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Review

20 Years of Mre11 Biology: No End in Sight

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The Mre11 nuclease has been the subject of intensive investigation for the past 20 years because of the central role that Mre11/Rad50 complexes play in genome maintenance. The last two decades of work on this complex has led to a much deeper understanding of the structure, biochemical activities, and regulation of Mre11/Rad50 complexes from archaea, bacteria, and eukaryotic cells. This review will discuss some of the important findings over recent years that have illuminated roles for the Mre11 nuclease in these different contexts as well as the insights from structural biology that have helped us to understand its mechanisms of action.

Double-strand breaks in DNA constitute a unique type of DNA lesion that is potentially lethal to eukaryotic cells if essential genetic material is lost at the break site, yet double-strand breaks are also important in many biological processes. Severing of both strands of DNA generates an essential intermediate that is required for homologous recombination preceding sexual reproduction in eukaryotic organisms and for programmed gene rearrangements in the vertebrate immune system. Naturally occurring double-stranded ends of chromosomes also comprise the telomere structure that affects many aspects of genome integrity and aging, and signaling induced at double-strand break sites is an important component of DNA-damage-induced checkpoint control of growth. The importance of double-strand breaks is highlighted by the evolution of protein complexes that specifically recognize this lesion within seconds of its appearance in cells. One of the primary complexes responsible for the recognition, repair, and signaling of double-strand breaks in eukaryotes is composed of the Mre11, Rad50, and Nbs1/Xrs2 proteins (MRN/X), of which Mre11 and Rad50 constitute the catalytic components.

Mre11, the nuclease component of MRN/X, is conserved in all organisms, but the importance of this protein in processes related to DNA double-strand break repair and recombination was initially most evident in genetic studies in *S. cerevisiae*. Early studies in budding yeast showed an absolute requirement for Mre11 and its nuclease activity in the processing of covalent Spo11-bound breaks that initiate homologous recombination during meiosis (Ajimura et al., 1993; Moreau et al., 1999; Nairz and Klein, 1997; Ogawa et al., 1995; Usui et al., 1998). We now know that Mre11 complexes initiate the processing of DNA double-strand breaks in many cellular contexts outside of meiosis, that Mre11 can remove a variety of nucleic acid and protein blocks on DNA ends, and that Mre11 nuclease activity can also have deleterious effects on DNA replication intermediates in certain pathological situations.

Insights from Structural Analysis of Mre11/Rad50 Complexes

Archaea Mre11 from *P. furiosus* was first glimpsed at atomic resolution in 2001, which showed a dimeric Mre11 catalytic domain with an L-shaped DNA-binding groove leading to an active site with two bound metal ions (Hopfner et al., 2001), consistent with other members of the protein phosphatase 2B family of phosphoesterases (Fernandez et al., 2011). The binding groove appeared large enough to accommodate single-stranded DNA, but not a double-stranded DNA duplex, an observation borne out in a later co-crystal structure of Mre11 bound to synapsed DNA ends and separately to a partially unwound DNA duplex (Williams et al., 2008). Several structures of Mre11 in complex with Rad50 core domains from bacteria and archaea have also illuminated the interactions between the nuclease and its invariant cofactor, Rad50 (Lammens et al., 2011; Lim et al., 2011; Möckel et al., 2012; Williams et al., 2011). Interestingly, these studies have indicated that when the SMC-like Rad50 core domains are bound to ATP, the catalytic domains of Mre11 are completely occluded (Figure 1). Later studies have shown that Mre11 is also physically separated from the DNA duplex, which is bound in a groove on the opposite, top surface of the Rad50 catalytic head domains (Liu et al., 2016; Seifert et al., 2016). For the Mre11 catalytic domains to contact the DNA, the core Walker A/Walker B ATP-binding domains of Rad50 must presumably detach from the ATP-binding domains of the other Rad50 monomer coincident with ATP hydrolysis. Currently, however, we do not know the mechanism for DNA end recognition by Mre11 within an MRN or Mre11/Rad50 (MR) complex, and we do not know how Mre11 engages a DNA end within its active site.

Biochemical analysis of Mre11 nuclease activity confirms that ATP hydrolysis by Rad50 is a prerequisite for Mre11/Rad50-mediated nuclease activity on double-stranded DNA (Connelly et al., 1997; Deshpande et al., 2017; Herdendorf et al., 2011; Hopfner et al., 2000a; Paull and Gellert, 1999; Trujillo and Sung, 2001). However, single-stranded DNA with secondary structure, such as M13 phage DNA, can be cleaved by Mre11/Rad50 complexes in the absence of ATP (Connelly and Leach, 1996; Herdendorf et al., 2011; Hopfner et al., 2000a), and eukaryotic Mre11 exhibits 3′ to 5′ exonuclease activity in the absence of Rad50 (Paull and Gellert, 1998; Trujillo et al., 1998), indicating that Rad50-catalyzed ATP hydrolysis is not essential for all

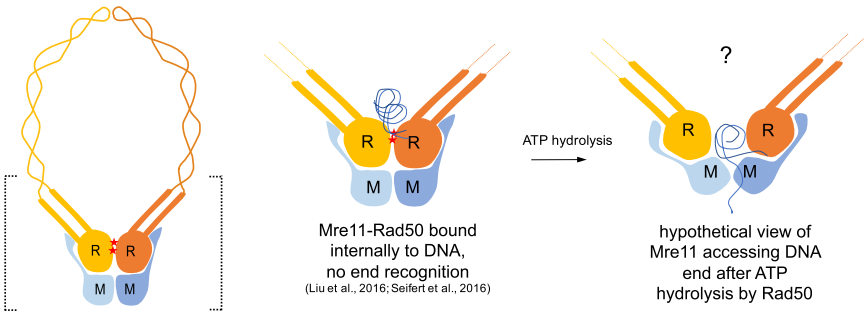

Figure 1. Schematic Diagram of an Mre11/Rad50 Complex Bound to DNA

Left: an Mre11/Rad50 complex is shown (Nbs1/Xrs2 not pictured here) with two molecules of ATP (red stars). Middle: Mre11 (M) dimer bound to Rad50 (R) dimer with a double-stranded DNA molecule bound on the top surface of Rad50 (DNA helix coming out of the page), based on structures of Mre11/Rad50 complexes (Liu et al., 2016; Seifert et al., 2016). Right: hypothetical model of Mre11/Rad50 after ATP hydrolysis with Mre11 accessing DNA for nuclease attack. See text for additional details.

Mre11 functions. T4 bacteriophage Mre11 (gp47) also has been shown to require ATP hydrolysis by Rad50 (gp46) for exonuclease activity, but not for the removal of the first nucleotide (Herdendorf et al., 2011), also suggesting that the requirement of ATP hydrolysis for Mre11 nuclease activity is likely related to the conformation of the active site on DNA and how it is restrained in some circumstances by association with Rad50.

In eukaryotes, the third component of the complex, Nbs1(Nibrin) in mammals and fission yeast and Xrs2 in budding yeast, regulates the catalytic activities of Mre11/Rad50. Nbs1 binds to Mre11 through an Mre11-interacting region in the C terminus, which is crystallized with *S. pombe* Mre11 on the surface of the phosphodiesterase domain and at the Mre11 dimer interface (Schiller et al., 2012). Human Nbs1 is required to promote Mre11 endonuclease activity on blocked DNA ends and hairpin substrates and is important for the stimulatory effect of DNA ends on the rate of Rad50 ATP hydrolysis (Deshpande et al., 2016, 2017; Paull and Gellert, 1999). Nbs1 also restrains MR-catalyzed 3′ to 5′ exonuclease activity on open DNA ends while promoting 3′ to 5′ exonuclease activity at protein-blocked ends (Deshpande et al., 2016); thus, Nbs1 is a key determinant of the outcomes of Mre11 activity. While the N-terminal forkhead-associated (FHA) and BRCA1 C-terminal (BRCT) domains of Nbs1/Xrs2 are important for association with Mdc1, CtBP-interacting protein (CtIP)/Ctp1, and other factors in the DNA damage response (Chapman and Jackson, 2008; Dodson et al., 2010; Lloyd et al., 2009; Melander et al., 2008; Spycher et al., 2008; Wang et al., 2013; Williams et al., 2009), the Mre11-interacting region of Nbs1 is necessary and sufficient for cell viability in the absence of the rest of the Nbs1 polypeptide (Kim et al., 2017). In contrast, the yeast Xrs2 protein does not play as essential a role in budding yeast as Nbs1 does in human cells; fusion of a nuclear localization signal to Mre11 (normally provided through the Xrs2 protein) bypasses the requirement for Xrs2 in MRX-mediated end resection, DNA damage survival, hairpin resolution, and meiosis (Oh et al., 2016).

Melting of the DNA duplex, as seen in structures of Mre11 bound to DNA ends (Williams et al., 2008), allows the enzyme to accommodate a single-stranded DNA or a 3′ end of a DNA strand. Human Mre11, when bound to Rad50, Nbs1, and ATP, was later shown to induce an unwinding of ~15 bp at the ends of duplex DNA (Cannon et al., 2013), consistent with the idea that partial unwinding of the DNA duplex would be essential for access of DNA into the Mre11 active site. Evidence for this was also seen with the *M. jannaschii* enzyme, where structural analysis of the core of Mre11 bound to the core catalytic head domains of Rad50 combined with biochemical analysis of mutants supported a model in which rotation of the Rad50 nucleotide-binding domains generates unwinding of the helix and access to the Mre11 active sites (Liu et al., 2016).

Although we have many structures of the catalytic cores of Mre11 and Rad50 (Hopfner et al., 2000b, 2001; Lammens et al., 2011; Lim et al., 2011; Liu et al., 2016; Möckel et al., 2012; Park et al., 2011; Seifert et al., 2016; Williams et al., 2008, 2011), we currently do not have structural detail showing end recognition of DNA in the context of the complete Mre11/Rad50 complex or the intermediate in which Mre11/Rad50 accesses DNA after ATP hydrolysis. Furthermore, a recent structure of the core catalytic domain of human Mre11 revealed that there are significant differences between bacterial and archaea Mre11 and mammalian Mre11 (Park et al., 2011), and we do not have structural detail of the interaction between human Mre11 and Nbs1. Future structural analysis of the complete MRN complex is necessary to understand the functional regulation of its activities at a mechanistic level and decipher how it recognizes DNA ends.

DNA End Processing by Mre11 Complexes: Endonucleolytic Activity

Purified Mre11/Rad50 complexes from all organisms exhibit exonuclease activity *in vitro* on blunt or recessed 3′ ends in the 3′ to 5′ direction (Connelly et al., 1999; Herdendorf et al., 2011; Hopfner et al., 2000a; Paull and Gellert, 1998; Trujillo and Sung, 2001; Trujillo et al., 1998). For many years, the polarity of the exonuclease activity was difficult to reconcile with its known biological importance in promoting the 5′ to 3′ resection of DNA double-strand breaks. A critical step in unraveling this paradox was the observation that the processing of Spo11-induced breaks in *S. cerevisiae* occurs through an endonucleolytic cleavage of DNA adjacent to the Spo11 covalent conjugate and also subsequent 3′ to 5′ exonucleolytic degradation toward the conjugate (Garcia et al., 2011). This endo-then-exo model of DNA end resection (Figure 2) explained the existence of the 3′ to 5′ exonuclease activity and was also demonstrated *in vitro* with protein-blocked ends (Anand et al., 2016; Connelly et al., 2003; Deshpande et al., 2016; Reginato et al., 2017; Wang et al., 2017).

Remarkably, endonucleolytic incision of DNA ends followed by exonucleolytic degradation is observed *in vitro* only on DNA substrates containing a blocked end. The blocks used *in vitro* have generally consisted of a tightly bound or covalently bound

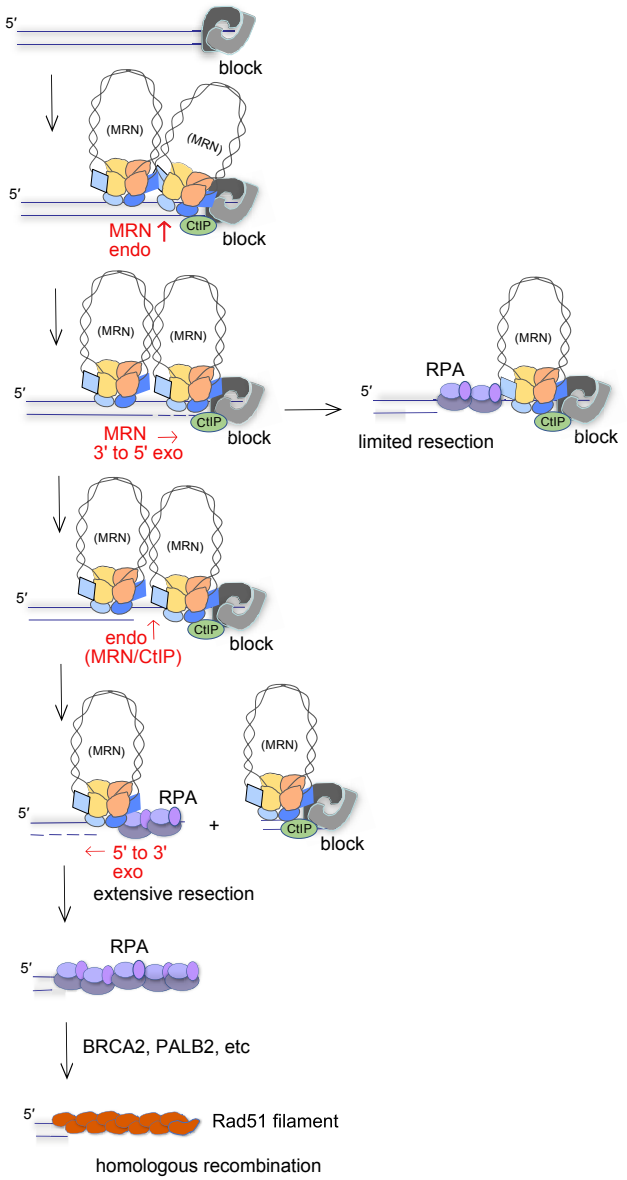

Figure 2. Schematic Diagram of Processing of Blocked DNA Ends for Homologous Recombination and the Role of MRN and CtIP in Processing

A DNA end with a covalent block or noncovalently bound protein, such as the Ku heterodimer, that is recognized by the MRN complex and CtIP protein. Endonucleolytic incision of the 5′ strand adjacent to the block is catalyzed by MRN (MRN endo). Subsequent 3′ to 5′ exonucleolytic degradation by MRN toward the break extends the single-stranded DNA gap (MRN 3′ to 5′ exo), and 5′ to 3′ degradation by Exo1 or Dna2 generates an extensive region of single-stranded DNA (5′ to 3′ exo). Removal of MRN and the block occurs in a reaction that is attributed to both MRN nuclease activity and CtIP (endo MRN/CtIP), which promotes extensive resection and finally the loading of Rad51. See text for additional details.

protein, as first demonstrated by Leach and colleagues, who showed that *E. coli* Mre11/Rad50 (suppressor of RecBC [Sbc] CD) generates an endonucleolytic cleavage on an avidin-bound DNA (Connelly et al., 2003). Similar endonucleolytic cutting was shown with *S. cerevisiae* Mre11/Rad50/Xrs2 (MRX) and with human MRN complexes adjacent to DNA ends containing biotin-streptavidin attachments, and in these cases, MRN(X) showed block-dependent endonuclease incision followed by 3′ to 5′ exonuclease degradation away from the nick site (Anand et al., 2016; Cannavo and Cejka, 2014; Deshpande et al., 2016; Reginato et al., 2017; Wang et al., 2017). Nucleases are generally specific for a particular DNA lesion or structure, but it is unusual for a nuclease to require a protein to be bound adjacent to a cleavage site. The identity of this protein seems to be irrelevant since streptavidin (bound to biotin moieties on the DNA) can promote endonucleolytic activity of Mre11 (Anand et al., 2016; Deshpande et al., 2016).

Although the endo-then-exo catalytic activities were initially demonstrated with heterologous blocked ends, eukaryotic MRN(X) complexes were also shown to recognize naturally occurring, non-covalent DNA-bound proteins as end blocks, most notably the non-homologous end joining (NHEJ) factor Ku (Myler et al., 2017; Reginato et al., 2017; Wang et al., 2017). In this case, the presence of the Sae2 (budding yeast) and CtIP (human ortholog of Sae2) proteins is stimulatory of Mre11 endonuclease activity in a manner that requires phosphorylation of Sae2/CtIP (Anand et al., 2016; Cannavo and Cejka, 2014). The phosphorylation requirement for Sae2/CtIP is important, because these include modifications by cyclin-dependent kinase (CDK) that are specific to S/G_2 phase cells, thus promoting MRN(X) endonucleolytic processing of protein-bound ends specifically in phases of the cell cycle when sister chromatids are present. Phosphorylation of CtIP by Plk3 during G_1 phase on the same sites as are phosphorylated by CDK during S/G_2 phase has also been reported (Barton et al., 2014); these modifications were shown to promote resection, deletions, and alternative NHEJ during G_1 phase. Consistent with this idea, CtIP has been widely reported to facilitate DNA repair in G_1 phase in mammalian cells (Averbeck et al., 2014; Barton et al., 2014; Biehs et al., 2017; Helmink et al., 2011; Quennet et al., 2011; Yun and Hiom, 2009).

Three independent groups have also shown that Sae2/CtIP proteins possess intrinsic structure-specific endonuclease activity on 5′ flap structures (Arora et al., 2017; Ghodke and Muniyappa, 2016; Lengsfeld et al., 2007; Makharashvili et al., 2014; Wang et al., 2014) This activity requires ATM phosphorylation of CtIP, but not CDK, phosphorylation and is important for survival in the presence of lesions such as covalent Top1 adducts (Chanut et al., 2016; Makharashvili et al., 2014). Since the intrinsic nuclease activity does not require phosphorylation of the CDK sites, this regulation is consistent with a function for the intrinsic nuclease activity that is not limited to S/G_2 phases of the cell cycle.

MRN recognition of DNA ends blocked by heterologous adducts suggests that specific recognition of an adduct is not absolutely required and that the complexes may initially bind at internal sites. This idea was proposed by studies of SbcCD (*E. coli* Mre11/Rad50) (Connelly et al., 2003) and was experimentally demonstrated using atomic force microscopy (de Jager et al., 2002) as well as single-molecule techniques that showed MRN diffusion on double-stranded DNA (Myler et al., 2017). Structures of Mre11/Rad50 catalytic domains bound to DNA, but not engaged with the DNA ends (Liu et al., 2016; Seifert

et al., 2016), are also consistent with a nonspecific DNA-binding mode that promotes internal sliding of the complex.

The endo-then-exo model of MRN initiation of double-strand break (DSB) resection also suggests the possibility that the two events may be uncoupled, such that endonucleolytic cleavage occurs but the subsequent 3′ to 5′ exonuclease activity does not or perhaps is significantly separated in time. If this occurs, then 5′ resection of the incised 5′ strand could initiate with the recruitment of downstream nucleases such as Exo1, but the adduct could remain at the double-strand break end. Evidence for this was first obtained in *S. pombe*, where MRN and Ctp1 (ortholog of Sae2 in fission yeast) are important for the initiation of resection; nevertheless, in the absence of Mre11 nuclease activity, inefficient resection could still occur while Ku is bound to the ends of the broken DNA (Langerak et al., 2011). A similar result with human cells in which double-strand breaks were induced during S phase using the Top1 poison camptothecin agrees with this view (Chanut et al., 2016). This study showed that Mre11 nuclease activity is important for removal of Ku from single-ended (camptothecin [CPT]-induced) double-strand breaks formed during replication and that Mre11 exonuclease activity together with CtIP intrinsic nuclease activity functions at a subset of these breaks. Interestingly, in cases where Ku removal is blocked, resection of the 5′ strand internal to the break still occurs, but Rad51 filament formation is inhibited. Taken together, these results indicate that the ultimate completion of resection to promote Rad51 filament formation and homologous recombination requires complete removal of the Ku adduct from the end, even though some measure of resection can occur without Ku removal. Observations of substantial resection in the absence of Mre11 or its nuclease activity in vertebrate cells have also suggested that in some cases resection does not absolutely require Mre11, but that CtIP can provide the primary resection-promoting activity (Hoa et al., 2015).

In budding yeast, separation-of-function mutations in *SAE2* were identified that eliminate either Sae2 promotion of Mre11 endonuclease activity or the intrinsic nuclease activity of the Sae2 protein (Arora et al., 2017). Mutations that block stimulation of Mre11 activity confer DNA damage sensitivity, while mutations that block Sae2 nuclease only produce sensitivity in combination with mutations that eliminate Mre11 nuclease function; thus, in yeast, the role of Sae2 in promoting Mre11 activity is the primary function in survival of DNA damage. Nevertheless, both types of mutations eliminate the function of Sae2 during meiosis, so the specialized series of reactions that occur during prophase I specifically requires both functions. Overall, it is clear that Sae2 activities do directly regulate Mre11 nuclease functions but are also playing nonredundant roles in DNA repair.

The importance of Ku as a block to MRN(X)-mediated resection is also clear in both budding and fission yeasts, where a number of groups have shown that deletion of Ku partially alleviates the DNA damage sensitivity of strains expressing Mre11-nuclease-deficient alleles (Foster et al., 2011; Langerak et al., 2011; Mimitou and Symington, 2010; Tomita et al., 2003; Wasko et al., 2009; Wu et al., 2008). Reconstitution of resection using recombinant MRX, Exo1, and Ku likewise shows a strong inhibitory of role of Ku in blocking resection of linearized DNA by Exo1 *in vitro* that can be dramatically reduced by MRX (Nicolette et al., 2010; Shim et al., 2010). In budding yeast, the MRX complex also is important for NHEJ (Moore and Haber, 1996) and interacts with components of the end-joining machinery, although this role is not recapitulated in vertebrate cells (Yamaguchi-Iwai et al., 1999).

DNA End Processing by Mre11 Complexes: The Roles of Endonuclease versus Exonuclease Functions

Further evidence for the importance of the Mre11 endonucleolytic incision was provided using small-molecule inhibitors of Mre11 specific for endonucleolytic activity or exonucleolytic activity (Shibata et al., 2014). The exonuclease inhibitors were designed based on the mirin compound (Dupré et al., 2008) isolated in a screen for molecules that inhibit Mre11 function in *Xenopus* egg extracts. In a co-crystal structure with *T. maritima* Mre11, mirin was found to bind to a site in Mre11 that restricts rotation of the phosphate backbone necessary for 3′ to 5′ exonucleolytic degradation of double-stranded DNA (Shibata et al., 2014). The endonuclease inhibitor of Mre11, in contrast, was designed to block a single-stranded DNA-binding groove in Mre11. In human cells in G_2 phase, inhibition of Mre11 endonuclease activity blocked homologous recombination but did not inhibit NHEJ-mediated repair of the breaks, implying that no resection had been initiated (Shibata et al., 2014). In contrast, inhibition of Mre11 exonuclease activity under the same circumstances precluded both homologous recombination and NHEJ, suggesting that resection initiated by endonuclease activity prevents subsequent repair of the processed ends through NHEJ.

Interestingly, Mre11 nuclease activity has also been found to be important for the repair of double-strand breaks in G_1 phase of the cell cycle, during which long-range resection does not occur but short-range processing of ends can precede NHEJ (Biehs et al., 2017). In this case, Mre11 endonuclease activity is not required but inhibition of exonuclease activity reduces the efficiency of repair. How this processing is initiated in the absence of endonuclease activity, however, is not clear. Mre11 nuclease activity also acts at uncapped telomeres in a manner that promotes ligase-4-dependent NHEJ (Deng et al., 2009), another indication that MRN is not limited to the processing of breaks for homologous recombination. In mammalian cells, MRN and CtIP function in processing of breaks prior to single-stranded annealing and alternative end joining, in some cases contributing to chromosomal translocations (Bennardo et al., 2008, 2009; Clerici et al., 2005; Gunn et al., 2011; Lee and Lee, 2007; Lee-Theilen et al., 2011; Zhang and Jasin, 2011).

Pathological Outcomes of MRN(X) Occupancy at DNA Lesions

Analysis of the kinetics of binding and dissociation of DNA repair factors at DNA ends *in vivo* in *S. cerevisiae* first showed that MRX and Sae2 are associated with sites of double-strand breaks very rapidly after their introduction (Lisby et al., 2004). The dissociation of MRX and Sae2 from double-strand break ends was shown to coincide exactly with the first appearance of Rad51, suggesting that there is a defined transition between these two states. In addition, deletion of *SAE2* in yeast generates a prolonged occupancy of MRX at break sites (Lisby et al., 2004),

suggesting that one of the roles of the Sae2 protein could be to remove MRX from breaks. Experimental support for this hypothesis came with the identification of hypomorphic mutations in Mre11 that partially alleviate the DNA damage sensitivity of *sae2* null alleles (Chen et al., 2015; Puddu et al., 2015). These mutants were shown to have deficits in DNA binding, implying that the prolonged presence of high levels of MRX complex at DNA ends actually restricts homologous recombination and successful repair. One of these hypomorphic mutations in Mre11 was also shown to alleviate the DNA damage sensitivity of *sae2* mutants impaired in either intrinsic catalytic activity or the ability to promote Mre11 endonuclease (Arora et al., 2017); thus, the roles of Sae2 at double-strand break ends are intimately connected with the removal of MRX. These examples in yeast indicate the potential for pathological activities of MRX in specific mutant contexts.

Another recent example of Mre11 acting in a manner that appears to be antagonistic to error-free genome maintenance is the observation that Mre11 degrades nascent DNA strands during replication under fork-stalling conditions, particularly when factors such as BRCA2, Rad51, Pax transactivation domain-interacting protein (PTIP), or Werner syndrome (WRN) are absent (Hashimoto et al., 2010; Iannascoli et al., 2015; Kim et al., 2014; Kolinjivadi et al., 2017; Lemaçon et al., 2017; Mijic et al., 2017; Ray Chaudhuri et al., 2016; Schlacher et al., 2011; Taglialatela et al., 2017; Vallerga et al., 2015; Ying et al., 2012). In this case, stalled forks generate a situation in which nascent strands are degraded by Mre11 (likely on regressed ends). Direct protection of nascent strands by Rad51 filaments is proposed as the mechanism that blocks this outcome in normal cells.

Given that the 3′ to 5′ exonuclease activity of human MRN at an open end or a 3′ recessed strand is normally strongly inhibited by Nbs1, it is not clear why Mre11 would act at a nascent strand during replication, even in the absence of recombinase loading (Deshpande et al., 2016). It could be that nascent strands in the absence of homologous recombination factors are uniquely susceptible to Mre11 degradation, but the mechanistic basis of this is not yet understood. Since MRN has been shown to be present at active replication forks (Maser et al., 2001; Sirbu et al., 2011) and prevent the formation of double-strand breaks during replication (Costanzo et al., 2001), it is important to understand in future work what the characteristics of its substrates are and how the complex is regulated in normal cells to prevent loss of replication intermediates. In budding yeast, replication protein A (RPA) has been shown to be important for the recruitment of MRX to stalled replication forks (Seeber et al., 2016), so it will be informative to determine how MRN recognizes replication intermediates in mammalian cells and whether RPA is similarly integral to this process.

While the activities of Mre11 at sites of double-strand breaks are rapidly becoming clear, we have comparatively less understanding of how its functions are regulated at non-double-strand break sites. From the work on SbcCD in *E. coli*, we know that Mre11/Rad50 complexes can act to process secondary structures such as hairpin loops at sites of inverted repeats during replication (Connelly et al., 1998; Eykelenboom et al., 2008). Ultimately, this processing results in double-strand breaks created by SbcCD, which are resolved by recombination. In budding yeast, Mre11 nuclease activity and Sae2 were shown to be essential for the processing of hairpin structures created by inverted repeats in the genome or by fold-back of single-stranded DNA intermediates (Lobachev et al., 2002; Rattray et al., 2001). In these cases, one could perhaps envision the hairpin structure as analogous to a protein lesion in that it blocks diffusion of MRN(X) on DNA, and bacterial, yeast, and human Mre11 complexes have all been shown to cleave hairpin structures *in vitro* (Connelly et al., 1998; Lengsfeld et al., 2007; Paull and Gellert, 1999; Trujillo and Sung, 2001). CtIP and MRN nuclease function have also been shown to be required for processing of inverted repeats and fragile sites in human cells (Wang et al., 2014), consistent with data from other organisms, although we still have much to learn about the roles of MRN in genome maintenance during S phase.

Mre11-Nuclease-Deficient Phenotypes in Vertebrates

Mutation of the active site of Mre11 in budding yeast generates modest sensitivity to ionizing radiation but is overall a subtle phenotype compared to the complete loss of either Mre11 or Rad50 (Bressan et al., 1998; Moreau et al., 1999). Thus, the early embryonic inviability of mice expressing nuclease-dead Mre11 was surprising, as was the observation that loss of Mre11 nuclease activity is lethal in the mouse as well as in vertebrate cells in culture (Buis et al., 2008; Hoa et al., 2015, 2016; Yamaguchi-Iwai et al., 1998). This phenotype was not due to an effect on ATM activation, a conclusion also supported by work *in vitro* with purified components (Lee et al., 2013). In the absence of Mre11 nuclease activity, mammalian cells rapidly generate spontaneous chromosomal abnormalities, including chromatid breaks, translocations, dicentric chromosomes, and radial structures, as well as a dramatic decrease in the initiation of homologous recombination after ionizing radiation exposure (Buis et al., 2008; Hoa et al., 2015, 2016). In addition, recent insight into the nature of the essential activities of Mre11 was provided in a study showing that human TK6 cells and chicken DT40 cells lacking Mre11 nuclease activity accumulate covalent Top2 adducts, a lesion not generally seen except in cells treated with Top2 poisons. Overexpression of Tdp2, an enzyme specialized for the direct removal of Top2-DNA lesions, delays and partially suppresses the lethality of the Mre11-nuclease-deficient cells, indicating that this specific protein-DNA adduct contributes to the striking phenotype (Hoa et al., 2016). Processing of covalent Top2-DNA adducts has not yet been demonstrated *in vitro*, but this genetic evidence suggests that MRN likely recognizes these as protein adducts on DNA ends and acts to process and remove the adduct.

Concluding Remarks

While our understanding of Mre11 biochemistry and biology has advanced tremendously over the past 20 years, its importance as a central hub that senses, processes, and signals double-strand breaks in eukaryotes is only growing with each new piece of the puzzle that is solved. We still have much more to learn about the complex and its regulation *in vivo*, most notably its substrates and regulation during replication in eukaryotic cells and how these activities are altered in pathological contexts. Data from diverse organisms have contributed to our current

understanding of Mre11 nuclease function and how the endo-then-exo activities act to process DNA ends with protein adducts. *In vitro*, we have a wealth of structural detail of the core catalytic domains of Mre11 and Rad50 from several species; however, we do not know at a mechanistic level how Nbs1(Xrs2) controls all of the catalytic activities of the core components, how ATP hydrolysis by Rad50 controls the conformations of the other components, or how the complete complex actually engages with a double-strand break end. Lastly, detailed understanding of how MRN(X) processes a Spo11 lesion, arguably the biological event that has been the most insightful in terms of understanding this complex, has not yet been achieved, as it remains a technical challenge in the field. The next 20 years may bring answers to these difficult but important questions.

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REFERENCES

Ajimura, M., Leem, S.H., and Ogawa, H. (1993). Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. Genetics *133*, 51–66.

Anand, R., Ranjha, L., Cannavo, E., and Cejka, P. (2016). Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. Mol. Cell *64*, 940–950.

Arora, S., Deshpande, R.A., Budd, M., Campbell, J., Revere, A., Zhang, X., Schmidt, K.H., and Paull, T.T. (2017). Genetic separation of Sae2 nuclease activity from Mre11 nuclease functions in budding yeast. Mol. Cell. Biol. *37*, e00156–e00157.

Averbeck, N.B., Ringel, O., Herrlitz, M., Jakob, B., Durante, M., and Taucher-Scholz, G. (2014). DNA end resection is needed for the repair of complex lesions in G1-phase human cells. Cell Cycle *13*, 2509–2516.

Barton, O., Naumann, S.C., Diemer-Biehs, R., Künzel, J., Steinlage, M., Conrad, S., Makharashvili, N., Wang, J., Feng, L., Lopez, B.S., et al. (2014). Polo-like kinase 3 regulates CtIP during DNA double-strand break repair in G1. J. Cell Biol. *206*, 877–894.

Bennardo, N., Cheng, A., Huang, N., and Stark, J.M. (2008). Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. PLoS Genet. *4*, e1000110.

Bennardo, N., Gunn, A., Cheng, A., Hasty, P., and Stark, J.M. (2009). Limiting the persistence of a chromosome break diminishes its mutagenic potential. PLoS Genet. *5*, e1000683.

Biehs, R., Steinlage, M., Barton, O., Juhasz, S., Kunzel, J., Spies, J., Shibata, A., Jeggo, P.A., and Lobrich, M. (2017). DNA double-strand break resection occurs during non-homologous end joining in G1 but is distinct from resection during homologous recombination. Mol. Cell *65*, 671–684.e675.

Bressan, D.A., Olivares, H.A., Nelms, B.E., and Petrini, J.H. (1998). Alteration of N-terminal phosphoesterase signature motifs inactivates Saccharomyces cerevisiae Mre11. Genetics *150*, 591–600.

Buis, J., Wu, Y., Deng, Y., Leddon, J., Westfield, G., Eckersdorff, M., Sekiguchi, J.M., Chang, S., and Ferguson, D.O. (2008). Mre11 nuclease activity has essential roles in DNA repair and genomic stability distinct from ATM activation. Cell *135*, 85–96.

Cannavo, E., and Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. Nature *514*, 122–125.

Cannon, B., Kuhnlein, J., Yang, S.H., Cheng, A., Schindler, D., Stark, J.M., Russell, R., and Paull, T.T. (2013). Visualization of local DNA unwinding by Mre11/Rad50/Nbs1 using single-molecule FRET. Proc. Natl. Acad. Sci. USA *110*, 18868–18873.

Chanut, P., Britton, S., Coates, J., Jackson, S.P., and Calsou, P. (2016). Coordinated nuclease activities counteract Ku at single-ended DNA double-strand breaks. Nat. Commun. *7*, 12889.

Chapman, J.R., and Jackson, S.P. (2008). Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. EMBO Rep. *9*, 795–801.

Chen, H., Donnianni, R.A., Handa, N., Deng, S.K., Oh, J., Timashev, L.A., Kowalczykowski, S.C., and Symington, L.S. (2015). Sae2 promotes DNA damage resistance by removing the Mre11-Rad50-Xrs2 complex from DNA and attenuating Rad53 signaling. Proc. Natl. Acad. Sci. USA *112*, E1880–E1887.

Clerici, M., Mantiero, D., Lucchini, G., and Longhese, M.P. (2005). The Saccharomyces cerevisiae Sae2 protein promotes resection and bridging of double strand break ends. J. Biol. Chem. *280*, 38631–38638.

Connelly, J.C., and Leach, D.R. (1996). The sbcC and sbcD genes of Escherichia coli encode a nuclease involved in palindrome inviability and genetic recombination. Genes Cells *1*, 285–291.

Connelly, J.C., de Leau, E.S., Okely, E.A., and Leach, D.R. (1997). Overexpression, purification, and characterization of the SbcCD protein from *Escherichia coli*. J. Biol. Chem. *272*, 19819–19826.

Connelly, J.C., Kirkham, L.A., and Leach, D.R. (1998). The SbcCD nuclease of Escherichia coli is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. Proc. Natl. Acad. Sci. USA *95*, 7969–7974.

Connelly, J.C., de Leau, E.S., and Leach, D.R.F. (1999). DNA cleavage and degradation by the SbcCD protein complex from Escherichia coli. Nucleic Acids Res. *27*, 1039–1046.

Connelly, J.C., de Leau, E.S., and Leach, D.R. (2003). Nucleolytic processing of a protein-bound DNA end by the E. coli SbcCD (MR) complex. DNA Repair (Amst.) *2*, 795–807.

Costanzo, V., Robertson, K., Bibikova, M., Kim, E., Grieco, D., Gottesman, M., Carroll, D., and Gautier, J. (2001). Mre11 protein complex prevents double-strand break accumulation during chromosomal DNA replication. Mol. Cell *8*, 137–147.

de Jager, M., Wyman, C., van Gent, D.C., and Kanaar, R. (2002). DNA end-binding specificity of human Rad50/Mre11 is influenced by ATP. Nucleic Acids Res. *30*, 4425–4431.

Deng, Y., Guo, X., Ferguson, D.O., and Chang, S. (2009). Multiple roles for MRE11 at uncapped telomeres. Nature *460*, 914–918.

Deshpande, R.A., Lee, J.H., Arora, S., and Paull, T.T. (2016). Nbs1 converts the human Mre11/Rad50 nuclease complex into an endo/exonuclease machine specific for protein-DNA adducts. Mol. Cell *64*, 593–606.

Deshpande, R.A., Lee, J.H., and Paull, T.T. (2017). Rad50 ATPase activity is regulated by DNA ends and requires coordination of both active sites. Nucleic Acids Res. *45*, 5255–5268.

Dodson, G.E., Limbo, O., Nieto, D., and Russell, P. (2010). Phosphorylation-regulated binding of Ctp1 to Nbs1 is critical for repair of DNA double-strand breaks. Cell Cycle *9*, 1516–1522.

Dupré, A., Boyer-Chatenet, L., Sattler, R.M., Modi, A.P., Lee, J.H., Nicolette, M.L., Kopelovich, L., Jasin, M., Baer, R., Paull, T.T., and Gautier, J. (2008). A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex. Nat. Chem. Biol. *4*, 119–125.

Eykelenboom, J.K., Blackwood, J.K., Okely, E., and Leach, D.R.F. (2008). SbcCD causes a double-strand break at a DNA palindrome in the Escherichia coli chromosome. Mol. Cell *29*, 644–651.

Fernandez, F.J., Lopez-Estepa, M., and Vega, M.C. (2011). Nucleases of metallo-beta-lactamase and protein phosphatase families in DNA repair. In DNA Repair: On the Pathways to Fixing DNA Damage and Errors, F. Storici, ed. (Intech).

Foster, S.S., Balestrini, A., and Petrini, J.H. (2011). Functional interplay of the Mre11 nuclease and Ku in the response to replication-associated DNA damage. Mol. Cell. Biol. *31*, 4379–4389.

Garcia, V., Phelps, S.E., Gray, S., and Neale, M.J. (2011). Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. Nature *479*, 241–244.

Ghodke, I., and Muniyappa, K. (2016). Genetic and biochemical evidences reveal novel insights into the mechanism underlying *Saccharomyces cerevisiae* Sae2-mediated abrogation of DNA replication stress. J. Biosci. *41*, 615–641.

Gunn, A., Bennardo, N., Cheng, A., and Stark, J.M. (2011). Correct end use during end joining of multiple chromosomal double strand breaks is influenced by repair protein RAD50, DNA-dependent protein kinase DNA-PKcs, and transcription context. J. Biol. Chem. *286*, 42470–42482.

Hashimoto, Y., Ray Chaudhuri, A., Lopes, M., and Costanzo, V. (2010). Rad51 protects nascent DNA from Mre11-dependent degradation and promotes continuous DNA synthesis. Nat. Struct. Mol. Biol. *17*, 1305–1311.

Helmink, B.A., Tubbs, A.T., Dorsett, Y., Bednarski, J.J., Walker, L.M., Feng, Z., Sharma, G.G., McKinnon, P.J., Zhang, J., Bassing, C.H., and Sleckman, B.P. (2011). H2AX prevents CtIP-mediated DNA end resection and aberrant repair in G1-phase lymphocytes. Nature *469*, 245–249.

Herdendorf, T.J., Albrecht, D.W., Benkovic, S.J., and Nelson, S.W. (2011). Biochemical characterization of bacteriophage T4 Mre11-Rad50 complex. J. Biol. Chem. *286*, 2382–2392.

Hoa, N.N., Akagawa, R., Yamasaki, T., Hirota, K., Sasa, K., Natsume, T., Kobayashi, J., Sakuma, T., Yamamoto, T., Komatsu, K., et al. (2015). Relative contribution of four nucleases, CtIP, Dna2, Exo1 and Mre11, to the initial step of DNA double-strand break repair by homologous recombination in both the chicken DT40 and human TK6 cell lines. Genes Cells *20*, 1059–1076.

Hoa, N.N., Shimizu, T., Zhou, Z.W., Wang, Z.Q., Deshpande, R.A., Paull, T.T., Akter, S., Tsuda, M., Furuta, R., Tsutsui, K., et al. (2016). Mre11 is essential for the removal of lethal topoisomerase 2 covalent cleavage complexes. Mol. Cell *64*, 580–592.

Hopfner, K.P., Karcher, A., Shin, D., Fairley, C., Tainer, J.A., and Carney, J.P. (2000a). Mre11 and Rad50 from Pyrococcus furiosus: cloning and biochemical characterization reveal an evolutionarily conserved multiprotein machine. J. Bacteriol. *182*, 6036–6041.

Hopfner, K.P., Karcher, A., Shin, D.S., Craig, L., Arthur, L.M., Carney, J.P., and Tainer, J.A. (2000b). Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell *101*, 789–800.

Hopfner, K.P., Karcher, A., Craig, L., Woo, T.T., Carney, J.P., and Tainer, J.A. (2001). Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell *105*, 473–485.

Iannascoli, C., Palermo, V., Murfuni, I., Franchitto, A., and Pichierri, P. (2015). The WRN exonuclease domain protects nascent strands from pathological MRE11/EXO1-dependent degradation. Nucleic Acids Res. *43*, 9788–9803.

Kim, T.M., Son, M.Y., Dodds, S., Hu, L., and Hasty, P. (2014). Deletion of BRCA2 exon 27 causes defects in response to both stalled and collapsed replication forks. Mutat. Res. *766-767*, 66–72.

Kim, J.H., Grosbart, M., Anand, R., Wyman, C., Cejka, P., and Petrini, J.H.J. (2017). The Mre11-Nbs1 interface is essential for viability and tumor suppression. Cell Rep. *18*, 496–507.

Kolinjivadi, A.M., Sannino, V., De Antoni, A., Zadorozhny, K., Kilkenny, M., Techer, H., Baldi, G., Shen, R., Ciccia, A., Pellegrini, L., et al. (2017). Smarcal1-mediated fork reversal triggers Mre11-dependent degradation of nascent DNA in the absence of Brca2 and stable Rad51 nucleofilaments. Mol. Cell *67*, 867–881.e867.

Lammens, K., Bemeleit, D.J., Möckel, C., Clausing, E., Schele, A., Hartung, S., Schiller, C.B., Lucas, M., Angermüller, C., Söding, J., et al. (2011). The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. Cell *145*, 54–66.

Langerak, P., Mejia-Ramirez, E., Limbo, O., and Russell, P. (2011). Release of Ku and MRN from DNA ends by Mre11 nuclease activity and Ctp1 is required for homologous recombination repair of double-strand breaks. PLoS Genet. *7*, e1002271.

Lee, K., and Lee, S.E. (2007). Saccharomyces cerevisiae Sae2- and Tel1-dependent single-strand DNA formation at DNA break promotes microhomology-mediated end joining. Genetics *176*, 2003–2014.

Lee, J.H., Mand, M.R., Deshpande, R.A., Kinoshita, E., Yang, S.H., Wyman, C., and Paull, T.T. (2013). Ataxia telangiectasia-mutated (ATM) kinase activity is regulated by ATP-driven conformational changes in the Mre11/Rad50/Nbs1 (MRN) complex. J. Biol. Chem. *288*, 12840–12851.

Lee-Theilen, M., Matthews, A.J., Kelly, D., Zheng, S., and Chaudhuri, J. (2011). CtIP promotes microhomology-mediated alternative end joining during class-switch recombination. Nat. Struct. Mol. Biol. *18*, 75–79.

Lemaçon, D., Jackson, J., Quinet, A., Brickner, J.R., Li, S., Yazinski, S., You, Z., Ira, G., Zou, L., Mosammaparast, N., and Vindigni, A. (2017). MRE11 and EXO1 nucleases degrade reversed forks and elicit MUS81-dependent fork rescue in BRCA2-deficient cells. Nat. Commun. *8*, 860.

Lengsfeld, B.M., Rattray, A.J., Bhaskara, V., Ghirlando, R., and Paull, T.T. (2007). Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. Mol. Cell *28*, 638–651.

Lim, H.S., Kim, J.S., Park, Y.B., Gwon, G.H., and Cho, Y. (2011). Crystal structure of the Mre11-Rad50-ATPγS complex: understanding the interplay between Mre11 and Rad50. Genes Dev. *25*, 1091–1104.

Lisby, M., Barlow, J.H., Burgess, R.C., and Rothstein, R. (2004). Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell *118*, 699–713.

Liu, Y., Sung, S., Kim, Y., Li, F., Gwon, G., Jo, A., Kim, A.K., Kim, T., Song, O.K., Lee, S.E., and Cho, Y. (2016). ATP-dependent DNA binding, unwinding, and resection by the Mre11/Rad50 complex. EMBO J. *35*, 743–758.

Lloyd, J., Chapman, J.R., Clapperton, J.A., Haire, L.F., Hartsuiker, E., Li, J., Carr, A.M., Jackson, S.P., and Smerdon, S.J. (2009). A supramodular FHA/BRCT-repeat architecture mediates Nbs1 adaptor function in response to DNA damage. Cell *139*, 100–111.

Lobachev, K.S., Gordenin, D.A., and Resnick, M.A. (2002). The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. Cell *108*, 183–193.

Makharashvili, N., Tubbs, A.T., Yang, S.H., Wang, H., Barton, O., Zhou, Y., Deshpande, R.A., Lee, J.H., Lobrich, M., Sleckman, B.P., et al. (2014). Catalytic and noncatalytic roles of the CtIP endonuclease in double-strand break end resection. Mol. Cell *54*, 1022–1033.

Maser, R.S., Mirzoeva, O.K., Wells, J., Olivares, H., Williams, B.R., Zinkel, R.A., Farnham, P.J., and Petrini, J.H. (2001). Mre11 complex and DNA replication: linkage to E2F and sites of DNA synthesis. Mol. Cell. Biol. *21*, 6006–6016.

Melander, F., Bekker-Jensen, S., Falck, J., Bartek, J., Mailand, N., and Lukas, J. (2008). Phosphorylation of SDT repeats in the MDC1 N terminus triggers retention of NBS1 at the DNA damage-modified chromatin. J. Cell Biol. *181*, 213–226.

Mijic, S., Zellweger, R., Chappidi, N., Berti, M., Jacobs, K., Mutreja, K., Ursich, S., Ray Chaudhuri, A., Nussenzweig, A., Janscak, P., and Lopes, M. (2017). Replication fork reversal triggers fork degradation in BRCA2-defective cells. Nat. Commun. *8*, 859.

Mimitou, E.P., and Symington, L.S. (2010). Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. EMBO J. *29*, 3358–3369.

Möckel, C., Lammens, K., Schele, A., and Hopfner, K.P. (2012). ATP driven structural changes of the bacterial Mre11:Rad50 catalytic head complex. Nucleic Acids Res. *40*, 914–927.

Moore, J.K., and Haber, J.E. (1996). Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *16*, 2164–2173.

Moreau, S., Ferguson, J.R., and Symington, L.S. (1999). The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. Mol. Cell. Biol. *19*, 556–566.

Myler, L.R., Gallardo, I.F., Soniat, M.M., Deshpande, R.A., Gonzalez, X.B., Kim, Y., Paull, T.T., and Finkelstein, I.J. (2017). Single-molecule imaging reveals how Mre11-Rad50-Nbs1 initiates DNA break repair. Mol. Cell *67*, 891–898.e894.

Nairz, K., and Klein, F. (1997). mre11S–a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. *11*, 2272–2290.

Nicolette, M.L., Lee, K., Guo, Z., Rani, M., Chow, J.M., Lee, S.E., and Paull, T.T. (2010). Mre11-Rad50-Xrs2 and Sae2 promote 5′ strand resection of DNA double-strand breaks. Nat. Struct. Mol. Biol. *17*, 1478–1485.

Ogawa, H., Johzuka, K., Nakagawa, T., Leem, S.H., and Hagihara, A.H. (1995). Functions of the yeast meiotic recombination genes, MRE11 and MRE2. Adv. Biophys. *31*, 67–76.

Oh, J., Al-Zain, A., Cannavo, E., Cejka, P., and Symington, L.S. (2016). Xrs2 dependent and independent functions of the Mre11-Rad50 complex. Mol. Cell *64*, 405–415.

Park, Y.B., Chae, J., Kim, Y.C., and Cho, Y. (2011). Crystal structure of human Mre11: understanding tumorigenic mutations. Structure *19*, 1591–1602.

Paull, T.T., and Gellert, M. (1998). The 3′ to 5′ exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. Mol. Cell *1*, 969–979.

Paull, T.T., and Gellert, M. (1999). Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. Genes Dev. *13*, 1276–1288.

Puddu, F., Oelschlaegel, T., Guerini, I., Geisler, N.J., Niu, H., Herzog, M., Salguero, I., Ochoa-Montaño, B., Viré, E., Sung, P., et al. (2015). Synthetic viability genomic screening defines Sae2 function in DNA repair. EMBO J. *34*, 1509–1522.

Quennet, V., Beucher, A., Barton, O., Takeda, S., and Löbrich, M. (2011). CtIP and MRN promote non-homologous end-joining of etoposide-induced DNA double-strand breaks in G1. Nucleic Acids Res. *39*, 2144–2152.

Rattray, A.J., McGill, C.B., Shafer, B.K., and Strathern, J.N. (2001). Fidelity of mitotic double-strand-break repair in Saccharomyces cerevisiae: a role for SAE2/COM1. Genetics *158*, 109–122.

Ray Chaudhuri, A., Callen, E., Ding, X., Gogola, E., Duarte, A.A., Lee, J.E., Wong, N., Lafarga, V., Calvo, J.A., Panzarino, N.J., et al. (2016). Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature *535*, 382–387.

Reginato, G., Cannavo, E., and Cejka, P. (2017). Physiological protein blocks direct the Mre11-Rad50-Xrs2 and Sae2 nuclease complex to initiate DNA end resection. Genes Dev. *31*, 2325–2330.

Schiller, C.B., Lammens, K., Guerini, I., Coordes, B., Feldmann, H., Schlauderer, F., Möckel, C., Schele, A., Strässer, K., Jackson, S.P., and Hopfner, K.P. (2012). Structure of Mre11-Nbs1 complex yields insights into ataxia-telangiectasia-like disease mutations and DNA damage signaling. Nat. Struct. Mol. Biol. *19*, 693–700.

Schlacher, K., Christ, N., Siaud, N., Egashira, A., Wu, H., and Jasin, M. (2011). Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell *145*, 529–542.

Seeber, A., Hegnauer, A.M., Hustedt, N., Deshpande, I., Poli, J., Eglinger, J., Pasero, P., Gut, H., Shinohara, M., Hopfner, K.P., et al. (2016). RPA mediates recruitment of MRX to forks and double-strand breaks to hold sister chromatids together. Mol. Cell *64*, 951–966.

Seifert, F.U., Lammens, K., Stoehr, G., Kessler, B., and Hopfner, K.P. (2016). Structural mechanism of ATP-dependent DNA binding and DNA end bridging by eukaryotic Rad50. EMBO J. *35*, 759–772.

Shibata, A., Moiani, D., Arvai, A.S., Perry, J., Harding, S.M., Genois, M.M., Maity, R., van Rossum-Fikkert, S., Kertokalio, A., Romoli, F., et al. (2014). DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. Mol. Cell *53*, 7–18.

Shim, E.Y., Chung, W.H., Nicolette, M.L., Zhang, Y., Davis, M., Zhu, Z., Paull, T.T., Ira, G., and Lee, S.E. (2010). Saccharomyces cerevisiae Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. EMBO J. *29*, 3370–3380.

Sirbu, B.M., Couch, F.B., Feigerle, J.T., Bhaskara, S., Hiebert, S.W., and Cortez, D. (2011). Analysis of protein dynamics at active, stalled, and collapsed replication forks. Genes Dev. *25*, 1320–1327.

Spycher, C., Miller, E.S., Townsend, K., Pavic, L., Morrice, N.A., Janscak, P., Stewart, G.S., and Stucki, M. (2008). Constitutive phosphorylation of MDC1 physically links the MRE11-RAD50-NBS1 complex to damaged chromatin. J. Cell Biol. *181*, 227–240.

Taglialatela, A., Alvarez, S., Leuzzi, G., Sannino, V., Ranjha, L., Huang, J.W., Madubata, C., Anand, R., Levy, B., Rabadan, R., et al. (2017). Restoration of replication fork stability in BRCA1- and BRCA2-deficient cells by inactivation of SNF2-family fork remodelers. Mol. Cell *68*, 414–430.e418.

Tomita, K., Matsuura, A., Caspari, T., Carr, A.M., Akamatsu, Y., Iwasaki, H., Mizuno, K., Ohta, K., Uritani, M., Ushimaru, T., et al. (2003). Competition between the Rad50 complex and the Ku heterodimer reveals a role for Exo1 in processing double-strand breaks but not telomeres. Mol. Cell. Biol. *23*, 5186–5197.

Trujillo, K.M., and Sung, P. (2001). DNA structure-specific nuclease activities in the Saccharomyces cerevisiae Rad50*Mre11 complex. J. Biol. Chem. *276*, 35458–35464.

Trujillo, K.M., Yuan, S.S., Lee, E.Y., and Sung, P. (1998). Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. J. Biol. Chem. *273*, 21447–21450.

Usui, T., Ohta, T., Oshiumi, H., Tomizawa, J., Ogawa, H., and Ogawa, T. (1998). Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell *95*, 705–716.

Vallerga, M.B., Mansilla, S.F., Federico, M.B., Bertolin, A.P., and Gottifredi, V. (2015). Rad51 recombinase prevents Mre11 nuclease-dependent degradation and excessive PrimPol-mediated elongation of nascent DNA after UV irradiation. Proc. Natl. Acad. Sci. USA *112*, E6624–E6633.

Wang, H., Shi, L.Z., Wong, C.C., Han, X., Hwang, P.Y., Truong, L.N., Zhu, Q., Shao, Z., Chen, D.J., Berns, M.W., et al. (2013). The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. PLoS Genet. *9*, e1003277.

Wang, H., Li, Y., Truong, L.N., Shi, L.Z., Hwang, P.Y., He, J., Do, J., Cho, M.J., Li, H., Negrete, A., et al. (2014). CtIP maintains stability at common fragile sites and inverted repeats by end resection-independent endonuclease activity. Mol. Cell *54*, 1012–1021.

Wang, W., Daley, J.M., Kwon, Y., Krasner, D.S., and Sung, P. (2017). Plasticity of the Mre11-Rad50-Xrs2-Sae2 nuclease ensemble in the processing of DNA-bound obstacles. Genes Dev. *31*, 2331–2336.

Wasko, B.M., Holland, C.L., Resnick, M.A., and Lewis, L.K. (2009). Inhibition of DNA double-strand break repair by the Ku heterodimer in mrx mutants of Saccharomyces cerevisiae. DNA Repair (Amst.) *8*, 162–169.

Williams, R.S., Moncalian, G., Williams, J.S., Yamada, Y., Limbo, O., Shin, D.S., Groocock, L.M., Cahill, D., Hitomi, C., Guenther, G., et al. (2008). Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. Cell *135*, 97–109.

Williams, R.S., Dodson, G.E., Limbo, O., Yamada, Y., Williams, J.S., Guenther, G., Classen, S., Glover, J.N., Iwasaki, H., Russell, P., and Tainer, J.A. (2009). Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. Cell *139*, 87–99.

Williams, G.J., Williams, R.S., Williams, J.S., Moncalian, G., Arvai, A.S., Limbo, O., Guenther, G., SilDas, S., Hammel, M., Russell, P., and Tainer, J.A. (2011). ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. Nat. Struct. Mol. Biol. *18*, 423–431.

Wu, D., Topper, L.M., and Wilson, T.E. (2008). Recruitment and dissociation of nonhomologous end joining proteins at a DNA double-strand break in Saccharomyces cerevisiae. Genetics *178*, 1237–1249.

Yamaguchi-Iwai, Y., Sonoda, E., Buerstedde, J.M., Bezzubova, O., Morrison, C., Takata, M., Shinohara, A., and Takeda, S. (1998). Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in RAD52. Mol. Cell. Biol. *18*, 6430–6435.

Yamaguchi-Iwai, Y., Sonoda, E., Sasaki, M.S., Morrison, C., Haraguchi, T., Hiraoka, Y., Yamashita, Y.M., Yagi, T., Takata, M., Price, C., et al. (1999). Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells. EMBO J. *18*, 6619–6629.

Ying, S., Hamdy, F.C., and Helleday, T. (2012). Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1. Cancer Res. *72*, 2814–2821.

Yun, M.H., and Hiom, K. (2009). CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature *459*, 460–463.

Zhang, Y., and Jasin, M. (2011). An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. Nat. Struct. Mol. Biol. *18*, 80–84.