate to regulate resection, in particular in the chromatin context and during DNA replication, is an important issue for future studies.

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End Resection at Double-Strand Breaks: Mechanism and Regulation

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