Functions and regulation of the MRX complex at DNA double-strand breaks

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ABSTRACT DNA double-strand breaks (DSBs) pose a serious threat to genome stability and cell survival. Cells possess mechanisms that recognize DSBs and promote their repair through either homologous recombination (HR) or non-homologous end joining (NHEJ). The evolutionarily conserved Mre11-Rad50-Xrs2 (MRX) complex plays a central role in the cellular response to DSBs, as it is implicated in controlling end resection and in maintaining the DSB ends tethered to each other. Furthermore, it is responsible for DSB signaling by activating the checkpoint kinase Tel1 that, in turn, supports MRX function in a positive feedback loop. The present review focuses mainly on recent works in the budding yeast Saccharomyces cerevisiae to highlight structure and regulation of MRX as well as its interplays with Tel1.

INTRODUCTION DNA double-strand breaks (DSBs) are cytotoxic lesions that threaten genomic integrity. Misrepair of DSBs often leads to chromosome rearrangements and loss of genetic information that can result in cell death or oncogenic transformation. DSBs can arise spontaneously in eukaryotic cells either during DNA replication or as intermediates in programmed recombination events, such as meiosis and immune system development. They can also be induced by exposure to DNA-damaging agents used in cancer therapies, including ionizing radiation and topoisomerase poisons.

Cells have evolved two main mechanisms to repair DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ directly religates the two broken DNA ends with no or minimal base pairing at the junction [1]. HR uses intact homologous duplex DNA sequences (sister chromatids or homologous chromosomes) as templates for repairing DSBs in an error-free manner [2, 3]. In order to repair DSBs by HR, the 5’ strands of each DSB end has to be nucleolytically degraded, in a process referred to as resection [4, 5]. 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 the recombinase Rad51 to form a right-handed helical filament that searches for DNA homologous sequences and catalyzes invasion of the duplex DNA molecules [2, 3]. Initiation of resection not only channels DSB repair to HR but irreversibly inhibits NHEJ, indicating that this process is critical for discriminating between homology-dependent and end-joining repair of DSBs.

The highly conserved MRX/MRN complex (Mre11-Rad50-Xrs2 in yeast; Mre11-Rad50-Nbs1 in mammals) is among the first protein complexes that are recruited at DSBs [6]. MRX plays an important role in controlling end resection and in maintaining the DSB ends tethered to each other for their repair by NHEJ or HR [4, 5, 7]. Furthermore, it is implicated in the recruitment and activation...
interaction modules, among which there is a conserved region within the C-terminus that is responsible for the DNA binding and processing core of MRX [18-22] (Fig. 1). The Rad50 coiled-coil region protrudes from the head domain and its base interacts with two α helices of Mre11 located carboxy-terminal to the nuclease core domain [23-26]. At the apex of the coiled-coil, where the N-terminal and C-terminal regions fold back on themselves, a CXXC motif creates a zinc-mediated hook that allows the nuclease active sites that can access DNA to initiate DSB resection. The ATP-bound state of Rad50 negatively regulates MRX nuclease activity by masking the Mre11 nuclease sites. ATP hydrolysis by Rad50 causes conformational changes of both Rad50 and Mre11, resulting in disengagement of Rad50 dimer and exposure of the Mre11 active sites that can access DNA to initiate DSB resection. The Mre11 nuclease sites are indicated by yellow stars. ATP and ADP are indicated by purple and pink dots, respectively.

**ARCHITECTURE OF THE MRX COMPLEX**

The Mre11 subunit of MRX contains five conserved phosphoesterase motifs that are essential for both 3'-5' double-stranded DNA (dsDNA) exonuclease and ssDNA endonuclease activities [10-16]. Mre11 interacts with Rad50, whose domain organization is similar to that of the structural maintenance of chromosomes (SMC) family of proteins [17]. Rad50 is an ATPase belonging to the ABC ATPase superfamily that is characterized by the ATP-binding motives Walker A and B located at the amino- and carboxy-terminal regions of the protein. These motives associate together, with the intervening sequence forming a long antiparallel coiled-coil. TwoRad50 ATPase domains interact with two Mre11 nucleosome peptides to form a “head” domain, which is the DNA binding and processing core of MRX [18-22] (Fig. 1). The Rad50 coiled-coil region protrudes from the head domain and its base interacts with two α helices of Mre11 located carboxy-terminal to the nuclease core domain [23-26].

While Mre11 and Rad50 are conserved in bacteria and archaea, only eukaryotes possess Xrs2 (Nbs1 in mammals), which is responsible for nuclear localization of Mre11 [33]. The addition of a nuclear localization signal to Mre11 can partially suppress the hypersensitivity to DNA damaging agents of Xrs2-deficient cells [33], suggesting that localization of Mre11 into the nucleus is one of the main functions of Xrs2. Xrs2/Nbs1 contains a variety of protein-protein interaction modules, among which there is a conserved region within the C-terminus that is responsible for the interaction with Tel1/ATM [34, 35].

**FUNCTIONAL DYNAMICS OF THE MRX COMPLEX**

The MRX complex plays a central role in signaling, processing and repairing of DSBs. Structural studies have shown that these diverse functions are regulated by the ATP binding and hydrolysis activity of Rad50 that induce conformational changes of both Rad50 and Mre11. In the presence of ATP, Mre11 and Rad50 adopt a “closed” conformation, in which Rad50 head domains dimerize and occlude the nuclease active site of Mre11 (Fig. 1) [24, 26]. ATP hydrolysis drives the rotation of the two nucleotide binding domains of Rad50, leading to disengagement of the Rad50 dimer and DNA melting, so that the Mre11 active sites can access DNA to initiate DSB resection [23, 36] (Fig. 1). Point mutations that stabilize the ATP-bound conformation of Rad50 result in both reduced Mre11 nuclelease activity and increased DNA binding and end-tethering [37]. By contrast, mutations that increase ATP hydrolysis enhance both Mre11 nuclease and end-resection activities [37]. These data suggest that the ATP-bound state is required for DNA binding and tethering, whereas release from the ATP-bound state by ATP hydrolysis is necessary to allow access to DNA of the Mre11 nuclease active site and subsequent DSB resection (Fig. 1).

Rad50 has a slow ATP hydrolysis rate [38], suggesting that either MRX exists mostly in the ATP-bound state or other proteins can promote ATP hydrolysis within the cell. In *S. cerevisiae*, MRX is known to interact with Rif2, which is recruited to telomeric DNA ends by Rap1 and negatively regulates telomerase action [39-42]. Rif2 was recently found to be recruited also to intrachromosomal DSBs in a manner partially dependent on MRX [43]. Interestingly, Rif2 enhances the ATP hydrolysis activity of the MRX complex [43]. Furthermore, rif2Δ cells show an increased efficiency of both end-tethering and NHEJ compared to wild type cells, indicating that Rif2 counteracts end-tethering and DSB repair by NHEJ [43]. These observations suggest that Rif2 can modulate the choice between HR and NHEJ by promoting the transition of the MRX complex from a closed state, required for tethering, to an open state that is competent for DSB resection. Interestingly, Rif2 is known to promote the transition of the MRX complex from a closed state, required for tethering, to an open state that is competent for DSB resection.
to counteract NHEJ at telomeres [44]. Whether this Rif2 function depends on a Rif2-mediated regulation of MRX conformational changes is an interesting question that remains to be addressed.

**MRX IN END-RESECTION**

The first step in HR is the degradation of the 5' DNA strands on either side of the DSB through a process termed resection [4, 5]. Several lines of evidence indicate that the MRX complex functions together with the Sae2 protein (CtIP in mammals) in the processing of the DSB ends. In fact, *S. cerevisiae* mutants lacking Sae2 or any component of the MRX/MRN complex delay resection of an endonuclease-induced DSB by acting in the same epistasis group [45-48]. Furthermore, both Sae2 and MRX play a unique role in the processing of hairpin-containing DNA structures [49-51]. Finally, *sae2Δ, mre11* nuclease defective mutants and a class of separation-of-function *rad50* and *mre11* alleles, named *rad50Δs* and *mre11Δs*, allow formation of meiotic DSBs but prevent their resection [11, 52-56].

However, Mre11 has a 3'-5' dsDNA exonuclease activity, whose polarity is opposite to that required to generate the 3'-ended ssDNA at the DSB ends [12, 13]. Mre11 possesses also a weak endonuclease activity on 5'-terminated dsDNA strands [16, 57, 58], which is strongly stimulated by Sae2 and by a protein block at the DNA ends [59]. Furthermore, *sae2Δ* and *mre11* nuclease mutants are defective in the removal of covalent adducts, such as Spo11 (the trans-esterase that generates meiotic DSBs and remains covalently bound to the 5' strands of the ensuing breaks) [60-63] or hairpin-containing DNA structures, from DNA ends [49-51]. The same mutants are also hypersensitive to both camptothecin, which extends the half-life of DNA-topoisomerase cleavage complexes, and ionizing radiations, which can generate chemically complex DSBs [64-66]. Altogether, these observations suggest that the MRX complex initiates DNA resection by creating a nick that provides an internal entry site for nucleases capable of degrading DNA in a 5'-3' direction (Fig. 2).

These nucleases comprise the 5'-3' exonucleases Exo1 and the endonuclease Dna2, which control two partially overlapping pathways [67, 68]. While Exo1 is able to release mononucleotide products from a dsDNA end [69-71], Dna2-mediated resection needs the RecQ helicase Sgs1 (BLM in humans) that unwinds double-stranded DNA in a 3'-5' polarity [72-74]. Noteworthy, the MRX complex not only provides an entry site for Dna2 and Exo1, but it has also a structural role in allowing their recruitment to the DSB [75], thus explaining why *mre11Δ* cells show a resection defect more severe than *sae2Δ* or *mre11* nuclease defective mutants. In any case, *mre11Δ* cells are severely defective in DSB resection when the break is present in the G2 phase of the cell cycle, whereas they slow down resection only of two fold when the break occurs when they are exponentially growing [45-48]. This observation, together with the finding that a DSB is processed more efficiently during S phase than in G2 [76], suggests that ongoing DNA replication can partially bypass MRX requirement in DSB resection.

Both Sae2 and Dna2 have been shown to be targets of cyclin-dependent kinase (Cdk1 in yeast)-Clb complexes [77, 78], which allow DSB resection to take place only during the S and G2 phases of the cell cycle when sister chromatids or homologous chromosomes are present as repair templates [79, 80]. Substitution of Sae2 S267 with a non-phosphorylatable residue impairs DSB processing, whereas the same process takes place when Sae2 S267 is replaced by a residue mimicking constitutive phosphorylation [77]. Similarly, substitution of three Cdk1 consensus sites of Dna2 with alanines reduces extensive resection [78]. Notably, the lack of any subunit of the Ku complex allows DSB processing.
resection in G1-arrested cells when Cdk1-CIb activity is low [76, 81], suggesting that Cdk1-CIb activity can relieve the inhibitory effect exerted by Ku on Sae2. The finding that this resection in G1-arrested Ku-deficient cells is limited to the break-proximal sequence [76, 81] indicates that Cdk1 is still required to activate proteins involved in extensive DSB resection such as Dna2.

Altogether, these data support a model, where MRX is recruited to the DSB ends in the ATP-bound state. In this configuration, MRX maintains the DSB ends tethered to each other to allow DSB repair by NHEJ (Fig. 2). Upon ATP hydrolysis by Rad50 and Cdk1-mediated phosphorylation events, MRX and Sae2 provide an initial incision of the 5’ strand at a certain distance from the DSB end. As first proposed by Garcia et al. [82], this initial cleavage is followed by bidirectional resection using the Mre11 3’-5’ exonuclease and the 5’-3’ nuclease activities of Exo1 or Dna2-Sgs1 (Fig. 2).

**ACTIVATION OF Tel1 BY MRX**

In addition to promoting end-resection and to maintaining the DSB ends tethered to each other, MRX/MRN is required to activate Tel1, which is a member of the evolutionary conserved phosphoinositide 3-kinase-related protein kinase (PIKK) family and whose mammalian ortholog is called ATM (Ataxia Telangiectasia Mutated) [8]. The PIKK enzymes are large serine/threonine protein kinases characterized by N-terminal HEAT repeat domains and by C-terminal kinase domains [83]. The C-terminal kinase domain of ATM is flanked by two regions called FAT (FRAP, ATM, TRRAP) and FATC (FAT C-terminus), which both participate in the regulation of the kinase activity [84].

Tel1/ATM is a master regulator of the DNA damage response in both yeast and mammals, where it coordinates checkpoint activation and DNA repair in response to DNA DSBs and oxidative stress [85, 86]. Bisallelic mutations in ATM result in ataxia telangiectasia (AT), an autosomal recessive inherited disease characterized by cerebellar degeneration, strong predisposition to malignancy, growth retardation, radiosensitivity, immune deficiencies, and premature aging [87-89]. The functional interaction between MRX/MRN and Tel1/ATM is supported by the finding that bisallelic mutation in the *MRE11* gene causes a genetic syndrome, called ataxia-telangiectasia-like disease (ATLD), whose clinical phenotypes are nearly indistinguishable from AT [90, 91]. ATLD cells exhibit reduced activation of ATM by DSBs, suggesting that MRN is required for optimal ATM activation following DSB induction, thus explaining the AT-like phenotype of ATLD patients.

Subsequent studies have revealed that MRX/MRN drives the localization of Tel1/ATM to the site of damage through direct interaction between Tel1/ATM with Xrs2/Nbs1 [35, 92-94] (Fig. 3). The Tel1 kinase activity is stimulated by MRX binding to DNA-protein complexes at DSBs [95] and the purified MRX/MRN complex increases the catalytic activity of Tel1/ATM in the presence of DNA fragments [96-98], suggesting that MRX/MRN also controls Tel1/ATM catalytic activity through an unknown mechanism.

Tel1 was originally identified in *S. cerevisiae* by screen for genes involved in telomere length maintenance [99-101]. In addition to its role in DSB repair, Tel1 is required to maintain telomere length by promoting telomerase recruitment through phosphorylation events [102]. Deletion of any subunit of the MRX complex causes telomere shortening similar to that caused by the lack of Tel1 or both Tel1 and Rad50, indicating that Tel1 acts in the same pathway of MRX in telomere length maintenance [103]. As it is observed at DSBs, Tel1 binding to telomeres is dependent on an interaction between Tel1 and the carboxyl terminus of the Xrs2 subunit of the MRX complex [104, 105]. Tel1 association to telomeres is counteracted by Rif2, which is known to inhibit telomerase-dependent telomere elongation [39, 40]. Co-immunoprecipitation experiments show that the C terminus of Xrs2 interacts with Rif2 [41]. As Tel1 also binds this Xrs2 region [41], Rif2 may reduce Tel1 association to telomeres by interfering with MRX-Tel1 interaction.
tion. Further support for a Tel1-Rif2 competition comes from the recent finding that a hypermorphic allele of Tel1 (Tel1-hy909) is capable to overcome the inhibitory activity of Rif2 on MRX [42]. The Rif2 function in modulating MRX-Tel1 interaction is not restricted to telomeres. In fact, the lack of Rif2 increases the association of MRX also to intrachromosomal DNA ends in a Tel1-dependent manner [43] (Fig. 3). Consistent with a direct role of this protein at DSBs, Rif2 can bind DNA ends both in vitro and in vivo [43], although the amount of Rif2 bound at a DSB flanked by telomeric repeats is higher than that found at a DSB that does not contain telomeric sequences [41-43].

In S. cerevisiae, Tel1 signaling activity is disrupted when the DSB ends are subjected to 5’-3’ exonucleolytic degradation [106] (Fig. 3). Similarly, ATM activation in mammals is triggered by blunt ends or short overhangs and is inhibited by long overhangs of 3’ or 5’ ssDNA [107]. Interestingly, the lack of either Sae2 or Mre11 nuclease activity enhances Tel1/ATM activation at DSBs by increasing MRX persistence at DSBs [47, 108], suggesting that Sae2 and Mre11 nucleases can inhibit Tel1/ATM signaling activity. As the mammalian counterpart of MRX has been shown to bind ssDNA at DSBs [109], the slowing down of DSB resection might lead to MRX persistence at DSBs by increasing the stability of these DNA structures. Alternatively, ssDNA near the dsDNA junction may break to form a second DSB that activates MRX-Tel1, as suggested in [110].

Attenuation of Tel1/ATM signaling by nuclease-mediated DSB resection is accompanied by activation of the checkpoint kinase Mec1 (ATR in mammals), which is another member of the phosphoinositide 3-kinase-related protein kinase family [8] (Fig. 3). In both yeast and mammals, all Mec1/ATR functions at DSBs depend on the interaction with Ddc2 (ATRIP in mammals), which mediates Mec1/ATR recruitment on RPA-coated ssDNA 3’ overhangs [111-114].

**REGULATION OF MRX BY Tel1**

Recent data have shown that Tel1, once recruited to DSBs by MRX, supports MRX function in a positive feedback loop (Fig. 3). In fact, a screen for S. cerevisiae mutants that require Tel1 to survive to genotoxic treatments recently showed that a mutation in Rad50 (rad50-V1269M) makes tel1Δ cells hypersensitive to DNA damaging agents [43]. The rad50-V1269M mutation impairs MRX association at DSBs that is further reduced by the lack of Tel1, indicating that Tel1 promotes/stabilizes MRX association to the DSB. Interestingly, Tel1 exerts this function independently of its kinase activity [43], suggesting that it plays a structural role in promoting/stabilizing MRX retention to DSBs. Similarly, the lack of Tel1, but not of its kinase activity, was shown to impair MRX association also at DNA ends flanked by telomeric DNA repeats [43].

Although the rad50-V1269M mutation resides in the globular domain of Rad50, rad50-V1269M tel1Δ cells are severely defective in the maintenance of the DSB ends tethered to each other [43], suggesting that the Rad50 hook and globular domains function interdependently.

Consistent with this hypothesis, it has been shown that proper association to DNA of the globular domain can induce parallel orientation of the Rad50 coiled-coil domains that favours intercomplex association needed for DNA tethering [22]. Furthermore, mutations in the hook domain that reduce its dimerization state affect the MRX functions specified by the globular domain, including Tel1/ATM activation, Rad50-Mre11 interaction, NHEJ and DSB resection [115]. Altogether these results support a model wherein Tel1, once loaded at DSBs by MRX, exerts a positive feedback by promoting an end-specific association of MRX with DNA (Fig. 3). This Tel1-mediated regulation of DNA-MRX retention is important to allow the establishment of a productive MRX intercomplex association that is needed to maintain DNA ends in close proximity.

Defects in maintaining the DSB ends tethered to each other in tel1Δ rad50-V1269M cells affect DSB repair not only by NHEJ, but also by HR [43]. During HR, the 5’ ends at the DSB are degraded to yield 3’ ssDNA tails that invade an intact homologous duplex DNA to prime DNA synthesis. After ligation of the newly synthesized DNA to the resected 5’ strands, a double Holliday junction intermediate is generated and can be resolved by endonucleolytic cleavage to produce non-crossover (NCO) or crossover (CO) products. However, in mitotic cells, the invading strand is often displaced after limited synthesis and anneals to the 3’ ssDNA tail at the other end of the DSB. After fill-in synthesis and ligation, this mechanism, called synthesis-dependent strand annealing (SDSA), generates exclusively NCO products and explain the lower incidence of associated COs during mitotic DSB repair [116-118]. Interestingly, tel1Δ rad50-V1269M cells are specifically impaired in SDSA [43], suggesting that the tethering activity exerted by MRX can be particularly important to promote the annealing of the displaced strand to the 3’ ssDNA tail at the other end of the DSB. By contrast, this function can be escaped when the second DSB end is already captured by the D-loop and the DNA intermediate is stabilized by the formation of a double Holliday junction.

**CONCLUSION**

Overall, the MRX complex emerges as a multifunctional enzyme involved in a number of activities that include sensing and processing of the DSB ends. Furthermore, its association with Tel1 links DSB sensing with signaling by the checkpoint machinery to coordinate DSB repair with cell cycle progression. These functions are regulated by ATP binding and hydrolysis activities of Rad50 that provide intrinsic dynamics and flexibility properties. Furthermore, the Tel1 protein itself, once recruited at DNA ends by MRX, supports the end-tethering activity of the MRX complex by facilitating its proper association to DNA ends. These observations reveal the complex architecture that characterizes the activity of MRX and Tel1 in DNA-damage response, maintenance of genetic stability and cell cycle regulation.
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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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