Current Genetics
https://doi.org/10.1007/s00294-018-0861-5

REVIEW

Structure–function relationships of the Mre11 protein in the control of DNA end bridging and processing

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Received: 22 May 2018 / Revised: 15 June 2018 / Accepted: 15 June 2018

Abstract

The evolutionarily conserved Mre11–Rad50–Xrs2 (MRX) complex cooperates with the Sae2 protein in initiating resection of DNA double-strand breaks (DSBs) and in maintaining the DSB ends tethered to each other for their accurate repair. How these MRX–Sae2 functions contribute to DNA damage resistance is not understood. By taking advantage of *mre11* alleles that suppress the hypersensitivity of *sae2*Δ cells to genotoxic agents, we have recently found that Mre11 can be divided in two structurally distinct domains that support resistance to genotoxic agents by mediating different processes. While the Mre11 N-terminal domain impacts on the resection activity of long-range resection nucleases by mediating MRX and Tel1/ATM association to DNA DSBs, the C-terminus influences the MRX-tethering activity by its virtue to interact with Rad50. Given the evolutionary conservation of the MRX complex, our results have implications for understanding the consequences of its dysfunctions in human diseases.

Keywords DNA damage · Mre11 · Rad50 · Resection · Sae2 · Tel1

DNA double-strand breaks (DSBs) are cytotoxic lesions that can arise spontaneously during DNA replication or can be induced by exposure to radiation or chemicals, such as chemotherapeutic agents used in cancer therapies. Failure to repair them can result in genome instability that is a well-known hallmark of cancer cells.

DSBs can be repaired by either of two mechanisms: non-homologous end-joining (NHEJ) and homologous recombination (HR). NHEJ acts by directly ligating DNA ends with very minimal or no base pairing at the junction (Mehta and Haber 2014; Durdíková and Chovanec 2017). This process requires the binding to DNA ends of the Ku70–80 heterodimer (hereafter referred to as Ku), followed by ligation of the broken DNA ends by the DNA ligase IV (Dnl4/Lig4 in *Saccharomyces cerevisiae*) complex. In *S. cerevisiae*, DSB repair by NHEJ requires also the evolutionarily conserved MRX/MRN complex (Mre11–Rad50 in prokaryotes; Mre11–Rad50–Xrs2 in yeast; MRE11–RAD50–NBS1 in mammals). Interestingly, the *S. cerevisiae* requirement for MRX in NHEJ-mediated repair of DSBs generated by nucleases is dispensable in both *S. pombe* and mammalian cells, although this complex is required for unusual NHEJ reactions in *S. pombe* (Runge and Li 2018).

In contrast to NHEJ, HR uses intact homologous duplex DNA sequences as templates for accurate repair (Daley et al. 2014; Mehta and Haber 2014). In HR, the 5′ DNA strands on both sides of the DSB are nucleolytically degraded through a process termed resection (Villa et al. 2016). The resulting 3′-ended single-stranded DNA (ssDNA) tails are first coated by the ssDNA binding complex replication protein A (RPA), which is then replaced by Rad51 to form a nucleofilament that searches for DNA homologous sequences and catalyzes strand invasion (Daley et al. 2014; Mehta and Haber 2014).

The MRX/MRN complex functions together with the Sae2 protein (CtIP in mammals) in initiation of DSB resection (Gobbini et al. 2016). In addition, both *S. cerevisiae* MRX and Sae2 have architectural DNA scaffolding activities that play important roles in maintaining the DSB ends tethered to each other for their repair (Kaye et al. 2004; Lobachev et al. 2004; Clerici et al. 2005; Lee et al. 2008; Nakai et al. 2011). The eukaryote-specific Xrs2 (NBS1 in

Communicated by M. Kupiec.

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Published online: 19 June 2018

mammals) protein, containing FHA and BRCT motifs but otherwise largely disordered, flexibly links the MRX complex to Tel1 (ATM in mammals) checkpoint kinase (Nakada et al. 2003; Lee and Paull 2007; Williams et al. 2009).

The N-terminal domain of Mre11 possesses endonuclease and 3′–5′ exonuclease activities in vitro (Paull and Gellert 1998; Trujillo et al. 1998). Rad50 is an ATPase that is characterized by the ATP-binding motives Walker A and B located at the N- and C-terminal regions of the protein. Upon ATP binding, these motives associate intermolecularly and together with two Mre11 proteins (Hopfner et al. 2001; Moncalian et al. 2004; Williams et al. 2008), with the sequence in between forming a long antiparallel coiled-coil, whose apex contains a CXXC motif that can dimerize and tether together MRX complexes at their bound DNA ends (de Jager et al. 2001; Hopfner et al. 2002; Lobachev et al. 2004; Wiltzius et al. 2005; Hohl et al. 2011; Seifert et al. 2016). Structural studies have shown that ATP binding and hydrolysis activities of Rad50 are crucial to regulate DNA binding, nuclease and tethering functions of the MRX complex. In the presence of ATP, the two Rad50 ATPase domains dimerize and occlude the active site of Mre11 dimer (Lim et al. 2011; Möckel et al. 2012). This ATP-bound conformation has been proposed to be important for DNA binding and tethering activities of the complex (Deshpande et al. 2014). ATP hydrolysis drives the rotation of the two nucleotide-binding domains of Rad50 and the disengagement of Rad50 dimer, which allows DNA to access the Mre11 active sites and the MRX engagement in DSB resection (Lammens et al. 2011; Lim et al. 2011; Deshpande et al. 2014).

Studies in budding yeast indicate that MRX initiates DSB resection by catalyzing an endonucleolytic cleavage in the 5′-terminated strands at both DSB ends. This reaction is stimulated by the Sae2 protein (Cannavo and Cejka 2014), whose protein level in the cell is controlled by multiple and poorly understood mechanisms (Robert et al. 2011; Leshets et al. 2018). The resulting nick generates an entry site for the Mre11 exonuclease that degrades in the 3′–5′ direction toward the DSB end, whereas 5′–3′ degradation from the gap is carried out by either Exo1 or the combined activities of the Sgs1 helicase and the Dna2 endonuclease (Mimitou and Symington 2008; Zhu et al. 2008; Cejka et al. 2010; Niu et al. 2010; Garcia et al. 2011; Nimonkar et al. 2011; Shibata et al. 2014; Reginato et al. 2017; Wang et al. 2017). In addition, MRX has also a structural role in allowing Sgs1 and Exo1 recruitment to the DSB, independently of both Mre11 nuclease activity and Sae2 (Nicolette et al. 2010; Shim et al. 2010; Cejka et al. 2010; Niu et al. 2010; Cannavo et al. 2013).

The lack of any MRX subunit or of Sae2 increases the sensitivity to phleomycin, which generates chemically complex DNA termini, and to camptothecin (CPT), which reversibly traps the topoisomerase Top1 on nicked DNA intermediates thus extending the half-life of topoisomerase 1-DNA cleavable complexes (Top1ccs) (Pommier et al. 2016). To understand how the functions of MRX–Sae2 in DSB resection and end-tethering contribute to DNA damage resistance, we searched for and characterized *S. cerevisiae mre11* mutations that suppress the *sae2*Δ hypersensitivity to phleomycin and/or to CPT (Cassani et al. 2018). The functional and structural characterization of these mutant variants revealed the existence of structurally distinct Mre11 domains that support resistance to genotoxic agents by mediating different processes.

We found that the *mre11-H98Y, mre11-K292E* and *mre11-R389C* mutations restore *sae2*Δ resistance to both CPT and phleomycin and also increase the resection efficiency of *sae2*Δ cells. By contrast, the *mre11-R522H* and *mre11-N631Y* mutations restore resistance only to phleomycin and bypass the function of Sae2 in end-tethering but not in end-resection. These findings indicate that Sae2–MRX function in end-resection appears to be more important to repair CPT rather than phleomycin-induced DNA lesions, which instead rely on the tethering activity of MRX–Sae2 for their repair.

Structural studies have shown that Mre11 can be divided into an N-terminal core domain, which comprises the phosphodiesterase and the capping domains, and a C-terminal domain, which binds the Rad50 coiled-coils (Lim et al. 2011; Lammens et al. 2011; Williams et al. 2011). These two domains are linked by an extended connecting loop (C-linker). Notably, the *mre11-H98Y, mre11-K292E* and *mre11-R389C* mutations restoring CPT resistance in *sae2*Δ cells are located in the Mre11 N-terminus and upstream the C-linker, in a region which has been extensively characterized as far as protein structure is concerned (Fig. 1). Furthermore, independent searches for *mre11* mutations that bypass Sae2 function in CPT resistance identified changes in H37, Q70, T74, L77, L89, E101 and P110 residues (Chen et al. 2015; Puddu et al. 2015), again all located in Mre11 N-terminus (Fig. 1). By contrast, both the *mre11-R522H* and *mre11-N631Y* mutations specifically bypassing Sae2 function in end-tethering are located in the Mre11 C-terminus, downstream the C-linker (Fig. 2). This finding indicates that the C-linker divides Mre11 in two architecturally distinct modules that support resistance to DNA damage by controlling specific sets of MRX functions.

MRX has at least two roles in DSB resection: (1) it catalyzes the endonucleolytic cleavage of the 5′-terminated strands at both DNA ends in a Sae2-dependent manner; (2) it promotes the association of the downstream nucleases Exo1 and Sgs1–Dna2 in a Sae2-independent manner (Gobbini et al. 2016). The Mre11-H98Y, Mre11-K292E and Mre11-R389C mutant variants do not bypass the requirement for Sae2 in the activation of Mre11 endonuclease, indicating that the suppression of *sae2*Δ CPT sensitivity is not due to a

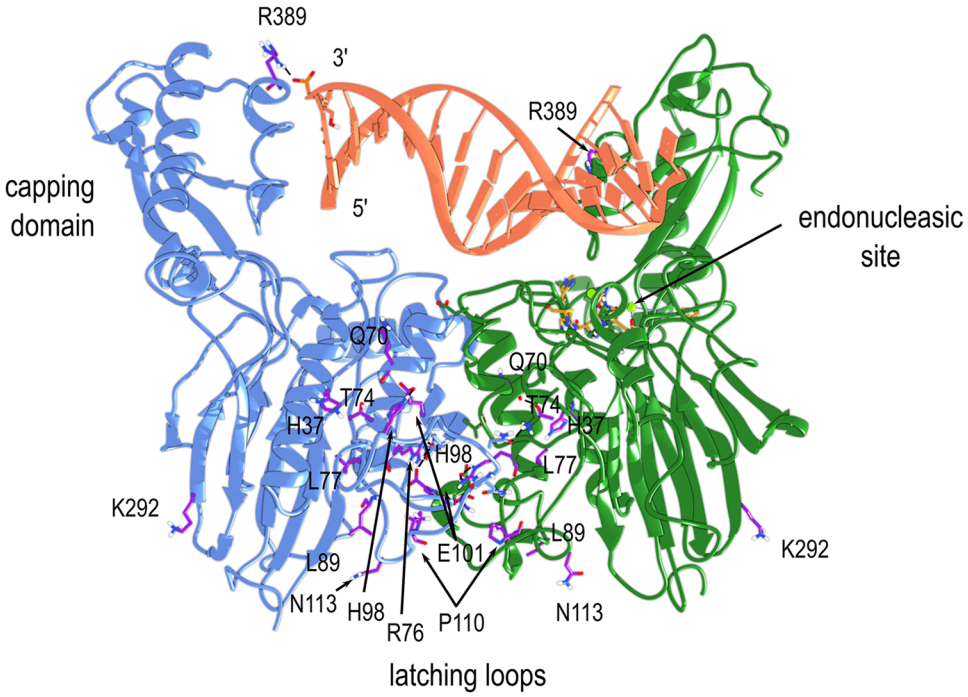

Fig. 1 N-terminal region of Mre11 with dsDNA. The mutations identified as suppressors of both the CPT and phleomycin hypersensitivity of *sae2*Δ cells are mapped (in purple) in the N-terminal region of *S. cerevisiae* Mre11 on the structural model described in Cassani et al. (2018), where a strand of dsDNA (in orange) is bound to *S. cerevisiae* Mre11 dimer. The two Mre11 monomers are shown in light blue and green. Residues involved in the endonuclease activity are shown in yellow. Other residues involved in Mre11 dimer stabilization by hydrogen bonds (black dashed lines) are shown on the latching loops

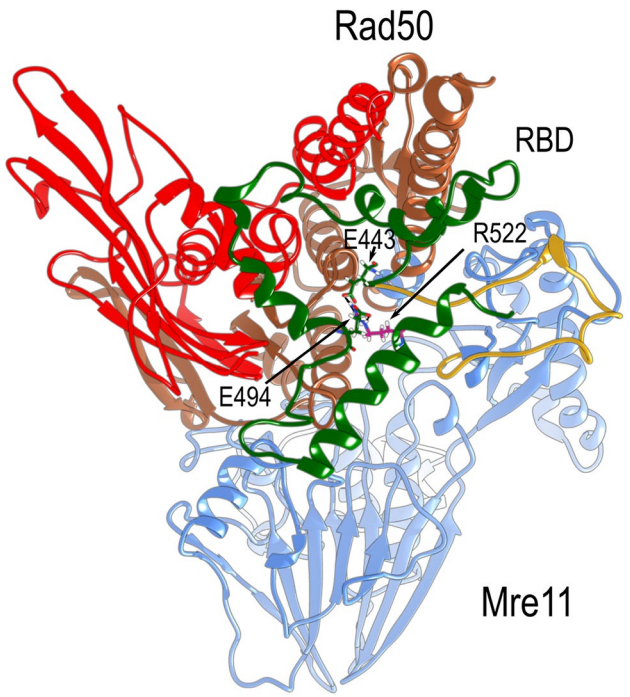

Fig. 2 Rad50-binding domain (RBD) of Mre11. The position of R522 residue (in purple), localized in the RBD region of *S. cerevisiae* Mre11, is shown on a structural model of ScMre11 and Rad50 heterodimer described in Cassani et al. (2018). Rad50 N-terminal lobe is in red, Rad50 C-terminal lobe in brown, RBD is in green. Mre11 N-terminal region is in light blue and the connecting C-linker is in yellow. Residues interacting with R522 are highlighted

more efficient endonucleolytic removal of Top1ccs. Rather, Mre11-H98Y, Mre11-K292E and Mre11-R389C increase the efficiency of DSB resection of *sae2*Δ cells by potentiating the Sgs1-Dna2 resection machinery. This finding suggests that they restore CPT resistance by facilitating the processing and the repair of DNA DSBs that are generated from the encountering of Top1ccs that are reversibly trapped by CPT with DNA replication or transcription. The primary cause of the resection defect in *sae2*Δ cells is an increased Rad9 association at DSBs that is due to an enhanced Tel1 signalling activity (Usui et al. 2001; Clerici et al. 2006; Ferrari et al. 2015; Gobbini et al. 2015). Rad9 binding at DSBs in turn reduces the resection activity of Sgs1–Dna2 (Gobbini et al. 2015; Bonetti et al. 2015). Mre11-H98Y, Mre11-K292E and Mre11-R389C bind poorly to DNA ends and this defect causes decreased Tel1 association to DSBs. As a consequence, diminished Tel1 persistence leads to a decreased amount of Rad9 bound at DSBs and therefore to a relief of Sgs1–Dna2 inhibition.

Our structural studies by homology modeling and molecular dynamics of *S. cerevisiae* Mre11–Rad50 indicate that H98Y and R389C amino acid substitutions reduce MRX association to DNA by altering different MRX properties. In particular, H98 is located in the eukaryotic-specific latching loop, which not only binds Xrs2, but also substantially extends the Mre11 dimer interface (Schiller et al. 2012) (Fig. 1). The H98Y substitution does not affect the binding of Mre11 with Xrs2. Rather, this mutation reduces MRX association to DNA ends by impairing Mre11 dimer formation, which is required for MRX–DNA association

(Williams et al. 2008). Several of the other *mre11* mutations previously identified as suppressors of *sae2*Δ CPT hypersensitivity, targeting H37, Q70, T74, L77, L89, E101 and P110 residues, map in this region (Fig. 1), suggesting that they can decrease Tel1 association to DNA by impairing Mre11–Xrs2 interaction and/or Mre11 dimer formation. Consistently, the *S. cerevisiae* R76A (corresponding to R80A on human MRE11; Park et al. 2011) and N113S mutations, which both map in this Mre11 region (Fig. 1), were reported to abolish Mre11 binding to Xrs2, with the former impeding also Mre11 dimerization (Schiller et al. 2012). MRE11 mutations in humans result in the ataxia telangiectasia-like disease (ATLD), which is characterized by radiation hypersensitivity, chromosome instability, neurological defects and cancer predisposition only in certain kindreds (Stewart et al. 1999). Interestingly, the hMRE11 N117S mutation (equivalent to *S. cerevisiae* Mre11 N113S) found in ATLD3/4 patients impairs Mre11–Nbs1 interaction and telomere length maintenance, consistent with a defect in recruiting ATM (Schiller et al. 2012).

By contrast, the R389 residue directly contacts the phosphodiesteric bridge of the 3′ DNA terminus and its substitution with C can reduce Mre11–DNA association by eliminating the positive charge (Fig. 1). However, as the R389 residue also contacts Rad50 in the closed tetrameric complex, we cannot exclude that the mutation, by eliminating a salt bridge that needs to be broken during the ATP-driven rotation of the Rad50 subunits, could favor the conformational transition of Mre11–Rad50 to an open configuration that is known to possess a reduced DNA-binding affinity (Deshpande et al. 2014).

The *mre11-R522H* and *mre11-N631Y* mutations, which are located at the C-terminus, bypass Sae2 function in end-tethering but not in end-resection, suggesting that the ssDNA generated in *sae2*Δ cells is enough to repair phleomycin-induced DNA lesions (Jinks-Robertson et al. 1993; Ira and Haber 2002). We also found that the $M^{R522H}RX$ mutant complex possesses an increased efficiency of DNA tethering per se. This enhanced end-tethering activity does not restore *sae2*Δ phleomycin resistance by increasing NHEJ efficiency. In the major HR pathway, the 3′-ended ssDNA tail invades the duplex homologous DNA region creating a D-loop structure that can be extended by DNA synthesis to form a double Holliday junction intermediate. Alternatively, the newly synthesized strand can dissociate from the D-loop and eventually pairs with the resected 3′ ssDNA on the other side of the DSB, resulting in noncrossover outcome in a process called synthesis-dependent strand annealing (SDSA) (Mehta and Haber 2014). Interestingly, we found that *sae2*Δ cells are specifically defective in DSB repair by SDSA and the *mre11-R522H* mutation suppresses this defect. This finding suggests that the end-tethering activity of MRX is particularly important for SDSA-mediated DSB repair, possibly because it facilitates the annealing of the displaced strand to the other DSB end.

Structural studies have shown that, in the presence of ATP, the Rad50 dimer associates with Mre11 via both the Mre11-capping domain and the Rad50-binding domain (RBD). In prokaryotes, the RBD is constituted by two helices that interact with the Rad50 coiled-coil base through a conserved hydrophobic surface patch (Williams et al. 2011). This is conserved in eukaryotic Mre11 RBD, but additional motifs are present that take contact with Rad50 (Seifert et al. 2016; Fig. 2). The R522 residue is located in the RBD domain according to a model we have recently shown (Cassani et al. 2018), and its substitution to H might confer more mobility to the whole RBD by disrupting some of its interactions (Fig. 2). As Mre11-R522H possesses an increased tethering activity by itself, the substitution of R522 with H might destabilize the Mre11–Rad50 interaction only after ATP hydrolysis, when the Mre11-capping domain dissociates from Rad50 and the RBD becomes essential to maintain the Mre11–Rad50 interaction (Lim et al. 2011). As a consequence, a defect in the stabilization of the interaction between Mre11 and ADP-bound Rad50 could lead to increased association at DSBs of MRX in its ATP-bound conformation, and therefore to a more efficient DNA end-tethering.

In summary, our results reveal that the C-linker divides Mre11 into two structurally distinct domains that mediate different MRX functions at DNA DSBs. While MRX abundance at DNA DSBs and its ability to promote the resection activity of the downstream nucleases is under the control of the Mre11 N terminus, the C-terminus influences the MRX-tethering activity by mediating Mre11–Rad50 interaction. These modules and their functions contribute differently in supporting DNA damage resistance depending on the genotoxic agents. Since the MRE11 alleles causing ATLD are partial loss-of-function mutations, our findings can be important to understand the different ATLD clinical outcomes to achieve accurate prognosis and treatment.

Acknowledgements We thank all members of the Longhese lab for helpful discussions. This work was supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) (IG Grant 19783) and Progetti di Ricerca di Interesse Nazionale (PRIN) 2015 to M. P. L. C. C. was supported by a fellowship from Fondazione Italiana Ricerca sul Cancro (FIRC).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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