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Mechanism and Regulation of DNA End Resection in Eukaryotes

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Abstract

The repair of DNA double-strand breaks (DSBs) by homologous recombination (HR) is initiated by nucleolytic degradation of the 5' terminated strands in a process termed end resection. End resection generates 3' single-stranded DNA tails, substrates for Rad51 to catalyze homologous pairing and exchange of DNA strands, and for activation of the DNA damage checkpoint. The commonly accepted view is that end resection occurs by a two-step mechanism. In the first step, Sae2/CtIP activates the Mre11-Rad50-Xrs2/Nbs1 (MRX/N) complex to endonucleolytically cleave the 5'-terminated DNA strands close to break ends, and in the second step Exo1 and/or Dna2 nucleases extend the resected tracts to produce long 3'-ssDNA-tailed intermediates. Initiation of resection commits a cell to repair a DSB by HR because long ssDNA overhangs are poor substrates for non-homologous end joining (NHEJ). Thus, the initiation of end resection has emerged as a critical control point for repair pathway choice. Here, I review recent studies on the mechanism of end resection and how this process is regulated to ensure the most appropriate repair outcome.

Keywords

DNA repair; recombination; double-strand break; end joining; Mre11; Sae2/CtIP; Exo1; Dna2

Introduction

DNA double-strand breaks (DSBs) can form as a consequence of normal cell metabolism or by action of exogenous agents, such as ionizing radiation (IR) or chemotherapeutic drugs. In addition, DSBs are intermediates in several programmed recombination events. Failure to repair a chromosomal DSB leads to loss of genetic information or even cell death, and inaccurate repair can cause mutagenesis or chromosome rearrangements. Indeed, defects in DSB repair are associated with developmental, immunological and neurological disorders, and can accelerate tumorigenesis (O'Driscoll, 2012).

Typically, DSBs are repaired by an end joining mechanism or by homologous recombination (HR). Non-homologous end joining (NHEJ) involves the direct ligation of DNA ends with

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little or no homology and is generally considered to be an error-prone process because nucleotides can be lost or gained at the ends prior to ligation (Chiruvella, Liang, and Wilson, 2013). Furthermore, in cells with multiple DSBs on different chromosomes, end joining can result in chromosome translocations. Classical NHEJ requires the Ku70-Ku80 heterodimer (hereafter referred to as Ku), which protects ends from degradation, and the DNA ligase IV (Dnl4/Lig4) complex to ligate ends (Chiruvella, Liang, and Wilson, 2013). NHEJ predominates in the G1 phase of the cell cycle when end resection is less active (Figure 1). Alternative Ku and Dnl4/Lig4 independent joining mechanisms (alt-NHEJ) can repair DSBs at a lower frequency than NHEJ. One form of alt-NHEJ involves alignment of ends through very short sequence homologies (generally <18 nt) internal to the ends that are revealed by end resection and is termed microhomology-mediated end joining (MMEJ) (Sfeir and Symington, 2015). If long (>200 bp) direct repeats flank the DSB, then repair can occur by annealing of the complementary 3'-ssDNA tails formed by end resection in a mechanism referred to as single-strand annealing (SSA) (Paques and Haber, 1999). In several respects SSA and MMEJ are similar, they differ by the extent of sequence homology and requirement for a dedicated annealing protein for SSA (Rad52) resulting in a higher efficiency of repair than MMEJ. SSA and MMEJ are mutagenic repair processes because they are associated with deletions and chromosome translocations (Symington and Gautier, 2011). Since SSA requires sufficient resection to expose long repeats it has frequently been used as read out for extensive resection (Karanja et al., 2012; Mimitou and Symington, 2008; Zhu et al., 2008).

HR requires a homologous DNA duplex as a template for repair of the broken chromosome, and consequently HR is restricted to the S and G2 phases of the cell cycle when a sister chromatid is present and kept in close proximity by Cohesin (Figure 1) (Symington, Rothstein, and Lisby, 2014). A chromosome homolog or ectopic sequence can be used as repair template, but there is a strong bias to use the sister chromatid (Kadyk and Hartwell, 1992). HR initiates by nucleolytic degradation of the 5'-terminated strands to generate 3' single-stranded DNA (ssDNA) tails, a process termed end resection (Paques and Haber, 1999; Mimitou and Symington, 2009). The 3' ssDNA tails are initially bound by the ubiquitous and abundant ssDNA binding protein, Replication Protein A (RPA) (Wang and Haber, 2004; Lisby et al., 2004). The recombination mediators, yeast Rad52 or human BRCA2, replace RPA with the conserved Rad51 recombinase (San Filippo, Sung, and Klein, 2008). Once Rad51 nucleates on ssDNA it can cooperatively bind to form a right-handed helical filament, displacing RPA. The Rad51 nucleoprotein filament is essential for the homology search and pairing of the ssDNA bound by Rad51 with the complementary strand of the donor duplex (San Filippo, Sung, and Klein, 2008). The 3' end of the invading strand can then be extended by DNA synthesis, followed by resolution of branched DNA structures, DNA synthesis and ligation. The reader is referred to several excellent reviews of the late steps of HR that are not described here (West et al., 2015; Bizard and Hickson, 2014).

Two-step mechanism for end resection

The nucleolytic activities responsible for end resection in eukaryotes were identified, and have been best characterized, in *Saccharomyces cerevisiae* (Mimitou and Symington, 2009). Here, I review these studies and refer to other model systems where findings have been

confirmed or differ from budding yeast. Physical analysis of resection intermediates generated at naturally occurring DSBs during meiosis and at an HO endonuclease-induced DSB in somatic cells demonstrated that ends are processed to generate 3' ssDNA tails (Sun, Treco, and Szostak, 1991; White and Haber, 1990). In meiosis, a dimer of the topoisomerase II-like protein Spo11 generates DSBs by covalent attachment to the 5' ends at recombination hotspots (Keeney, Giroux, and Kleckner, 1997; Bergerat et al., 1997). Spo11 is then removed along with a short oligonucleotide by an endonucleolytic mechanism requiring the Mre11-Rad50-Xrs2 (MRX) complex and Sae2 (Figure 2) (Mimitou and Symington, 2009; Neale, Pan, and Keeney, 2005; Garcia et al., 2011). The size of the oligonucleotide attached to Spo11 is shorter than the average length of 3' ssDNA tails in meiosis (~850 nt) suggesting MRX and Sae2 are responsible for the initial clipping to remove Spo11 prior to more extensive resection (Neale, Pan, and Keeney, 2005; Zakharyevich et al., 2010). The initiation of resection at an HO endonuclease-induced DSB is greatly delayed in *mre11*, *rad50* and *xrs2* mutants, but processing eventually occurs indicating that other nucleases can act directly on ends that lack chemical modifications (Mimitou and Symington, 2009). Once resection initiates in *mre11* or *rad50* mutants, it proceeds at around 4 kb/h, the same rate as wild-type cells (Zhu et al., 2008). Exo1 and Dna2 were identified as redundant nucleases required for extensive resection of HO-induced DSBs (Zhu et al., 2008). Dna2 is an endonuclease that requires Sgs1 helicase and the Sgs1-interacting proteins, Top3 and Rmi1 (STR complex), for resection of dsDNA (Zhu et al., 2008). In the *exo1 sgs1* mutant, resection initiates but the ssDNA tracts are short (~100–600 nt) (Mimitou and Symington, 2008; Zhu et al., 2008; Gravel et al., 2008). Elimination of MRX or Sae2 in the *exo1 sgs1* background blocks resection and is cell lethal, highlighting the importance of DNA end processing for chromosome maintenance (Mimitou and Symington, 2008).

Initiation of end resection by Mre11-Rad50-Xrs2/Nbs1 and Sae2

MRE11, *RAD50* and *XRS2* were identified by their roles in resistance to ionizing radiation (IR) and meiotic recombination (Mimitou and Symington, 2009). Null mutation of any of the genes confers a similar phenotype and the mutations are epistatic, consistent with them functioning in the same pathway. Subsequent showed the proteins form a stable complex (Carney et al., 1998; Usui et al., 1998). In addition to its important role in the initiation of HR, MRX/N is essential for NHEJ in budding yeast, and is partially required for NHEJ in mammals (Dinkelmann et al., 2009; Boulton and Jackson, 1998; Rass et al., 2009; Xie, Kwok, and Scully, 2009). Thus, MRX/N plays a pivotal role in repair pathway selection.

Mre11 and Rad50 are conserved in all phylogenetic kingdoms and even in some bacteriophages. Mre11 has phosphodiesterase motifs in the N-terminal region of the protein, and displays Mn²⁺-dependent 3'-5' dsDNA exonuclease and ssDNA endonuclease activities *in vitro* (Usui et al., 1998; Furuse et al., 1998; Moreau, Ferguson, and Symington, 1999; Paull and Gellert, 1998). Mutation of conserved residues within the phosphodiesterase motifs eliminates nuclease activity *in vitro* and confers a separation of function *in vivo* (Usui et al., 1998; Furuse et al., 1998; Moreau, Ferguson, and Symington, 1999; Bressan et al., 1998). Mre11 nuclease-defective mutants (termed *mre11-nd* here) are unable to repair meiotic DSBs because Spo11 is retained at the 5' ends; however, HO-induced DSBs are processed with close to normal kinetics and *mre11-nd* cells exhibit intermediate sensitivity

to IR, relative to *mre11* and wild-type cells (Moreau, Ferguson, and Symington, 1999; Krogh et al., 2005; Llorente and Symington, 2004). Lobachev and colleagues showed that closely spaced inverted Alu repeats stimulate mitotic recombination and this stimulation requires the Mre11 nuclease, Sae2 and other components of the MRX complex (Lobachev, Gordenin, and Resnick, 2002). The inverted repeats are thought to extrude to form a hairpin or cruciform structure that is cleaved at the base by a resolvase, or to form a fold back structure when a nearby DSB is resected. The tip of the foldback or hairpin structure would then be cleaved by MRX creating a DSB to be repaired by HR (Figure 2). The MR complex and *E. coli* SbcCD (SbcC is the homolog of Rad50, and SbcD of Mre11) cleave hairpin DNA structures *in vitro* consistent with their role in hairpin cleavage in cells (Connelly, Kirkham, and Leach, 1998; Paull and Gellert, 1999; Trujillo and Sung, 2001). While the MRX complex is required for NHEJ, DNA damage checkpoint signaling and telomere length homeostasis in yeast, the *mre11-nd* mutants are normal for these functions (Moreau, Ferguson, and Symington, 1999). In *Schizosaccharomyces pombe* and mouse cells, *mre11-nd* mutations confer greater defects in resection, HR and resistance to DNA damaging agents than is observed in budding yeast (Buis et al., 2008; Langerak et al., 2011; Williams et al., 2008). The reason for this difference is unknown, but could be due to lower compensating activity by Dna2 and/or Exo1.

Most of the *mre11-nd* mutations eliminate both exo- and endonuclease activities *in vitro*; however, the *Pyrococcus furiosus* *mre11*-H52S mutation eliminates only the exonuclease activity (Williams et al., 2008). The separation of activities is less distinct for the *S. cerevisiae* *mre11*-H59S protein (equivalent to Pfmre11-H52S), which exhibits slightly impaired endonuclease activity in addition to reduced exonuclease activity (Garcia et al., 2011). The *mre11-H59S* mutation confers an interesting phenotype during meiosis: Spo11-oligonucleotides are released but show increased size heterogeneity as compared with wild-type cells (Garcia et al., 2011). This phenotype could stem from MRX cleaving further from ends than originally proposed with the short Spo11-oligonucleotides resulting from Mre11 3'-5' exonuclease activity initiated at the nick (Figure 2), an appealing model because it provides a rationale for the conserved Mre11 3'-5' exonuclease activity.

Rad50 is a member of the SMC family of proteins, characterized by nucleotide-binding motifs at the N- and C-termini of the protein separated by long coiled-coil domains (Alani, Subbiah, and Kleckner, 1989). The coiled coils fold on themselves to create a nucleotide-binding domain (NBD) at one end of the polypeptide and the other end has a Zn²⁺-hook dimerization motif (Hopfner et al., 2002). A dimer of Mre11 interacts with the NBDs of a Rad50 dimer to form a DNA-binding head domain (Hopfner, 2014). ATP binding and hydrolysis induce large conformational changes to Rad50 that influence the Mre11 nuclease activity (Lammens et al., 2011; Lim et al., 2011; Williams et al., 2011). The Rad50 NBDs bind ATP at their interface and engage the dsDNA-binding cleft of Mre11, partially occluding the nuclease active site. In this conformation the Mre11 3'-5' exonuclease is inhibited while the endonuclease activity is unaffected. Conserved residues in the Rad50 NBD are essential for Rad50 function *in vivo* and for activation of the Mre11 endonuclease activity *in vitro* (Alani, Padmore, and Kleckner, 1990; Cannavo and Cejka, 2014). A class of *rad50* mutants (*rad50s*) is deficient in Spo11 removal and hairpin cleavage *in vivo*, but shows much higher resistance to DNA damaging agents than the *rad50* mutant, a similar

phenotype to *mre11-nd* mutants (Lobachev, Gordenin, and Resnick, 2002; Alani, Padmore, and Kleckner, 1990). The *rad50s* mutations are close to the NBD on the surface of the protein suggesting they might be important for protein-protein interactions.

Xrs2/Nbs1 is less conserved than Mre11 and Rad50 and has only been identified in eukaryotes (Stracker and Petrini, 2011). Hypomorphic mutations of *NBS1* are found in Nijmegen breakage syndrome (NBS), which is characterized by cellular radiosensitivity, immune deficiency and cancer proneness, similar to individuals with Ataxia telangiectasia (A-T) (Carney et al., 1998; Stracker and Petrini, 2011; McKinnon and Caldecott, 2007). Similarly, hypomorphic alleles of human *RAD50* and *MRE11* are associated with NBS-like and AT-like disorders (Stewart et al., 1999; Waltes et al., 2009). Xrs2/Nbs1 is required for nuclear localization of Mre11 and has a number of protein-protein interaction motifs, suggesting it functions as a chaperone and scaffold (Carney et al., 1998; Stracker and Petrini, 2011; Nakada, Matsumoto, and Sugimoto, 2003; Tsukamoto et al., 2005). The C-terminal region of Xrs2/Nbs1 contains Mre11 and Tel1/ATM binding motifs (Nakada, Matsumoto, and Sugimoto, 2003; Tsukamoto et al., 2005; Falck, Coates, and Jackson, 2005; You et al., 2005). Mutations of residues within the Mre11 interaction motif that prevent binding to Mre11 confer a phenotype indistinguishable from *xrs2* and *mre11* null mutations (Tsukamoto et al., 2005). Interestingly, expression of a 35 amino acid peptide encompassing the Mre11 interaction motif fused to a nuclear localization sequence in the *xrs2* mutant restores partial resistance to DNA damaging agents, suggesting some of the repair functions of MR are independent of Xrs2 (Tsukamoto et al., 2005). *S. pombe* Nbs1 binds to sequences in the N-terminal region of Mre11, including an insertion loop between nuclease motifs II and III that is absent from the bacterial and archaeal proteins (Schiller et al., 2012). Mutations of contact residues within the loop result in reduced Mre11 nuclear localization and are associated with NBS-like disorder in humans. Deletion of the Tel1 interaction motif results in a phenotype similar to *tel1* mutants, including a defect in DNA damage signaling and short telomeres (Nakada, Matsumoto, and Sugimoto, 2003). The N-terminal region of Nbs1 contains motifs associated with binding to phosphorylated proteins: a forkhead associated (FHA) domain and a tandem breast cancer associated C-terminal (BRCT) domain; Xrs2 has only the FHA domain. Several phosphorylated DNA repair and checkpoint proteins bind to the Nbs1/Xrs2 FHA domain, including CtIP/Ctp1/Sae2, Lif1, and Mcd1 (Chapman and Jackson, 2008; Lloyd et al., 2009; Williams et al., 2009; Palmboos et al., 2008; Wang et al., 2013).

Like Xrs2, Sae2 is poorly conserved and is only present in eukaryotes. The *S. pombe* Ctp1 and vertebrate CtIP (CtIP is also known as RBBP8) proteins are thought to be the functional orthologs of Sae2 (Limbo et al., 2007; Sartori et al., 2007; Akamatsu et al., 2008). These proteins have an N-terminal coiled-coil multimerization domain that is essential for function, and are regulated by cyclin-dependent kinase (CDK)- and DNA damage checkpoint kinase-dependent phosphorylation (Andres et al., 2015; Davies et al., 2015; Baroni et al., 2004; Huertas et al., 2008; Huertas and Jackson, 2009; Wang et al., 2012; Kim et al., 2008). The *sae2* mutant exhibits a similar phenotype to *rad50s* and *mre11-nd* mutants, including defects in Spo11 removal and hairpin opening *in vivo*, consistent with Sae2 activating the Mre11 endonuclease (Lobachev, Gordenin, and Resnick, 2002; McKee and Kleckner, 1997; Prinz, Amon, and Klein, 1997; Rattray et al., 2005). The role of Sae2 in hairpin cleavage is

puzzling because MR or MRX/N can cleave hairpin structures *in vitro* without Sae2 (Paull and Gellert, 1998; Paull and Gellert, 1999; Trujillo and Sung, 2001). Sae2 is likely to have additional cellular functions because *sae2* mutants exhibit higher sensitivity to DNA damaging agents than *mre11-nd* mutants, Mre11 persists at ends for longer in the absence of Sae2 than observed when the Mre11 nuclease is eliminated and the DNA damage checkpoint is hyper-activated in *sae2* mutants (Lisby et al., 2004; Clerici et al., 2006; Mimitou and Symington, 2010). The prolonged Mre11 retention at ends and checkpoint deactivation defects of *sae2* can be rescued by mutations in the N-terminal region of Mre11 that decrease DNA binding without affecting end resection, indicating that these defects are separable (Chen et al., 2015; Puddu et al., 2015). Although Sae2 and CtIP were reported to exhibit endonuclease activity *in vitro* (Lengsfeld et al., 2007; Makharashvili et al., 2014; Wang et al., 2014), the proteins lack homology to known nucleases and other groups have not found nuclease activity associated with highly purified Sae2 or Ctp1 (Cannavo and Cejka, 2014; Andres et al., 2015). Further investigation of these conflicting results is needed to clarify the role of Sae2/Ctp1/CtIP.

Because the polarity of the Mre11 exonuclease is opposite to that predicted for processing DSBs, interest has centered on the endonuclease activity of Mre11 and how it is activated to cleave dsDNA. MRN exhibits limited ATP-dependent melting activity *in vitro* and this could potentially generate the substrate for Mre11 cleavage (Cannon et al., 2013). In efforts to model the substrate for resection in meiotic cells, Cannavo and Cejka generated short linear duplexes with biotin/streptavidin blocks at 5' and/or 3' ends and were able to demonstrate MRX and Sae2 dependent cleavage of the 5' strand 15–20 nucleotides (nt) from the end (Cannavo and Cejka, 2014). The reaction was dependent on ATP, the Mre11 nuclease activity and phosphorylated Sae2. Variants of Rad50 deficient in ATP binding and/or hydrolysis were unable to support Sae2-stimulated MRX endonucleolytic cleavage. Furthermore, the MRX complex with the *rad50s* mutation (K81I) was also completely deficient for the endonuclease activity, consistent with *in vivo* studies. Over-expression of Sae2 has been shown to partially suppress the *rad50s* phenotype suggesting that Sae2 activates the Mre11 endonuclease via Rad50 (Clerici et al., 2005). Using plasmid-sized DNA substrates, a low level of MRX endonuclease activity was found in the absence of Sae2, in agreement with an earlier report of weak endonucleolytic activity for the archaeal MR complex (Cannavo and Cejka, 2014; Hopkins and Paull, 2008). How Sae2 activates MRX, and how the 5' strand is selected for cleavage are unanswered questions.

The MRN complex and CtIP are essential for early embryonic development and cell proliferation in mammalian cells (Chen et al., 2005; Luo et al., 1999; Xiao and Weaver, 1997; Zhu et al., 2001). In cells expressing conditional knock-out alleles or in which MRN components or CtIP have been depleted using short interfering RNAs (siRNA), DSB-induced RPA and Rad51 foci are greatly reduced (Buis et al., 2008; Sartori et al., 2007). Furthermore, a physical method to detect ssDNA formed by resection of site-specific DSBs was recently developed in human cells and verified the important roles of Mre11 and CtIP in resection (Zhou et al., 2014).

Extensive resection by Exo1 and Dna2

Although Mre11 nuclease and Sae2 are essential for processing meiosis-specific DSBs and hairpins, resection of DSBs produced by HO and I-SceI endonucleases still occurs in their absence (Llorente and Symington, 2004; Clerici et al., 2005). Exo1 is member of the Rad2/XPG family of nucleases and was first identified as a meiosis induced 5'-3' dsDNA exonuclease (Szankasi and Smith, 1992; Szankasi and Smith, 1995). The *exo1* mutant shows only mild sensitivity to DNA damaging agents and normal kinetics of resection initiation of an HO-induced DSB, but exhibits a partial defect in extensive resection (Mimitou and Symington, 2008; Llorente and Symington, 2004). Resection of endonuclease-induced DSBs is further reduced in the *exo1 mre11* mutant compared to *mre11*, and *EXO1* overexpression partially suppresses the *mre11* resection initiation defect (Lee et al., 2002; Moreau, Morgan, and Symington, 2001; Tsubouchi and Ogawa, 2000). Elimination of Ku suppresses the resection initiation defect of the *mre11*, *mre11-nd* and *sae2 /ctp1* mutants in an Exo1-dependent fashion suggesting Ku prevents access of Exo1 to DNA ends (Figure 3) (Langerak et al., 2011; Williams et al., 2008; Limbo et al., 2007; Mimitou and Symington, 2010; Shim et al., 2010; Foster, Balestrini, and Petrini, 2011). Biochemical studies with purified Ku and Exo1 support this idea (Shim et al., 2010; Krasner et al., 2015). Less Exo1 is localized to DNA ends in the absence of MRX as a result of increased Ku binding and possibly by a minor role for MRX in recruiting Exo1 (Shim et al., 2010; Clerici et al., 2008).

In the absence of Exo1 nuclease, meiotic DSBs are processed to form ssDNA tails of ~270 nt and the length of the ssDNA tails is unaffected by *sgs1* mutation (Zakharyevich et al., 2010). These data are consistent with the model that MRX initiates resection by cleaving the 5' strand ~270 nt from the end and further processing from the 5' end is carried out by Exo1 (Figure 2) (Garcia et al., 2011; Zakharyevich et al., 2010).

In vitro, purified Exo1 preferentially degrades dsDNA with a 5' recessed end (Cannavo, Cejka, and Kowalczykowski, 2013). Exo1 can also degrade from a nick in line with its role in MRX-initiated resection and mismatch repair (Szankasi and Smith, 1992; Modrich, 2006). MRX/N stimulates resection by Exo1 *in vitro*, even though the genetic studies indicate that Exo1 can function without MRX as long as Ku is absent (Mimitou and Symington, 2010; Cannavo, Cejka, and Kowalczykowski, 2013; Nicolette et al., 2010; Nimonkar et al., 2011). CtIP also physically interacts with EXO1 and negatively regulates its activity *in vitro* (Eid et al., 2010). The functional interaction between Exo1 and RPA is complex. Exo1 is recruited to DSBs in the absence of RPA and forms brighter foci; however, RPA is required for extensive resection by Exo1 *in vivo* (Chen, Lisby, and Symington, 2013; Myler et al., 2016). Independent *in vitro* studies using reconstituted proteins have reported that RPA could both stimulate and inhibit yeast Exo1 (Cannavo, Cejka, and Kowalczykowski, 2013; Nicolette et al., 2010), conflicting data have also been obtained with the human proteins (Nimonkar et al., 2011; Yang et al., 2013; Genschel and Modrich, 2003). The stimulatory role of RPA was proposed to occur by preventing non-specific interaction of Exo1 with ssDNA (Cannavo, Cejka, and Kowalczykowski, 2013). Single-molecule fluorescence imaging has shown that yeast and human Exo1 are both processive nucleases that are rapidly stripped from DNA by RPA attenuating resection (Myler et al., 2016).

Multiple cycles of binding and release would be required for extensive resection. Mammalian cells have another ssDNA binding complex, SOSS1, which contains a subunit related to *E. coli* SSB, hSSB1, shown to stimulate the resection activity of EXO1 (Yang et al., 2013). Human BLM helicase (the ortholog of yeast Sgs1) interacts with EXO1 and stimulates its nuclease activity *in vitro* in a helicase-independent manner (Nimonkar et al., 2008). To date, there is no evidence to suggest a similar interaction between yeast Exo1 and Sgs1.

Substantial resection of an HO-induced DSB occurs in the absence of Exo1 and Sae2 or the Mre11 nuclease indicating that at least one other nuclease is active. The *sgs1* and/or *dna2* mutations were shown to synergize with *exo1* to prevent extensive resection of endonuclease-induced DSBs (Mimitou and Symington, 2008; Zhu et al., 2008; Gravel et al., 2008). *DNA2* is essential for viability due to its role in Okazaki fragment processing (Bae et al., 2001), consequently, the *sgs1* mutation is more often used to study end resection. Dna2 has helicase and endonuclease activities, but only the nuclease is required for resection (Cejka et al., 2010; Niu et al., 2010). Instead, Dna2 relies on the Sgs1/BLM helicase to generate ssDNA for cleavage (Nimonkar et al., 2011; Cejka et al., 2010; Niu et al., 2010). The Sgs1 interacting partners, Top3 and Rmi1 are essential for Dna2-Sgs1 catalyzed resection in cells, and exhibit a stimulatory effect on the reaction *in vitro* by increasing Sgs1 recruitment to DNA ends (Zhu et al., 2008; Cejka et al., 2010; Niu et al., 2010). Unlike the decatenation activity of Top3, which is important for removing late recombination intermediates, resection does not require Top3 catalytic activity. MRX/N interacts with Sgs1/BLM and promotes its association with DNA ends resulting in more efficient DNA unwinding (Nimonkar et al., 2011; Cejka et al., 2010; Niu et al., 2010). In *Xenopus* egg extracts, DNA2 partners with WRN, another member of the RecQ family of helicases, to catalyze end resection (Liao, Toczylowski, and Yan, 2008; Toczylowski and Yan, 2006; Yan et al., 2011). *In vivo* studies support roles for both WRN and BLM in DNA2-catalyzed resection in mammalian cells (Gravel et al., 2008; Sturzenegger et al., 2014; Thangavel et al., 2015). Dna2 can cleave 5' or 3' flaps *in vitro*, but in the presence of RPA cleavage is directed to the 5' strand and the 3' strand is protected (Bae et al., 2001; Cejka et al., 2010; Niu et al., 2010). Dna2 fails to be recruited to DSBs when RPA is depleted from cells resulting in a block to extensive resection (Chen, Lisby, and Symington, 2013). RPA is also required to stabilize ssDNA generated by the Sgs1/BLM and WRN helicases, and is considered an obligate factor for resection by Dna2-STR (Nimonkar et al., 2011; Cejka et al., 2010; Niu et al., 2010; Yan et al., 2011).

Genetic studies suggest that Dna2-STR provides an alternative mechanism to initiate resection when Sae2 or the Mre11 nuclease is absent (Figure 3). The *sae2* mutation is lethal when combined with *sgs1*, and the lethality is suppressed if Ku is eliminated or if Exo1 is over expressed (Mimitou and Symington, 2010). Mutation of *MRE11* or *RAD50* does not cause lethality in the *sgs1* background, although cells grow very slowly, suggesting the *sae2 sgs1* lethality is not due solely to the end resection defect (Bernstein et al., 2013). Moreover, the *mre11 sae2 sgs1* triple mutant is viable suggesting it is retention of MRX at ends that causes lethality in *sae2 sgs1* cells (Hardy et al., 2014). The *mre11-nd sgs1* double mutant is viable, but grows slowly and early resection is greatly reduced (Mimitou and Symington, 2010; Shim et al., 2010; Budd and Campbell, 2009). In

yeast, MRX is required for Dna2 and Sgs1 localization to DSBs potentially explaining the greater resection defect observed for *mre11* compared with *mre11-nd* cells (Shim et al., 2010). Although Dna2 recruitment to DSBs is unaffected by *sae2*, a recent study showed that DNA2 localization to DSBs in vertebrate cells requires CtIP (Hoa et al., 2015). Furthermore, CtIP and Dna2 cooperate to promote end resection in *Xenopus* extracts (Peterson et al., 2013). The recruitment function could be by direct physical interaction between the MRN or CtIP and DNA2, or potentially by RPA bound to the short tracts of ssDNA formed by resection initiation or MRN-mediated duplex DNA melting. Thus, Dna2-STR is able to initiate end resection in the absence of MRX-Sae2 clipping and appears to be less inhibited by Ku binding to ends. By contrast, Exo1 initiates resection from ends poorly, except when Ku is absent. Notably, Dna2 requires a free 5' end for endonucleolytic cleavage explaining why it is unable to remove Spo11, but can function at an HO-induced DSB (Balakrishnan et al., 2010).

Physiological role of extensive resection

In the absence of a homologous template to direct repair, an endonuclease-induced DSB undergoes extensive resection, up to 50 kb from each end over 12 hours (Zhu et al., 2008). The physiological relevance of extensive resection by Exo1 and/or Dna2-STR is not obvious because the short ssDNA tails generated by MRX-Sae2 are sufficient for meiotic recombination (Zakharyevich et al., 2010). In mitotic cells, sister-chromatid repair of IR-induced DSBs is also unaffected by *exo1* and *sgs1* mutations, but the efficiency of ectopic recombination is reduced by ~30% (Zhu et al., 2008; Westmoreland and Resnick, 2016). The DNA damage checkpoint is defective in the *exo1 sgs1* mutant due to insufficient ssDNA to activate the Mec1/ATR kinase (Zhu et al., 2008; Gravel et al., 2008). Thus one possible explanation for the ectopic recombination defect is that pairing is slower from a non-sister template necessitating cell cycle arrest to allow more time for repair before chromosome segregation. There are pathological consequences of extensive resection: 3' ends become unstable over time and clustered mutations are generated due to the inherent lability of ssDNA compared with dsDNA (Zierhut and Diffley, 2008; Roberts et al., 2012). The 3' ssDNA tails would normally be bound and protected by RPA, but when RPA is depleted from cells the ssDNA forms secondary structures that can be attacked by structure-selective endonucleases, including MRX-Sae2 (Chen, Lisby, and Symington, 2013). The failure of MRX-Sae2 to remove foldback structures formed when RPA is dysfunctional can lead to palindromic gene amplification and more complex chromosome rearrangements (Deng et al., 2015).

Resection and the DNA damage checkpoint

The DNA damage checkpoint responds to DSBs, and to ssDNA formed by end resection or by replicative stress (Ciccio and Elledge, 2010). The sentinel kinases for the response are Tel1/ATM and Mec1/ATR, members of the phosphoinositide 3-kinase-related protein kinase (PIKK) family (Gobbini et al., 2013). The MRX/N complex functions as a sensor for DSBs and, after end binding, recruits and activates Tel1/ATM (Ciccio and Elledge, 2010). The RPA-coated ssDNA formed by end resection recruits Mec1-Ddc2/ATR-ATRIP (Zou and Elledge, 2003); in turn, the DNA damage checkpoint diminishes resection to prevent

excessive ssDNA formation (Symington and Gautier, 2011; You et al., 2009; Shiotani and Zou, 2009). In yeast, end resection is rapid and the primary checkpoint signal in response to DSBs is from the Mec1; however, if resection initiation is delayed, for example, in the *sae2* mutant, MRX is retained at ends for longer, Tel1 is hyper-activated and the *mec1* checkpoint defect is partially bypassed (Usui, Ogawa, and Petrini, 2001). The substrates for Mec1 and Tel1 are largely overlapping and include yeast H2A (H2AX in metazoans), the downstream effector kinases Rad53/CHK2 and Chk1, Rad9/53BP1, Sae2/CtIP, Dna2 and RPA (Baroni et al., 2004; Ciccica and Elledge, 2010; Gobbini et al., 2013; Brush et al., 1996; Chen et al., 2011). Phosphorylated H2A/H2AX (γ H2A/ γ H2AX) extends over large chromosomal domains and plays an important role in recruitment of other checkpoint proteins (Ciccica and Elledge, 2010).

The DNA damage checkpoint acts in both positive and negative ways to regulate end resection. The substitution with alanine of five of the Sae2 S/TQ sites, which conform to the consensus phosphorylation motif of Mec1 and Tel1, prevents DNA damage-dependent phosphorylation and the *sae2-5A* mutant is phenotypically similar to *sae2* (Baroni et al., 2004). Phosphorylation of Thr-90 of Sae2 mediates interaction with the FHA domains of Xrs2, Rad53 and Dun1, whereas Rad53 and Dun1 additionally interact with phosphorylated Thr-279 (Liang, Suhandynata, and Zhou, 2015). The *sae2-2AQ* mutant (T90A, T279A) is more resistant to DNA damaging agents than *sae2* and *sae2-5A*, but exhibits prolonged Rad53 activation, similar to the *sae2* mutant. Thr-859 of human CtIP (CtIP-T818 of *Xenopus*), equivalent to Sae2 Thr-279, is phosphorylated in response to DSBs and is required for CtIP association with chromatin, DNA end resection and HR repair (Wang et al., 2013; Peterson et al., 2013). Phosphorylation of *S. pombe* Ctp1 by casein kinase 2 is required for its interaction with the FHA domain of Nbs1 (Lloyd et al., 2009; Williams et al., 2009). Phosphorylation of Sae2 is suggested to alter the oligomeric state and solubility of Sae2 in response to DNA damage (Fu et al., 2014). Dna2 is also phosphorylated in response to DNA damage but the functional consequences of this modification are currently unknown (Chen et al., 2011).

Although the DNA damage checkpoint is important for resection initiation, it acts in a negative fashion to limit accumulation of long tracts of ssDNA in the genome (Figure 4). Rad9 is an adaptor protein that is phosphorylated by Mec1 and/or Tel1 and is required for activation of the Rad53 kinase (Harrison and Haber, 2006). Rad9 interacts with dimethylated histone H3 (H3-K79) and γ H2A in the vicinity of DSB sites forming a barrier to end resection by Exo1 and Dna2-STR (Chen et al., 2012; Lazzaro et al., 2008; Ngo and Lydall, 2015). Both chromatin modifications contribute to Rad9 recruitment and inhibition of end resection (Chen et al., 2012; Lazzaro et al., 2008; Eapen et al., 2012). Rad9 also prevents extensive resection by Exo1 and Dna2-STR at uncapped telomeres (Ngo et al., 2014; Ngo and Lydall, 2010). Elimination of Rad9 can suppress the *sae2* resection initiation defect by allowing more efficient recruitment of Dna2-STR to DSBs (Bonetti et al., 2015; Ferrari et al., 2015). Similarly, partial loss of function alleles of *TEL1* and *RAD53* that reduce Rad9 accumulation at DSBs can also suppress the *sae2* resection defect (Gobbini et al., 2015). Elimination of Tel1 causes a modest delay in resection initiation while the *mec1* mutant exhibits increased end resection due to reduced Rad9 recruitment (Clerici et al., 2014; Mantiero et al., 2007). The mechanism by which Rad9 inhibits resection is not fully

understood, but is dependent on chromatin association and oligomerization of Rad9 suggesting it forms a physical barrier to Exo1 and Dna2-STR (Ferrari et al., 2015). Activation of Rad53 could also contribute to the Rad9 inhibition of end resection (see below).

The 9-1-1 PCNA-like damage clamp binds to the junction of ssDNA and dsDNA at recessed 5' ends and contributes to Mec1 activation, and thereby Rad9 recruitment to DSBs. When the 9-1-1 complex is removed (e.g., by *mec3* mutation), extensive resection at DSBs is increased but not to the same extent as seen for the *rad9* mutation (Ngo and Lydall, 2015). The increase in long-range resection observed in the *mec3* mutant is *SGS1*, but not *EXO1* dependent, suggesting 9-1-1 facilitates resection by Exo1. At DSBs and uncapped telomeres, 9-1-1 contributes to recruitment and activity of Exo1 and Dna2-STR; however Dna2-STR is more inhibited by Rad9 at DSBs than at telomeres (Ngo and Lydall, 2015; Ngo et al., 2014). Consequently, extensive resection at uncapped telomeres shows greater dependence on 9-1-1, whereas at DSBs the stimulatory function of 9-1-1 is mainly through Exo1 (Figure 4). Moreover, the purified human 9-1-1 complex stimulates end resection by EXO1 and DNA2 *in vitro* (Ngo et al., 2014). PCNA also facilitates recruitment of human EXO1 to damaged sites and promotes processive degradation by EXO1 *in vitro* (Chen et al., 2013).

53BP1, the metazoan ortholog of Rad9, acts in a similar fashion to inhibit end resection at DSBs and at telomeres (Zimmermann and de Lange, 2014; Sfeir and de Lange, 2012). The increased resection observed in the absence of 53BP1 is dependent on CtIP, but this could be through the role of CtIP in recruiting DNA2 (Hoa et al., 2015). 53BP1 inhibits end resection via three effector proteins: RIF1, PTIP and REV7/MAD2L2 that interact with ATM-phosphorylated 53BP1 (Chapman et al., 2013; Di Virgilio et al., 2013; Escribano-Diaz et al., 2013; Feng et al., 2013; Zimmermann et al., 2013; Callen et al., 2013; Boersma et al., 2015; Xu et al., 2015).

Another mechanism to prevent long-range resection is through inhibition of Exo1 independently of Rad9/53BP1. In budding yeast, Rad53 phosphorylates Exo1 and this phosphorylation is proposed to inhibit its activity (Morin et al., 2008). ATR-dependent phosphorylation of EXO1 in mammalian cells triggers its ubiquitylation and subsequent degradation following fork stalling (El-Shemerly et al., 2005); it is not known whether a similar mechanism operates to regulate EXO1 activity at DSBs. The HELB 5'-3' ssDNA translocase was recently identified as a 53BP1-independent inhibitor of end resection in mammalian cells (Tkac et al., 2016). HELB is recruited to ssDNA by interacting with RPA and inhibits extensive resection by EXO1 and DNA2. Interestingly, HELB1 is exported from the nucleus during S phase, concomitant with the up-regulation of end resection. Expression of a variant of HELB lacking the nuclear export signal potently inhibited end resection, even in *53BP1*^{-/-} cells. Unlike 53BP1, HELB has no role in promoting NHEJ consistent with its function of inhibiting extensive resection and not resection initiation.

Cell cycle regulation of end resection

As noted in the Introduction, resection is reduced in G1 phase cells and is activated at the G1 to S transition to coordinate with DNA replication (Zierhut and Diffley, 2008; Ira et al.,

2004; Aylon, Liefshitz, and Kupiec, 2004; Barlow, Lisby, and Rothstein, 2008). In yeast, low resection in G1 is due to both reduced CDK activity and Ku binding to DNA ends (Clerici et al., 2008; Ira et al., 2004; Aylon, Liefshitz, and Kupiec, 2004). Inhibition of CDK in G2/M phase-arrested cells inhibits end resection whereas activation of CDK in G1 phase promotes resection (Clerici et al., 2008; Ira et al., 2004; Aylon, Liefshitz, and Kupiec, 2004).

Elimination of Ku in G1-arrested cells causes increased Mre11 and RPA association with DSBs and greater Mre11-dependent resection initiation, but long-range resection is not fully restored (Clerici et al., 2008; Barlow, Lisby, and Rothstein, 2008). By contrast, resection in G2-M phase arrested cells is unaffected by Ku. Inhibition of CDK in *yku80* (Ku is encoded by *YKU70* and *YKU80* in yeast) G1 or G2-M arrested cells prevents long-range resection, but not short-range resection suggesting Ku controls short-range resection by modulating MRX and Exo1 access to ends, whereas high CDK activity is required for resection initiation when Ku is present as well as for extensive end processing (Clerici et al., 2008). In contrast to *yku70*, the *rad9* mutant does not exhibit increased resection in G1-arrested cells; however, in the *yku70 rad9* double mutant both initiation and extensive resection can occur in G1-arrested cells (Trovati et al., 2011). Since Dna2 is not active in G1 cells (see below), resection under these circumstances is most likely due to Exo1 activity.

Some resection factors are constitutively expressed throughout the cell cycle while others are regulated transcriptionally and/or post-transcriptionally. Expression of *S. pombe* Ctp1 is induced in S-phase and in response to DNA damage, whereas Sae2 is constitutively expressed (Limbo et al., 2007; Akamatsu et al., 2008; Baroni et al., 2004). In budding yeast and human cells, CDK-dependent phosphorylation of Sae2/CtIP residue S267/T847 is required for end resection (Huertas et al., 2008; Huertas and Jackson, 2009; Manfrini et al., 2010). Expression of the phosphomimetic S267E/T847E mutant of Sae2/CtIP is able to partially rescue the block to resection in G1 phase cells consistent with this modification playing a major role in the cell cycle control of resection (Huertas et al., 2008; Huertas and Jackson, 2009). The *CtIP^{T847A/T847A}* homozygous mutation causes mouse embryonic lethality, suggesting that end resection is the essential function for CtIP in mammalian cells (Polato et al., 2014). CDK-dependent phosphorylation of CtIP is required for subsequent damage-induced phosphorylation by ATM and/or ATR ensuring activation of CtIP at the correct cell-cycle stage (Wang et al., 2013). Similarly, Nbs1-dependent DNA damage-induced phosphorylation of Ctp1 is eliminated by mutation of Ctp1 consensus casein kinase 2 sites (Lloyd et al., 2009). CDK-dependent phosphorylation of CtIP serine 327 is required for interaction with BRCA1, but this modification is not required for end resection or HR, and homozygous *CtIP^{S327A/S327A}* mice develop normally (Polato et al., 2014; Reczek et al., 2013; Nakamura et al., 2010). CDK also phosphorylates yeast Dna2 to promote nuclear entry, association with DSBs and extensive resection (Chen et al., 2011). Although there is no evidence for CDK-mediated regulation of Exo1 in yeast, human EXO1 is phosphorylated by CDK, and substitution of the target serine residues with alanine reduces RPA recruitment to DSBs and HR repair (Tomimatsu et al., 2014). Transcription of *EXO1* is elevated during meiosis in several organisms, consistent with its role in meiotic DSB processing (Zakharyevich et al., 2010; Szankasi and Smith, 1992; Digilio et al., 1996; Tishkoff et al., 1998).

Resection and repair pathway choice

NHEJ can occur throughout the cell cycle, but is favored in G1 cells when end resection activity is low; conversely, activation of end resection in S phase coordinates with HR. Although HR is considered to be a mostly accurate repair mechanism, this only applies to recombination between identical sister chromatids. Gene conversion between homologs carries the risk of local loss of heterozygosity (LOH), and if associated with a crossover can cause LOH from the point of exchange to the telomere. Furthermore, a crossover between repeats on different chromosomes results in translocation or more complex rearrangements. Thus, NHEJ might be considered a safer option than HR between non-sister chromatids, particularly in organisms with many repeated sequences.

Early evidence for competition between DSB repair pathways was obtained in mammalian cells where it was shown that elimination of Ku results in an increased frequency of DSB-induced HR between direct repeats (Pierce et al., 2001). Removal of Lig4 also increased HR, but not to the same extent as Ku, in agreement with the dual role of Ku in suppressing end resection and promoting NHEJ (Pierce et al., 2001). Even in G1-arrested yeast cells, Rad51-dependent gene conversion between ectopic repeats can be restored in the absence of Ku (Trovesi et al., 2011). Interestingly, crossovers were not detected in G1 *yku70* cells, indicating an additional layer of regulation by CDK beyond end resection. Elimination of Sae2 or the Mre11 nuclease results in a much higher frequency of NHEJ (Huertas et al., 2008; Deng et al., 2014; Lee and Lee, 2007). Similarly, a small molecule analog of mirin, which specifically inhibits the MRE11 endonuclease activity and prevents formation of IR-induced RPA foci, promotes NHEJ in human cells (Shibata et al., 2014; Dupre et al., 2008). On the other hand, elimination of Rad51 or Rad52 in yeast does not increase NHEJ (Deng et al., 2014; Lee and Lee, 2007), consistent with the view that once resection initiates cells are committed to HR and NHEJ is not an option.

Resection initiation also creates substrates for repair by mutagenic MMEJ. While this mechanism is inefficient in yeast, unless microhomologies of >14 nt are close to the ends, it is quite frequent in metazoan cells (Sfeir and Symington, 2015; Deng et al., 2014; Villarreal et al., 2012). In one study that directly compared different repair outcomes in mammalian cells, MMEJ was shown to occur at 10–20% of the frequency of gene conversion (Truong et al., 2013). Consistent with the requirement for resection initiation, MMEJ is dependent on Mre11 and CtIP, whereas EXO1 and BLM are dispensable (Truong et al., 2013; Bennardo et al., 2008). Resection initiation by Sae2 and Mre11 nuclease is not limiting for MMEJ in yeast, instead RPA inhibition of annealing between microhomologies accounts for the low frequency of MMEJ (Deng et al., 2014).

In yeast, removal of Ku from DNA ends is thought to result primarily from MRX/N and Sae2/Ctp1 clipping activity (Langerak et al., 2011; Mimitou and Symington, 2010). A recent study provided evidence that ubiquitylation of Ku is an additional mechanism to eject Ku from DSBs in mammalian S/G2 phase cells. RNF138, a ubiquitin E3 ligase, was identified in a screen for positive regulators of HR (Ismail et al., 2015). RNF138 is recruited to DNA damage sites where it stimulates end resection and promotes HR repair thereby contributing to cellular resistance to DNA damaging agents. Elimination of RNF138 results in decreased

ubiquitylation of Ku80 and retention of Ku at DSBs in S/G2 phase cells resulting in an increased frequency of NHEJ repair.

The tumor suppressors 53BP1 and BRCA1 play antagonistic roles in resection and repair pathway choice in mammalian cells. 53BP1 is enriched in the vicinity of DSBs in G1 phase cells via interaction with dimethylated histone H4 (H4-K20) and histone H2A ubiquitylated on K15. 53BP1 plays an important role in promoting NHEJ by preventing end resection and increasing chromosome mobility (Zimmermann and de Lange, 2014). As cells progress to S phase, 53BP1 is replaced by BRCA1, which interacts directly with MRN and CtIP (Chapman et al., 2012; Chen et al., 2008). Exactly how BRCA1 influences end resection is not well defined, but activation of end resection is concomitant with replacement of 53BP1 by BRCA1 on chromatin. Interestingly, the cell lethality, HR defects and chromosome 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). Indeed, removal of 53BP1 alleviates the embryonic lethality and tumorigenesis associated with BRCA1 deficiency. By contrast, removal of Ku does not reverse the cell and embryonic lethality of *BRCA1*^{-/-} cells even though it does partially restore Rad51 foci and decrease radial chromosome formation (Bunting et al., 2010; Bunting et al., 2012).

ATM sites in the N-terminal region of 53BP1 are essential for function suggesting 53BP1 promotion of NHEJ and inhibition of BRCA1 recruitment requires phosphorylation-dependent protein interactions (Chapman et al., 2012). RIF1 and PTIP were identified as downstream effectors of 53BP1 that are recruited to different subsets of ATM-dependent phosphorylation sites (Callen et al., 2013). RIF1 and PTIP both repress end resection, but promote NHEJ in different contexts. RIF1 is more important for physiological NHEJ (immunoglobulin class switch recombination [CSR]) and less effective in promoting pathological NHEJ that occurs in the absence of BRCA1 or at unprotected telomeres. On the other hand, PTIP is dispensable for CSR and appears to promote the toxic NHEJ events that result from BRCA1 deficiency. PTIP interacts with Artemis, a nuclease required for hairpin opening and end processing during NHEJ (Wang et al., 2014). Similar to PTIP, Artemis depletion partially rescued the PARP inhibitor sensitivity of BRCA1-deficient cells by restoring Rad51 foci. REV7 is an additional factor that acts downstream of 53BP1 to repress end resection and promote NHEJ. Like loss of 53BP1, REV7 deficiency restores HR to *BRCA1*^{-/-} cells (Boersma et al., 2015; Xu et al., 2015). The mechanism by which REV7 is recruited to DSBs is currently unknown, but does require the N-terminal ATM phosphorylation sites of 53BP1. Whether RIF1, PTIP and REV7 interact simultaneously with 53BP1 or form distinct sub-complexes with different activities remains to be determined.

BRCA1 interacts with BRCA2 and PALB2 to mediate RAD51 assembly at resected DSBs during S and G2 phases of the cell cycle. Elimination of 53BP1 allows BRCA1 recruitment in G1 phase cells, but not its binding partners PALB2 and BRCA2 (Escribano-Diaz et al., 2013; Orthwein et al., 2015). A recent study showed that PALB2 is ubiquitylated in G1 cells and this prevents its interaction with BRCA1 (Orthwein et al., 2015). Removal of the E3 ubiquitin ligase component, KEAP1, restores BRCA1-PALB2 interaction; however, KEAP1 is not cell cycle regulated raising the question of how the interaction between BRCA1 and

PALB2 is recovered in S-G2. The USP11 deubiquitylase, which had previously been identified as a positive regulator of HR (Wiltshire et al., 2010), is rapidly turned over in G1 phase cells and the HR defect caused by its deletion is reversed by removal of KEAP1 (Orthwein et al., 2015). Although BRCA1-PALB2 interaction was restored in G1 phase *53BP1*^{-/-} *KEAP1*^{-/-} cells, radiation-induced RAD51 foci were not detected suggesting there is insufficient ssDNA formed for productive RAD51 nucleoprotein filament formation. Indeed, expression of the phosphomimetic T847E mutant of CtIP in these cells resulted in formation of damage-induced RAD51 foci and partial restoration of HR during G1 (Orthwein et al., 2015).

Resection within the context of chromatin

As described above, the chromatin surrounding DSBs undergoes extensive modification and several different chromatin remodelers are recruited to DSBs. ATP-dependent chromatin remodelers translocate on dsDNA disrupting histone-DNA contacts by nucleosome sliding, eviction or by histone exchange. Genetic studies in budding yeast demonstrated an important role for the RSC complex in recruitment of the MRX complex to DSBs and early resection, while Fun30 is important for extensive resection (Chen et al., 2012; Eapen et al., 2012; Costelloe et al., 2012; Shim et al., 2007). 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). While Fun30 facilitates both extensive resection mechanisms, the phenotype of *fun30* is more similar to *exo1* and over-expression of Exo1 suppresses the DNA damage sensitivity of the *fun30* 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). Interestingly, the extensive resection defect of the *fun30* 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). 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). It will be of interest to examine end resection with nucleosomal substrates to determine whether the chromatin remodelers are required to move nucleosome prior to resection or allow resection in the context of nucleosomes.

Programmed versus accidental DSBs

A number of different cell types, including germ cells and developing lymphocytes, create DSBs in their genomes to initiate high frequency recombination. In meiosis, recombination intermediates physically link chromosome homologs to ensure their accurate segregation at the reductional division; recombination also contributes to diversity of haploid gametes (Kerr, Sarkar, and Arumugam, 2012). DSBs are introduced after S-phase when resection activity is high and are repaired solely by HR (Murakami and Keeney, 2014). The presence of Spo11 at DNA ends is a barrier to NHEJ and the coupling of Spo11 removal with creation of ssDNA tails ensures substrates are channeled to HR. In budding yeast, the removal of Spo11 oligos and Exo1-mediated resection are tightly coupled to DSB formation and consequently intermediates in the process are not detected, unless mutants are used

(Zakharyevich et al., 2010). By contrast, the initiation of resection at accidental DSBs is the rate-limiting step and plays a central role in repair pathway choice.

DSB-induced genome rearrangements are essential for diversification of antibody and T-cell receptor genes in B and T lymphocytes, respectively (Schatz and Swanson, 2011). RAG1 and RAG2 (RAG) proteins induce DSBs at recombination signal sequences flanking V, D and J segments in G1-phase lymphocytes when resection activity is low and are repaired exclusively by NHEJ (Schatz and Swanson, 2011). RAG creates hairpin intermediates at coding ends that require processing by DNA-PK and Artemis in order for end joining to proceed (Ma et al., 2002). The processing of hairpin ends and addition of untemplated nucleotides by terminal deoxynucleotidyltransferase contribute to variable region diversification. In the absence of classical NHEJ factors, MMEJ or HR fail to repair RAG-induced DSBs due to RAG association with ends. However, in certain RAG2 mutants, repair can be channeled to alternative pathways indicating that the RAG proteins collaborate with NHEJ factors to ensure the most appropriate repair outcome (Corneo et al., 2007). The examples of budding yeast meiosis and lymphocyte recombination highlight the importance of coupling between the enzymes that create programmed DSBs and the repair pathway utilized to repair them.

Concluding remarks

A great deal of progress has been made in identifying the nucleases responsible for end resection in eukaryotic cells. Reconstitution of the early and late steps of resection has significantly advanced our understanding of this critical process but some key questions remain, including how Sae2 activates the Mre11 endonuclease, the role of BRCA1 in end resection and how human EXO1 overcomes the inhibitory effect of RPA. Furthermore, reconstitution of resection with nucleosomal templates incorporating modified histones is needed to fully understand how chromatin-remodeling complexes facilitate end resection. Although the basic machinery catalyzing end resection is conserved, it has become apparent in recent years that the control of this process is more tightly regulated in mammalian cells than in yeast, presumably because of the hazard of inappropriate HR in organisms with vast amounts of repetitive DNA in their genomes. Understanding the mechanisms by which RIF1, PTIP and REV7 inhibit end resection is an important area of future investigation.

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References

1. O'Driscoll M. Diseases associated with defective responses to DNA damage. Cold Spring Harbor perspectives in biology. 2012;4.
2. Chiruvella KK, Liang Z, Wilson TE. Repair of double-strand breaks by end joining. Cold Spring Harbor perspectives in biology. 2013; 5:a012757. [PubMed: 23637284]
3. Sfeir A, Symington LS. Microhomology-Mediated End Joining: A Back-up Survival Mechanism or Dedicated Pathway? Trends Biochem Sci. 2015; 40:701–14. [PubMed: 26439531]
4. Paques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev. 1999; 63:349–404. [PubMed: 10357855]

5. Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. *Annu Rev Genet.* 2011; 45:247–71. [PubMed: 21910633]
6. Karanja KK, Cox SW, Duxin JP, Stewart SA, Campbell JL. DNA2 and EXO1 in replication-coupled, homology-directed repair and in the interplay between HDR and the FA/BRCA network. *Cell Cycle.* 2012; 11:3983–96. [PubMed: 22987153]
7. Mimitou EP, Symington LS. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature.* 2008; 455:770–4. [PubMed: 18806779]
8. Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell.* 2008; 134:981–94. [PubMed: 18805091]
9. Symington LS, Rothstein R, Lisby M. Mechanisms and regulation of mitotic recombination in *Saccharomyces cerevisiae*. *Genetics.* 2014; 198:795–835. [PubMed: 25381364]
10. Kadyk LC, Hartwell LH. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. *Genetics.* 1992; 132:387–402. [PubMed: 1427035]
11. Mimitou EP, Symington LS. DNA end resection: many nucleases make light work. *DNA Repair (Amst).* 2009; 8:983–95. [PubMed: 19473888]
12. Wang X, Haber JE. Role of *Saccharomyces* single-stranded DNA-binding protein RPA in the strand invasion step of double-strand break repair. *PLoS Biol.* 2004; 2:E21. [PubMed: 14737196]
13. Lisby M, Barlow JH, Burgess RC, Rothstein R. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell.* 2004; 118:699–713. [PubMed: 15369670]
14. San Filippo J, Sung P, Klein H. Mechanism of eukaryotic homologous recombination. *Annu Rev Biochem.* 2008; 77:229–57. [PubMed: 18275380]
15. West SC, Blanco MG, Chan YW, Matos J, Sarbajna S, Wyatt HD. Resolution of Recombination Intermediates: Mechanisms and Regulation. *Cold Spring Harb Symp Quant Biol.* 2015
16. Bizard AH, Hickson ID. The dissolution of double Holliday junctions. *Cold Spring Harbor perspectives in biology.* 2014; 6:a016477. [PubMed: 24984776]
17. Sun H, Treco D, Szostak JW. Extensive 3'-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. *Cell.* 1991; 64:1155–61. [PubMed: 2004421]
18. White CI, Haber JE. Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. *EMBO J.* 1990; 9:663–73. [PubMed: 2178924]
19. Keeney S, Giroux CN, Kleckner N. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell.* 1997; 88:375–84. [PubMed: 9039264]
20. Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P. An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature.* 1997; 386:414–7. [PubMed: 9121560]
21. Neale MJ, Pan J, Keeney S. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. *Nature.* 2005; 436:1053–7. [PubMed: 16107854]
22. Garcia V, Phelps SE, Gray S, Neale MJ. Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. *Nature.* 2011; 479:241–4. [PubMed: 22002605]
23. Zakharyevich K, Ma Y, Tang S, Hwang PY, Boiteux S, Hunter N. Temporally and biochemically distinct activities of Exo1 during meiosis: double-strand break resection and resolution of double Holliday junctions. *Mol Cell.* 2010; 40:1001–15. [PubMed: 21172664]
24. Gravel S, Chapman JR, Magill C, Jackson SP. DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev.* 2008; 22:2767–72. [PubMed: 18923075]
25. Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR 3rd, Hays L, Morgan WF, Petrini JH. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell.* 1998; 93:477–86. [PubMed: 9590181]
26. Usui T, Ohta T, Oshiumi H, Tomizawa J, Ogawa H, Ogawa T. Complex formation and functional versatility of Mre11 of budding yeast in recombination. *Cell.* 1998; 95:705–16. [PubMed: 9845372]

27. Dinkelmann M, Spehalski E, Stoneham T, Buis J, Wu Y, Sekiguchi JM, Ferguson DO. Multiple functions of MRN in end-joining pathways during isotype class switching. *Nat Struct Mol Biol.* 2009; 16:808–13. [PubMed: 19633670]
28. Boulton SJ, Jackson SP. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* 1998; 17:1819–28. [PubMed: 9501103]
29. Rass E, Grabarz A, Plo I, Gautier J, Bertrand P, Lopez BS. Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. *Nat Struct Mol Biol.* 2009; 16:819–24. [PubMed: 19633668]
30. Xie A, Kwok A, Scully R. Role of mammalian Mre11 in classical and alternative nonhomologous end joining. *Nat Struct Mol Biol.* 2009; 16:814–8. [PubMed: 19633669]
31. Furuse M, Nagase Y, Tsubouchi H, Murakami-Murofushi K, Shibata T, Ohta K. Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. *EMBO J.* 1998; 17:6412–25. [PubMed: 9799249]
32. Moreau S, Ferguson JR, Symington LS. The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol Cell Biol.* 1999; 19:556–66. [PubMed: 9858579]
33. Paull TT, Gellert M. The 3' to 5' exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. *Mol Cell.* 1998; 1:969–79. [PubMed: 9651580]
34. Bressan DA, Olivares HA, Nelms BE, Petrini JH. Alteration of N-terminal phosphoesterase signature motifs inactivates *Saccharomyces cerevisiae* Mre11. *Genetics.* 1998; 150:591–600. [PubMed: 9755192]
35. Krogh BO, Llorente B, Lam A, Symington LS. Mutations in Mre11 phosphoesterase motif I that impair *Saccharomyces cerevisiae* Mre11-Rad50-Xrs2 complex stability in addition to nuclease activity. *Genetics.* 2005; 171:1561–70. [PubMed: 16143598]
36. Llorente B, Symington LS. The Mre11 nuclease is not required for 5' to 3' resection at multiple HO-induced double-strand breaks. *Mol Cell Biol.* 2004; 24:9682–94. [PubMed: 15485933]
37. Lobachev KS, Gordenin DA, Resnick MA. The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. *Cell.* 2002; 108:183–93. [PubMed: 11832209]
38. Connelly JC, Kirkham LA, Leach DR. The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proc Natl Acad Sci U S A.* 1998; 95:7969–74. [PubMed: 9653124]
39. Paull TT, Gellert M. Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes Dev.* 1999; 13:1276–88. [PubMed: 10346816]
40. Trujillo KM, Sung P. DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50-Mre11 complex. *J Biol Chem.* 2001; 276:35458–64. [PubMed: 11454871]
41. Buis J, Wu Y, Deng Y, Leddon J, Westfield G, Eckersdorff M, Sekiguchi JM, Chang S, Ferguson DO. Mre11 nuclease activity has essential roles in DNA repair and genomic stability distinct from ATM activation. *Cell.* 2008; 135:85–96. [PubMed: 18854157]
42. Langerak P, Mejia-Ramirez E, Limbo O, Russell P. 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.* 2011; 7:e1002271. [PubMed: 21931565]
43. Williams RS, Moncalian G, Williams JS, Yamada Y, Limbo O, Shin DS, Grocock LM, Cahill D, Hitomi C, Guenther G, Moiani D, Carney JP, Russell P, Tainer JA. Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell.* 2008; 135:97–109. [PubMed: 18854158]
44. Alani E, Subbiah S, Kleckner N. The yeast RAD50 gene encodes a predicted 153-kD protein containing a purine nucleotide-binding domain and two large heptad-repeat regions. *Genetics.* 1989; 122:47–57. [PubMed: 2659437]
45. Hopfner KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT, Carney JP, Petrini JH, Tainer JA. The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature.* 2002; 418:562–6. [PubMed: 12152085]

46. Hopfner KP. ATP puts the brake on DNA double-strand break repair: a new study shows that ATP switches the Mre11-Rad50-Nbs1 repair factor between signaling and processing of DNA ends. *Bioessays*. 2014; 36:1170–8. [PubMed: 25213441]
47. Lammens K, Bemeleit DJ, Mockel C, Clausen E, Schele A, Hartung S, Schiller CB, Lucas M, Angermüller C, Soding J, Strasser K, Hopfner KP. The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. *Cell*. 2011; 145:54–66. [PubMed: 21458667]
48. Lim HS, Kim JS, Park YB, Gwon GH, Cho Y. Crystal structure of the Mre11-Rad50-ATPγS complex: understanding the interplay between Mre11 and Rad50. *Genes Dev*. 2011; 25:1091–104. [PubMed: 21511873]
49. Williams GJ, Williams RS, Williams JS, Moncalian G, Arvai AS, Limbo O, Guenther G, Sildas S, Hammel M, Russell P, Tainer JA. ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. *Nat Struct Mol Biol*. 2011
50. Alani E, Padmore R, Kleckner N. Analysis of wild-type and rad50 mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell*. 1990; 61:419–36. [PubMed: 2185891]
51. Cannavo E, Cejka P. Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. *Nature*. 2014; 514:122–5. [PubMed: 25231868]
52. Stracker TH, Petrini JH. The MRE11 complex: starting from the ends. *Nat Rev Mol Cell Biol*. 2011; 12:90–103. [PubMed: 21252998]
53. McKinnon PJ, Caldecott KW. DNA strand break repair and human genetic disease. *Annual review of genomics and human genetics*. 2007; 8:37–55.
54. Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell*. 1999; 99:577–87. [PubMed: 10612394]
55. Waltes R, Kalb R, Gatei M, Kijas AW, Stumm M, Soback A, Wieland B, Varon R, Lerenthal Y, Lavin MF, Schindler D, Dork T. Human RAD50 deficiency in a Nijmegen breakage syndrome-like disorder. *Am J Hum Genet*. 2009; 84:605–16. [PubMed: 19409520]
56. Nakada D, Matsumoto K, Sugimoto K. ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev*. 2003; 17:1957–62. [PubMed: 12923051]
57. Tsukamoto Y, Mitsuoka C, Terasawa M, Ogawa H, Ogawa T. Xrs2p regulates Mre11p translocation to the nucleus and plays a role in telomere elongation and meiotic recombination. *Mol Biol Cell*. 2005; 16:597–608. [PubMed: 15548595]
58. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*. 2005; 434:605–11. [PubMed: 15758953]
59. You Z, Chahwan C, Bailis J, Hunter T, Russell P. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Mol Cell Biol*. 2005; 25:5363–79. [PubMed: 15964794]
60. Schiller CB, Lammens K, Guerini I, Coords B, Feldmann H, Schlauderer F, Mockel C, Schele A, Strasser K, Jackson SP, Hopfner KP. Structure of Mre11-Nbs1 complex yields insights into ataxia-telangiectasia-like disease mutations and DNA damage signaling. *Nat Struct Mol Biol*. 2012; 19:693–700. [PubMed: 22705791]
61. Chapman JR, Jackson SP. Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. *EMBO Rep*. 2008; 9:795–801. [PubMed: 18583988]
62. Lloyd J, Chapman JR, Clapperton JA, Haire LF, Hartsuiker E, Li J, Carr AM, Jackson SP, Smerdon SJ. A supramodular FHA/BRCT-repeat architecture mediates Nbs1 adaptor function in response to DNA damage. *Cell*. 2009; 139:100–11. [PubMed: 19804756]
63. Williams RS, Dodson GE, Limbo O, Yamada Y, Williams JS, Guenther G, Classen S, Glover JN, Iwasaki H, Russell P, Tainer JA. Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. *Cell*. 2009; 139:87–99. [PubMed: 19804755]

64. Palmbo PL, Wu D, Daley JM, Wilson TE. Recruitment of *Saccharomyces cerevisiae* Dnl4-Lif1 complex to a double-strand break requires interactions with Yku80 and the Xrs2 FHA domain. *Genetics*. 2008; 180:1809–19. [PubMed: 18832348]
65. Wang H, Shi LZ, Wong CC, Han X, Hwang PY, Truong LN, Zhu Q, Shao Z, Chen DJ, Berns MW, Yates JR 3rd, Chen L, Wu X. The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. *PLoS Genet*. 2013; 9:e1003277. [PubMed: 23468639]
66. Limbo O, Chahwan C, Yamada Y, de Bruin RA, Wittenberg C, Russell P. Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. *Mol Cell*. 2007; 28:134–46. [PubMed: 17936710]
67. Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP. Human CtIP promotes DNA end resection. *Nature*. 2007; 450:509–14. [PubMed: 17965729]
68. Akamatsu Y, Murayama Y, Yamada T, Nakazaki T, Tsutsui Y, Ohta K, Iwasaki H. Molecular characterization of the role of the *Schizosaccharomyces pombe* *nip1+/ctp1+* gene in DNA double-strand break repair in association with the Mre11-Rad50-Nbs1 complex. *Mol Cell Biol*. 2008; 28:3639–51. [PubMed: 18378696]
69. Andres SN, Appel CD, Westmoreland JW, Williams JS, Nguyen Y, Robertson PD, Resnick MA, Williams RS. Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. *Nat Struct Mol Biol*. 2015; 22:158–66. [PubMed: 25580577]
70. Davies OR, Forment JV, Sun M, Belotserkovskaya R, Coates J, Galanty Y, Demir M, Morton CR, Rzechorzek NJ, Jackson SP, Pellegrini L. CtIP tetramer assembly is required for DNA-end resection and repair. *Nat Struct Mol Biol*. 2015; 22:150–7. [PubMed: 25558984]
71. Baroni E, Viscardi V, Cartagena-Lirola H, Lucchini G, Longhese MP. The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. *Mol Cell Biol*. 2004; 24:4151–65. [PubMed: 15121837]
72. Huertas P, Cortes-Ledesma F, Sartori AA, Aguilera A, Jackson SP. CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature*. 2008; 455:689–92. [PubMed: 18716619]
73. Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. *J Biol Chem*. 2009; 284:9558–65. [PubMed: 19202191]
74. Wang H, Shao Z, Shi LZ, Hwang PY, Truong LN, Berns MW, Chen DJ, Wu X. CtIP protein dimerization is critical for its recruitment to chromosomal DNA double-stranded breaks. *J Biol Chem*. 2012; 287:21471–80. [PubMed: 22544744]
75. Kim HS, Vijayakumar S, Reger M, Harrison JC, Haber JE, Weil C, Petrini JH. Functional interactions between Sae2 and the Mre11 complex. *Genetics*. 2008; 178:711–23. [PubMed: 18245357]
76. McKee AH, Kleckner N. A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene SAE2. *Genetics*. 1997; 146:797–816. [PubMed: 9215888]
77. Prinz S, Amon A, Klein F. Isolation of COM1, a new gene required to complete meiotic double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Genetics*. 1997; 146:781–95. [PubMed: 9215887]
78. Rattray AJ, Shafer BK, Neelam B, Strathern JN. A mechanism of palindromic gene amplification in *Saccharomyces cerevisiae*. *Genes Dev*. 2005; 19:1390–9. [PubMed: 15937224]
79. Clerici M, Mantiero D, Lucchini G, Longhese MP. The *Saccharomyces cerevisiae* Sae2 protein negatively regulates DNA damage checkpoint signalling. *EMBO Rep*. 2006; 7:212–8. [PubMed: 16374511]
80. Mimitou EP, Symington LS. Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. *EMBO J*. 2010; 29:3358–69. [PubMed: 20729809]
81. Chen H, Donnianni RA, Handa N, Deng SK, Oh J, Timashev LA, Kowalczykowski SC, Symington LS. Sae2 promotes DNA damage resistance by removing the Mre11-Rad50-Xrs2 complex from DNA and attenuating Rad53 signaling. *Proc Natl Acad Sci U S A*. 2015; 112:E1880–7. [PubMed: 25831494]

82. Puddu F, Oelschlaegel T, Guerini I, Geisler NJ, Niu H, Herzog M, Salguero I, Ochoa-Montano B, Vire E, Sung P, Adams DJ, Keane TM, Jackson SP. Synthetic viability genomic screening defines Sae2 function in DNA repair. *EMBO J.* 2015; 34:1509–22. [PubMed: 25899817]
83. Lengsfeld BM, Rattray AJ, Bhaskara V, Ghirlando R, Paull TT. Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. *Mol Cell.* 2007; 28:638–51. [PubMed: 18042458]
84. Makharashvili N, Tubbs AT, Yang SH, Wang H, Barton O, Zhou Y, Deshpande RA, Lee JH, Lobrich M, Sleckman BP, Wu X, Paull TT. Catalytic and noncatalytic roles of the CtIP endonuclease in double-strand break end resection. *Mol Cell.* 2014; 54:1022–33. [PubMed: 24837676]
85. Wang H, Li Y, Truong LN, Shi LZ, Hwang PY, He J, Do J, Cho MJ, Li H, Negrete A, Shiloach J, Berns MW, Shen B, Chen L, Wu X. CtIP maintains stability at common fragile sites and inverted repeats by end resection-independent endonuclease activity. *Mol Cell.* 2014; 54:1012–21. [PubMed: 24837675]
86. Cannon B, Kuhnlein J, Yang SH, Cheng A, Schindler D, Stark JM, Russell R, Paull TT. Visualization of local DNA unwinding by Mre11/Rad50/Nbs1 using single-molecule FRET. *Proc Natl Acad Sci U S A.* 2013; 110:18868–73. [PubMed: 24191051]
87. Clerici M, Mantiero D, Lucchini G, Longhese MP. The *Saccharomyces cerevisiae* Sae2 protein promotes resection and bridging of double strand break ends. *J Biol Chem.* 2005; 280:38631–8. [PubMed: 16162495]
88. Hopkins BB, Paull TT. The *P. furiosus* mre11/rad50 complex promotes 5' strand resection at a DNA double-strand break. *Cell.* 2008; 135:250–60. [PubMed: 18957200]
89. Chen PL, Liu F, Cai S, Lin X, Li A, Chen Y, Gu B, Lee EY, Lee WH. Inactivation of CtIP leads to early embryonic lethality mediated by G1 restraint and to tumorigenesis by haploid insufficiency. *Mol Cell Biol.* 2005; 25:3535–42. [PubMed: 15831459]
90. Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A, Petrini JH. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci U S A.* 1999; 96:7376–81. [PubMed: 10377422]
91. Xiao Y, Weaver DT. Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res.* 1997; 25:2985–91. [PubMed: 9224597]
92. Zhu J, Petersen S, Tessarollo L, Nussenzweig A. Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice. *Curr Biol.* 2001; 11:105–9. [PubMed: 11231126]
93. Zhou Y, Caron P, Legube G, Paull TT. Quantitation of DNA double-strand break resection intermediates in human cells. *Nucleic Acids Res.* 2014; 42:e19. [PubMed: 24362840]
94. Szankasi P, Smith GR. A DNA exonuclease induced during meiosis of *Schizosaccharomyces pombe*. *J Biol Chem.* 1992; 267:3014–23. [PubMed: 1737756]
95. Szankasi P, Smith GR. A role for exonuclease I from *S. pombe* in mutation avoidance and mismatch correction. *Science.* 1995; 267:1166–9. [PubMed: 7855597]
96. Lee SE, Bressan DA, Petrini JH, Haber JE. Complementation between N-terminal *Saccharomyces cerevisiae* mre11 alleles in DNA repair and telomere length maintenance. *DNA Repair (Amst).* 2002; 1:27–40. [PubMed: 12509295]
97. Moreau S, Morgan EA, Symington LS. Overlapping functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 nucleases in DNA metabolism. *Genetics.* 2001; 159:1423–33. [PubMed: 11779786]
98. Tsubouchi H, Ogawa H. Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in *Saccharomyces cerevisiae*. *Mol Biol Cell.* 2000; 11:2221–33. [PubMed: 10888664]
99. Shim EY, Chung WH, Nicolette ML, Zhang Y, Davis M, Zhu Z, Paull TT, Ira G, Lee SE. *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. *EMBO J.* 2010; 29:3370–80. [PubMed: 20834227]
100. Foster SS, Balestrini A, Petrini JH. Functional interplay of the Mre11 nuclease and Ku in the response to replication-associated DNA damage. *Mol Cell Biol.* 2011; 31:4379–89. [PubMed: 21876003]

101. Krasner DS, Daley JM, Sung P, Niu H. Interplay between Ku and Replication Protein A in the Restriction of Exo1-mediated DNA Break End Resection. *J Biol Chem*. 2015; 290:18806–16. [PubMed: 26067273]
102. Clerici M, Mantiero D, Guerini I, Lucchini G, Longhese MP. The Yku70-Yku80 complex contributes to regulate double-strand break processing and checkpoint activation during the cell cycle. *EMBO Rep*. 2008; 9:810–8. [PubMed: 18600234]
103. Cannavo E, Cejka P, Kowalczykowski SC. Relationship of DNA degradation by *Saccharomyces cerevisiae* Exonuclease 1 and its stimulation by RPA and Mre11-Rad50-Xrs2 to DNA end resection. *Proc Natl Acad Sci U S A*. 2013
104. Modrich P. Mechanisms in eukaryotic mismatch repair. *J Biol Chem*. 2006; 281:30305–9. [PubMed: 16905530]
105. Nicolette ML, Lee K, Guo Z, Rani M, Chow JM, Lee SE, Paull TT. Mre11-Rad50-Xrs2 and Sae2 promote 5' strand resection of DNA double-strand breaks. *Nat Struct Mol Biol*. 2010; 17:1478–85. [PubMed: 21102445]
106. Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C, Modrich P, Kowalczykowski SC. BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev*. 2011; 25:350–62. [PubMed: 21325134]
107. Eid W, Steger M, El-Shemerly M, Ferretti LP, Pena-Diaz J, Konig C, Valtorta E, Sartori AA, Ferrari S. DNA end resection by CtIP and exonuclease 1 prevents genomic instability. *EMBO Rep*. 2010; 11:962–8. [PubMed: 21052091]
108. Chen H, Lisby M, Symington LS. RPA coordinates DNA end resection and prevents formation of DNA hairpins. *Mol Cell*. 2013; 50:589–600. [PubMed: 23706822]
109. Myler LR, Gallardo IF, Zhou Y, Gong F, Yang SH, Wold MS, Miller KM, Paull TT, Finkelstein IJ. Single-molecule imaging reveals the mechanism of Exo1 regulation by single-stranded DNA binding proteins. *Proc Natl Acad Sci U S A*. 2016; 113:E1170–9. [PubMed: 26884156]
110. Yang SH, Zhou R, Campbell J, Chen J, Ha T, Paull TT. The SOSS1 single-stranded DNA binding complex promotes DNA end resection in concert with Exo1. *EMBO J*. 2013; 32:126–39. [PubMed: 23178594]
111. Genschel J, Modrich P. Mechanism of 5'-directed excision in human mismatch repair. *Mol Cell*. 2003; 12:1077–86. [PubMed: 14636568]
112. Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC. Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc Natl Acad Sci U S A*. 2008; 105:16906–11. [PubMed: 18971343]
113. Bae SH, Bae KH, Kim JA, Seo YS. RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. *Nature*. 2001; 412:456–61. [PubMed: 11473323]
114. Cejka P, Cannavo E, Polaczek P, Masuda-Sasa T, Pokharel S, Campbell JL, Kowalczykowski SC. DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. *Nature*. 2010; 467:112–6. [PubMed: 20811461]
115. Niu H, Chung WH, Zhu Z, Kwon Y, Zhao W, Chi P, Prakash R, Seong C, Liu D, Lu L, Ira G, Sung P. Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature*. 2010; 467:108–11. [PubMed: 20811460]
116. Liao S, Toczylowski T, Yan H. Identification of the *Xenopus* DNA2 protein as a major nuclease for the 5'→3' strand-specific processing of DNA ends. *Nucleic Acids Res*. 2008
117. Toczylowski T, Yan H. Mechanistic analysis of a DNA end processing pathway mediated by the *Xenopus* Werner syndrome protein. *J Biol Chem*. 2006; 281:33198–205. [PubMed: 16959775]
118. Yan H, Toczylowski T, McCane J, Chen C, Liao S. Replication protein A promotes 5'→3' end processing during homology-dependent DNA double-strand break repair. *J Cell Biol*. 2011; 192:251–61. [PubMed: 21263027]
119. Sturzenegger A, Burdova K, Kanagaraj R, Levikova M, Pinto C, Cejka P, Janscak P. DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA end resection in human cells. *J Biol Chem*. 2014; 289:27314–26. [PubMed: 25122754]
120. Thangavel S, Berti M, Levikova M, Pinto C, Gomathinayagam S, Vujanovic M, Zellweger R, Moore H, Lee EH, Hendrickson EA, Cejka P, Stewart S, Lopes M, Vindigni A. DNA2 drives

- processing and restart of reversed replication forks in human cells. *J Cell Biol.* 2015; 208:545–62. [PubMed: 25733713]
121. Bernstein KA, Mimitou EP, Mihalevic MJ, Chen H, Sunjaveric I, Symington LS, Rothstein R. Resection activity of the Sgs1 helicase alters the affinity of DNA ends for homologous recombination proteins in *Saccharomyces cerevisiae*. *Genetics.* 2013; 195:1241–51. [PubMed: 24097410]
 122. Hardy J, Churikov D, Geli V, Simon MN. Sgs1 and Sae2 promote telomere replication by limiting accumulation of ssDNA. *Nature communications.* 2014; 5:5004.
 123. Budd ME, Campbell JL. Interplay of Mre11 nuclease with Dna2 plus Sgs1 in Rad51-dependent recombinational repair. *PLoS One.* 2009; 4:e4267. [PubMed: 19165339]
 124. Hoa NN, Kobayashi J, Omura M, Hirakawa M, Yang SH, Komatsu K, Paull TT, Takeda S, Sasanuma H. BRCA1 and CtIP Are Both Required to Recruit Dna2 at Double-Strand Breaks in Homologous Recombination. *PLoS One.* 2015; 10:e0124495. [PubMed: 25909997]
 125. Peterson SE, Li Y, Wu-Baer F, Chait BT, Baer R, Yan H, Gottesman ME, Gautier J. Activation of DSB processing requires phosphorylation of CtIP by ATR. *Mol Cell.* 2013; 49:657–67. [PubMed: 23273981]
 126. Balakrishnan L, Polaczek P, Pokharel S, Campbell JL, Bambara RA. Dna2 exhibits a unique strand end-dependent helicase function. *J Biol Chem.* 2010; 285:38861–8. [PubMed: 20929864]
 127. Westmoreland JW, Resnick MA. Recombinational repair of radiation-induced double-strand breaks occurs in the absence of extensive resection. *Nucleic Acids Res.* 2016; 44:695–704. [PubMed: 26503252]
 128. Zierhut C, Diffley JF. Break dosage, cell cycle stage and DNA replication influence DNA double strand break response. *EMBO J.* 2008; 27:1875–85. [PubMed: 18511906]
 129. Roberts SA, Sterling J, Thompson C, Harris S, Mav D, Shah R, Klimczak LJ, Kryukov GV, Malc E, Mieczkowski PA, Resnick MA, Gordenin DA. Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. *Mol Cell.* 2012; 46:424–35. [PubMed: 22607975]
 130. Deng SK, Yin Y, Petes TD, Symington LS. Mre11-Sae2 and RPA Collaborate to Prevent Palindromic Gene Amplification. *Mol Cell.* 2015; 60:500–8. [PubMed: 26545079]
 131. Ciccio A, Elledge SJ. The DNA damage response: making it safe to play with knives. *Mol Cell.* 2010; 40:179–204. [PubMed: 20965415]
 132. Gobbin E, Cesena D, Galbiati A, Lockhart A, Longhese MP. Interplays between ATM/Tel1 and ATR/Mec1 in sensing and signaling DNA double-strand breaks. *DNA Repair (Amst).* 2013; 12:791–9. [PubMed: 23953933]
 133. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science.* 2003; 300:1542–8. [PubMed: 12791985]
 134. You Z, Shi LZ, Zhu Q, Wu P, Zhang YW, Basilio A, Tonnu N, Verma IM, Berns MW, Hunter T. CtIP links DNA double-strand break sensing to resection. *Mol Cell.* 2009; 36:954–69. [PubMed: 20064462]
 135. Shiotani B, Zou L. Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Mol Cell.* 2009; 33:547–58. [PubMed: 19285939]
 136. Usui T, Ogawa H, Petrini JH. A DNA damage response pathway controlled by Tel1 and the Mre11 complex. *Mol Cell.* 2001; 7:1255–66. [PubMed: 11430828]
 137. Brush GS, Morrow DM, Hieter P, Kelly TJ. The ATM homologue MEC1 is required for phosphorylation of replication protein A in yeast. *Proc Natl Acad Sci U S A.* 1996; 93:15075–80. [PubMed: 8986766]
 138. Chen X, Niu H, Chung WH, Zhu Z, Papusha A, Shim EY, Lee SE, Sung P, Ira G. Cell cycle regulation of DNA double-strand break end resection by Cdk1-dependent Dna2 phosphorylation. *Nat Struct Mol Biol.* 2011; 18:1015–9. [PubMed: 21841787]
 139. Liang J, Suhandynata RT, Zhou H. Phosphorylation of Sae2 Mediates Forkhead-associated (FHA) Domain-specific Interaction and Regulates Its DNA Repair Function. *J Biol Chem.* 2015; 290:10751–63. [PubMed: 25762720]

140. Fu Q, Chow J, Bernstein KA, Makharashvili N, Arora S, Lee CF, Person MD, Rothstein R, Paull TT. Phosphorylation-regulated transitions in an oligomeric state control the activity of the Sae2 DNA repair enzyme. *Mol Cell Biol.* 2014; 34:778–93. [PubMed: 24344201]
141. Harrison JC, Haber JE. Surviving the breakup: the DNA damage checkpoint. *Annu Rev Genet.* 2006; 40:209–35. [PubMed: 16805667]
142. Chen X, Cui D, Papusha A, Zhang X, Chu CD, Tang J, Chen K, Pan X, Ira G. The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. *Nature.* 2012; 489:576–80. [PubMed: 22960743]
143. Lazzaro F, Sapountzi V, Granata M, Pelliccioli A, Vaze M, Haber JE, Plevani P, Lydall D, Muzi-Falconi M. Histone methyltransferase Dot1 and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. *EMBO J.* 2008; 27:1502–12. [PubMed: 18418382]
144. Ngo GH, Lydall D. The 9-1-1 checkpoint clamp coordinates resection at DNA double strand breaks. *Nucleic Acids Res.* 2015; 43:5017–32. [PubMed: 25925573]
145. Eapen VV, Sugawara N, Tsabar M, Wu WH, Haber JE. The *Saccharomyces cerevisiae* chromatin remodeler Fun30 regulates DNA end resection and checkpoint deactivation. *Mol Cell Biol.* 2012; 32:4727–40. [PubMed: 23007155]
146. Ngo GH, Balakrishnan L, Dubarry M, Campbell JL, Lydall D. The 9-1-1 checkpoint clamp stimulates DNA resection by Dna2-Sgs1 and Exo1. *Nucleic Acids Res.* 2014; 42:10516–28. [PubMed: 25122752]
147. Ngo HP, Lydall D. Survival and growth of yeast without telomere capping by Cdc13 in the absence of Sgs1, Exo1, and Rad9. *PLoS Genet.* 2010; 6:e1001072. [PubMed: 20808892]
148. Bonetti D, Villa M, Gobbin E, Cassani C, Tedeschi G, Longhese MP. Escape of Sgs1 from Rad9 inhibition reduces the requirement for Sae2 and functional MRX in DNA end resection. *EMBO Rep.* 2015; 16:351–61. [PubMed: 25637499]
149. Ferrari M, Dibitetto D, De Gregorio G, Eapen VV, Rawal CC, Lazzaro F, Tsabar M, Marini F, Haber JE, Pelliccioli A. Functional interplay between the 53BP1-ortholog Rad9 and the Mre11 complex regulates resection, end-tethering and repair of a double-strand break. *PLoS Genet.* 2015; 11:e1004928. [PubMed: 25569305]
150. Gobbin E, Villa M, Gnugnoli M, Menin L, Clerici M, Longhese MP. Sae2 Function at DNA Double-Strand Breaks Is Bypassed by Dampening Tel1 or Rad53 Activity. *PLoS Genet.* 2015; 11:e1005685. [PubMed: 26584331]
151. Clerici M, Trovesi C, Galbiati A, Lucchini G, Longhese MP. Mec1/ATR regulates the generation of single-stranded DNA that attenuates Tel1/ATM signaling at DNA ends. *EMBO J.* 2014; 33:198–216. [PubMed: 24357557]
152. Mantiero D, Clerici M, Lucchini G, Longhese MP. Dual role for *Saccharomyces cerevisiae* Tel1 in the checkpoint response to double-strand breaks. *EMBO Rep.* 2007; 8:380–7. [PubMed: 17347674]
153. Chen X, Paudyal SC, Chin RI, You Z. PCNA promotes processive DNA end resection by Exo1. *Nucleic Acids Res.* 2013; 41:9325–38. [PubMed: 23939618]
154. Zimmermann M, de Lange T. 53BP1: pro choice in DNA repair. *Trends Cell Biol.* 2014; 24:108–17. [PubMed: 24094932]
155. Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. *Science.* 2012; 336:593–7. [PubMed: 22556254]
156. Chapman JR, Barral P, Vannier JB, Borel V, Steger M, Tomas-Loba A, Sartori AA, Adams IR, Batista FD, Boulton SJ. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol Cell.* 2013; 49:858–71. [PubMed: 23333305]
157. Di Virgilio M, Callen E, Yamane A, Zhang W, Jankovic M, Gitlin AD, Feldhahn N, Resch W, Oliveira TY, Chait BT, Nussenzweig A, Casellas R, Robbiani DF, Nussenzweig MC. Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science.* 2013; 339:711–5. [PubMed: 23306439]
158. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkac J, Cook MA, Rosebrock AP, Munro M, Canny MD, Xu D, Durocher D. A cell cycle-dependent regulatory

circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell*. 2013; 49:872–83. [PubMed: 23333306]

159. Feng L, Fong KW, Wang J, Wang W, Chen J. RIF1 counteracts BRCA1-mediated end resection during DNA repair. *J Biol Chem*. 2013; 288:11135–43. [PubMed: 23486525]
160. Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science*. 2013; 339:700–4. [PubMed: 23306437]
161. Callen E, Di Virgilio M, Kruhlak MJ, Nieto-Soler M, Wong N, Chen HT, Faryabi RB, Polato F, Santos M, Starnes LM, Wesemann DR, Lee JE, Tubbs A, Sleckman BP, Daniel JA, Ge K, Alt FW, Fernandez-Capetillo O, Nussenzweig MC, Nussenzweig A. 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell*. 2013; 153:1266–80. [PubMed: 23727112]
162. Boersma V, Moatti N, Segura-Bayona S, Peuscher MH, van der Torre J, Wevers BA, Orthwein A, Durocher D, Jacobs JJ. MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5' end resection. *Nature*. 2015; 521:537–40. [PubMed: 25799990]
163. Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, Bouwman P, Bartkova J, Gogola E, Warmerdam D, Barazas M, Jaspers JE, Watanabe K, Pieterse M, Kersbergen A, Sol W, Celie PH, Schouten PC, van den Broek B, Salman A, Nieuwland M, de Rink I, de Ronde J, Jalink K, Boulton SJ, Chen J, van Gent DC, Bartek J, Jonkers J, Borst P, Rottenberg S. REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature*. 2015; 521:541–4. [PubMed: 25799992]
164. Morin I, Ngo HP, Greenall A, Zubko MK, Morrice N, Lydall D. Checkpoint-dependent phosphorylation of Exo1 modulates the DNA damage response. *EMBO J*. 2008
165. El-Shemerly M, Janscak P, Hess D, Jiricny J, Ferrari S. Degradation of human exonuclease 1b upon DNA synthesis inhibition. *Cancer Res*. 2005; 65:3604–9. [PubMed: 15867354]
166. Tkac J, Xu G, Adhikary H, Young JT, Gallo D, Escibano-Diaz C, Krietsch J, Orthwein A, Munro M, Sol W, Al-Hakim A, Lin ZY, Jonkers J, Borst P, Brown GW, Gingras AC, Rottenberg S, Masson JY, Durocher D. HELB Is a Feedback Inhibitor of DNA End Resection. *Mol Cell*. 2016
167. Ira G, Pelliccioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, Liberi G, Bressan D, Wan L, Hollingsworth NM, Haber JE, Foiani M. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature*. 2004; 431:1011–7. [PubMed: 15496928]
168. Aylon Y, Liefshitz B, Kupiec M. The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle. *EMBO J*. 2004; 23:4868–75. [PubMed: 15549137]
169. Barlow JH, Lisby M, Rothstein R. Differential regulation of the cellular response to DNA double-strand breaks in G1. *Mol Cell*. 2008; 30:73–85. [PubMed: 18406328]
170. Trovesi C, Falcettoni M, Lucchini G, Clerici M, Longhese MP. Distinct Cdk1 requirements during single-strand annealing, noncrossover, and crossover recombination. *PLoS Genet*. 2011; 7:e1002263. [PubMed: 21901114]
171. Manfrini N, Guerini I, Citterio A, Lucchini G, Longhese MP. Processing of meiotic DNA double strand breaks requires cyclin-dependent kinase and multiple nucleases. *J Biol Chem*. 2010; 285:11628–37. [PubMed: 20150422]
172. Polato F, Callen E, Wong N, Faryabi R, Bunting S, Chen HT, Kozak M, Kruhlak MJ, Reczek CR, Lee WH, Ludwig T, Baer R, Feigenbaum L, Jackson S, Nussenzweig A. CtIP-mediated resection is essential for viability and can operate independently of BRCA1. *The Journal of experimental medicine*. 2014; 211:1027–36. [PubMed: 24842372]
173. Reczek CR, Szabolcs M, Stark JM, Ludwig T, Baer R. The interaction between CtIP and BRCA1 is not essential for resection-mediated DNA repair or tumor suppression. *J Cell Biol*. 2013; 201:693–707. [PubMed: 23712259]
174. Nakamura K, Kogame T, Oshiumi H, Shinohara A, Sumitomo Y, Agama K, Pommier Y, Tsutsui KM, Tsutsui K, Hartsuiker E, Ogi T, Takeda S, Taniguchi Y. Collaborative action of Brca1 and CtIP in elimination of covalent modifications from double-strand breaks to facilitate subsequent break repair. *PLoS Genet*. 2010; 6:e1000828. [PubMed: 20107609]

175. Tomimatsu N, Mukherjee B, Catherine Hardebeck M, Ilcheva M, Vanessa Camacho C, Louise Harris J, Porteus M, Llorente B, Khanna KK, Burma S. Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. *Nature communications*. 2014; 5:3561.
176. Digilio FA, Pannuti A, Lucchesi JC, Furia M, Polito LC. Tosca: a *Drosophila* gene encoding a nuclease specifically expressed in the female germline. *Dev Biol*. 1996; 178:90–100. [PubMed: 8812111]
177. Tishkoff DX, Amin NS, Viars CS, Arden KC, Kolodner RD. Identification of a human gene encoding a homologue of *Saccharomyces cerevisiae* EXO1, an exonuclease implicated in mismatch repair and recombination. *Cancer Res*. 1998; 58:5027–31. [PubMed: 9823303]
178. Pierce AJ, Hu P, Han M, Ellis N, Jasin M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. *Genes Dev*. 2001; 15:3237–42. [PubMed: 11751629]
179. Deng SK, Gibb B, de Almeida MJ, Greene EC, Symington LS. RPA antagonizes microhomology-mediated repair of DNA double-strand breaks. *Nat Struct Mol Biol*. 2014; 21:405–12. [PubMed: 24608368]
180. Lee K, Lee SE. *Saccharomyces cerevisiae* Sae2- and Tel1-dependent single-strand DNA formation at DNA break promotes microhomology-mediated end joining. *Genetics*. 2007; 176:2003–14. [PubMed: 17565964]
181. Shibata A, Moiani D, Arvai AS, Perry J, Harding SM, Genois MM, Maity R, van Rossum-Fikkert S, Kertokallio A, Romoli F, Ismail A, Ismalaj E, Petricci E, Neale MJ, Bristow RG, Masson JY, Wyman C, Jeggo PA, Tainer JA. DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. *Mol Cell*. 2014; 53:7–18. [PubMed: 24316220]
182. Dupre A, Boyer-Chatenet L, Sattler RM, Modi AP, Lee JH, Nicolette ML, Kopelovich L, Jasin M, Baer R, Paull TT, Gautier J. A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex. *Nat Chem Biol*. 2008; 4:119–25. [PubMed: 18176557]
183. Villarreal DD, Lee K, Deem A, Shim EY, Malkova A, Lee SE. Microhomology directs diverse DNA break repair pathways and chromosomal translocations. *PLoS Genet*. 2012; 8:e1003026. [PubMed: 23144625]
184. Truong LN, Li Y, Shi LZ, Hwang PY, He J, Wang H, Razavian N, Berns MW, Wu X. Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proc Natl Acad Sci U S A*. 2013; 110:7720–5. [PubMed: 23610439]
185. Bannardo N, Cheng A, Huang N, Stark JM. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. *PLoS Genet*. 2008; 4:e1000110. [PubMed: 18584027]
186. Ismail IH, Gagne JP, Genois MM, Strickfaden H, McDonald D, Xu Z, Poirier GG, Masson JY, Hendzel MJ. The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. *Nat Cell Biol*. 2015; 17:1446–57. [PubMed: 26502055]
187. Chapman JR, Sossick AJ, Boulton SJ, Jackson SP. BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. *J Cell Sci*. 2012; 125:3529–34. [PubMed: 22553214]
188. Chen L, Nievera CJ, Lee AY, Wu X. Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *J Biol Chem*. 2008; 283:7713–20. [PubMed: 18171670]
189. Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanassoulas M, Kulkarni A, Yang Q, Haffty BG, Tommiska J, Blomqvist C, Drapkin R, Adams DJ, Nevanlinna H, Bartek J, Tarsounas M, Ganesan S, Jonkers J. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat Struct Mol Biol*. 2010; 17:688–95. [PubMed: 20453858]
190. Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, Xu X, Deng CX, Finkel T, Nussenzweig M, Stark JM, Nussenzweig A. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell*. 2010; 141:243–54. [PubMed: 20362325]

191. Bunting SF, Callen E, Kozak ML, Kim JM, Wong N, Lopez-Contreras AJ, Ludwig T, Baer R, Faryabi RB, Malhowski A, Chen HT, Fernandez-Capetillo O, D'Andrea A, Nussenzweig A. BRCA1 functions independently of homologous recombination in DNA interstrand crosslink repair. *Mol Cell*. 2012; 46:125–35. [PubMed: 22445484]
192. Wang J, Aroumougame A, Lobrich M, Li Y, Chen D, Chen J, Gong Z. PTIP associates with Artemis to dictate DNA repair pathway choice. *Genes Dev*. 2014; 28:2693–8. [PubMed: 25512557]
193. Orthwein A, Noordermeer SM, Wilson MD, Landry S, Enchev RI, Sherker A, Munro M, Pinder J, Salsman J, Deltre G, Xia B, Peter M, Durocher D. A mechanism for the suppression of homologous recombination in G1 cells. *Nature*. 2015; 528:422–6. [PubMed: 26649820]
194. Wiltshire TD, Lovejoy CA, Wang T, Xia F, O'Connor MJ, Cortez D. Sensitivity to poly(ADP-ribose) polymerase (PARP) inhibition identifies ubiquitin-specific peptidase 11 (USP11) as a regulator of DNA double-strand break repair. *J Biol Chem*. 2010; 285:14565–71. [PubMed: 20233726]
195. Costelloe T, Louge R, Tomimatsu N, Mukherjee B, Martini E, Khadaroo B, Dubois K, Wiegant WW, Thierry A, Burma S, van Attikum H, Llorente B. The yeast Fun30 and human SMARCAD1 chromatin remodellers promote DNA end resection. *Nature*. 2012; 489:581–4. [PubMed: 22960744]
196. Shim EY, Hong SJ, Oum JH, Yanez Y, Zhang Y, Lee SE. RSC mobilizes nucleosomes to improve accessibility of repair machinery to the damaged chromatin. *Mol Cell Biol*. 2007; 27:1602–13. [PubMed: 17178837]
197. Tomimatsu N, Mukherjee B, Deland K, Kurimasa A, Bolderson E, Khanna KK, Burma S. Exo1 plays a major role in DNA end resection in humans and influences double-strand break repair and damage signaling decisions. *DNA Repair (Amst)*. 2012; 11:441–8. [PubMed: 22326273]
198. Adkins NL, Niu H, Sung P, Peterson CL. Nucleosome dynamics regulates DNA processing. *Nat Struct Mol Biol*. 2013; 20:836–42. [PubMed: 23728291]
199. Kerr GW, Sarkar S, Arumugam P. How to halve ploidy: lessons from budding yeast meiosis. *Cellular and molecular life sciences : CMLS*. 2012; 69:3037–51. [PubMed: 22481439]
200. Murakami H, Keeney S. Temporospatial coordination of meiotic DNA replication and recombination via DDK recruitment to replisomes. *Cell*. 2014; 158:861–73. [PubMed: 25126790]
201. Schatz DG, Swanson PC. V(D)J recombination: mechanisms of initiation. *Annu Rev Genet*. 2011; 45:167–202. [PubMed: 21854230]
202. Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell*. 2002; 108:781–94. [PubMed: 11955432]
203. Corneo B, Wendland RL, Deriano L, Cui X, Klein IA, Wong SY, Arnal S, Holub AJ, Weller GR, Pancake BA, Shah S, Brandt VL, Meek K, Roth DB. Rag mutations reveal robust alternative end joining. *Nature*. 2007; 449:483–6. [PubMed: 17898768]

Figure 1. Models for the repair of DSBs by NHEJ, MMEJ, SSA or HR

The direct ligation of ends by NHEJ is favored in G1 phase cells when end resection activity is low. Activation of end resection in S-G2 phase cells creates 3'-ssDNA overhangs that are initially bound by RPA, and then by Rad51 to catalyze homologous pairing and strand invasion (HR). Alternatively, annealing of microhomologies internal to the ends results in repair by MMEJ, or by SSA if longer direct repeats flank the DSB. Rad1-Rad10 nuclease (human XPF-ERCC1) cleaves the 3' flaps formed by annealing of repeats internal to the ends. Thick orange lines represent direct repeats flanking the DSB.

Figure 2. Activity of MRX at protein-bound and hairpin-capped ends

A. Phosphorylated Sae2 activates the Mre11 subunit of the MRX complex to cleave the 5'-terminated strand internal to the protein-bound end (Spo11 in meiotic cells). The nick acts as an entry site for the 3'-5' Mre11 exonuclease and 5'-3' Exo1 exonuclease for bidirectional resection. B. MRX can cleave the unpaired region of a DNA hairpin without Sae2, or potentially Sae2 could activate MRX cleavage at a distance from the end as proposed for protein-bound ends.

Figure 3. Resection initiation in the absence of Mre11 nuclease, Sae2 or Ku

Resection initiation at DSBs generated by endonucleases can occur by MRX-Sae2 cleavage, as envisioned for protein-bound ends (left panel). In the absence of the Mre11 nuclease or Sae2, Dna2-STR is the main nuclease for resection initiation, unless Ku is absent (middle panel). Exo1 can initiate resection from ends when Ku is eliminated (right panel).

Figure 4. DNA damage checkpoint regulation of end resection

MRX activates Tel1, which phosphorylates Sae2 and H2A. RPA binds to the ssDNA tail formed by MRX-Sae2 clipping and recruits Dna2-STR and Mec1-Ddc2. Mec1-Ddc2 is mainly responsible for phosphorylation of Rad9, Rad53 and H2A. The PCNA-like 9-1-1 complex binds at the ssDNA-dsDNA transition and activates Exo1 and Dna2. Rad9 binds to modified histones preventing extensive resection by Exo1 and Dna2. Rad9 also activates Rad53, which phosphorylates Exo1 to inhibit its activity.