



Review

Functional and structural insights into the MRX/MRN complex, a key player in recognition and repair of DNA double-strand breaks



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ABSTRACT

Chromosomal DNA double-strand breaks (DSBs) are potentially lethal DNA lesions that pose a significant threat to genome stability and therefore need to be repaired to preserve genome integrity. Eukaryotic cells possess two main mechanisms for repairing DSBs: non-homologous end-joining (NHEJ) and homologous recombination (HR). HR requires that the 5' terminated strands at both DNA ends are nucleolytically degraded by a concerted action of nucleases in a process termed DNA-end resection. This degradation leads to the formation of 3'-ended single-stranded DNA (ssDNA) ends that are essential to use homologous DNA sequences for repair. The evolutionarily conserved Mre11-Rad50-Xrs2/NBS1 complex (MRX/MRN) has enzymatic and structural activities to initiate DSB resection and to maintain the DSB ends tethered to each other for their repair. Furthermore, it is required to recruit and activate the protein kinase Tel1/ATM, which plays a key role in DSB signaling. All these functions depend on ATP-regulated DNA binding and nucleolytic activities of the complex. Several structures have been obtained in recent years for Mre11 and Rad50 subunits from archaea, and a few from the bacterial and eukaryotic orthologs. Nevertheless, the mechanism of activation of this protein complex is yet to be fully elucidated. In this review, we focused on recent biophysical and structural insights on the MRX complex and their interplay.

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1. Introduction

Chromosomal DNA double-strand breaks (DSBs) pose a significant threat to cell viability and genome stability because failure to repair them can lead to loss of genetic information and chromosome rearrangements. Although DSBs threaten genome integrity, germ cells deliberately sever both strands of their chromosomes

to initiate meiotic recombination that ensures proper homologous chromosome segregation [1]. Furthermore, DSBs are programmed recombination intermediates during antigen-receptor diversity in lymphocyte development [2].

Eukaryotic cells have evolved two main mechanisms to repair DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR) (Fig. 1). NHEJ allows direct rejoining of the

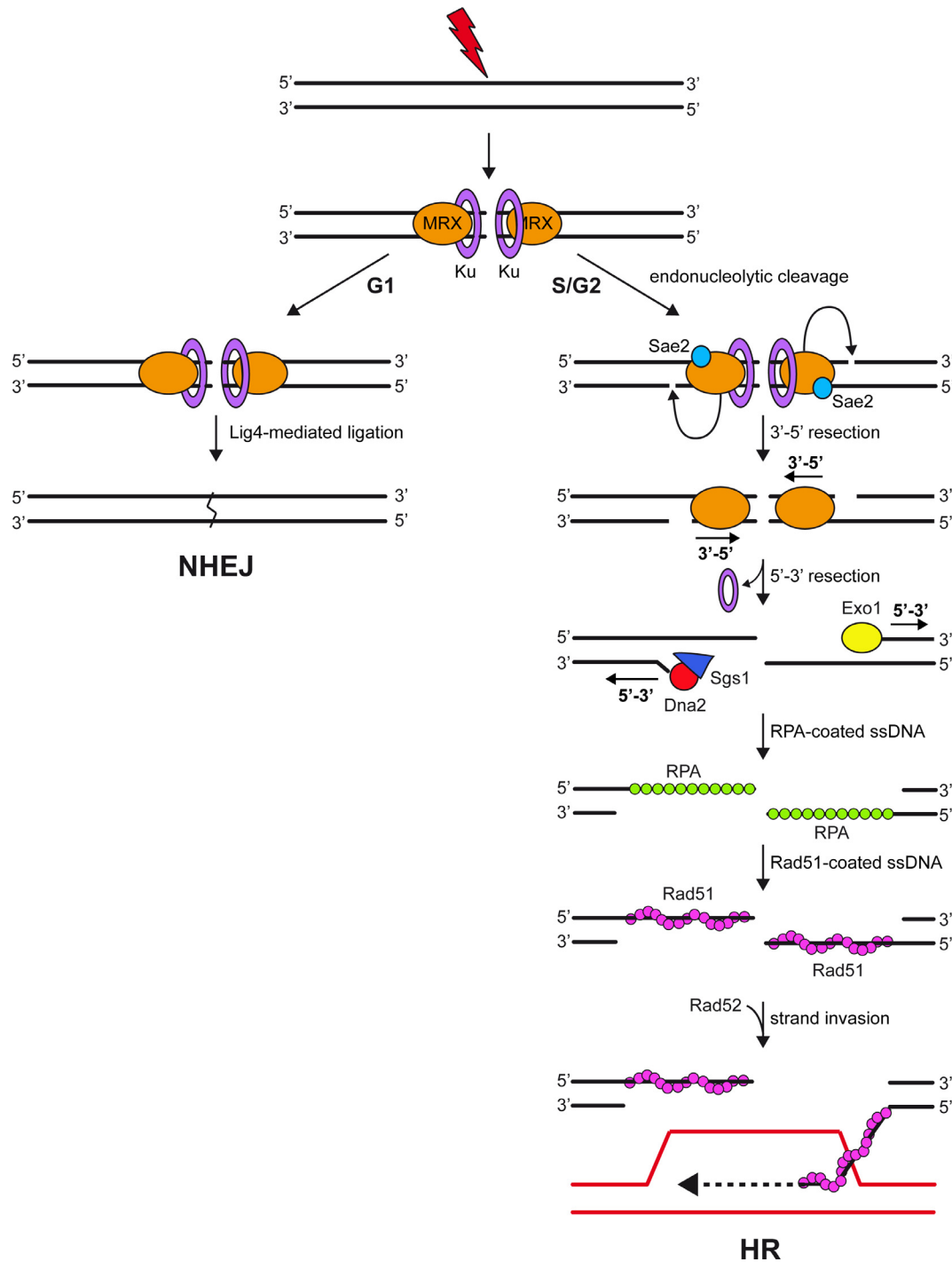


Fig. 1. Overview of the DSB repair mechanisms. DSBs are repaired by non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ directly rejoins the two DSB ends and occurs predominantly in the G1 phase of the cell cycle. HR utilizes homologous template and is active in S and G2. Once a DSB occurs, MRX and Ku are rapidly recruited to the DSB ends. After ATP hydrolysis by Rad50, Mre11 together with phosphorylated Sae2 catalyzes an endonucleolytic cleavage of the 5'-terminated strands at both DSB ends, followed by bidirectional resection catalyzed by Mre11 in the 3' to 5' direction and by Exo1 and Dna2-Sgs1 in the 5' to 3' direction. RPA binds to the 3'-ended ssDNA overhangs and is then replaced by Rad51. The Rad51-ssDNA complex promotes the homology search and strand invasion, pairing the invading 3' end with one strand of the donor duplex to template DNA synthesis (dashed line).

broken DNA ends with no or minimal base pairing at the junction and it operates predominantly in the G1 phase of the cell cycle [3]. By contrast, HR is the predominant repair pathway in the S and G2 phases of the cell cycle and it requires an undamaged homologous sequence (sister chromatids or homologous chromosomes) to serve as a template for repair of the broken DSB ends (Fig. 1) [4].

The initial step of NHEJ involves the binding to the DSB ends of the Ku70-Ku80 (Ku) heterodimer, which protects DNA ends from degradation and recruits additional NHEJ components such as the DNA ligase IV (Fig. 1) [3]. While NHEJ does not require extensive DSB end processing, HR initiates by nucleolytic degradation of the 5'-terminated strands at both DSB ends, in a process referred to as resection (Fig. 1) [5]. This degradation results in the generation of 3'-ended single-stranded DNA (ssDNA) ends, which are initially bound by the ssDNA binding protein complex Replication Protein A (RPA). RPA is then displaced by Rad51 to form a right-handed Rad51-ssDNA nucleoprotein filament that is essential for the homology search and pairing of the ssDNA with the complementary strand of the donor duplex DNA (Fig. 1) [4]. The 3'-terminated strand at the other side of the break anneals to the displaced strand. Extension by DNA synthesis and ligation generate a double Holliday junction that can be dissolved or resolved to yield intact duplex DNA molecules [4].

One of the primary protein complexes responsible for recognition, signaling and repair of DNA DSBs is the evolutionarily conserved Mre11-Rad50-Xrs2/NBS1 complex (MRX in *Saccharomyces cerevisiae*, MRN in humans). MRX/MRN is rapidly recruited to DSBs, where it initiates DSB resection and maintains the DSB ends tethered to each other for their repair [6]. Furthermore, it is required to recruit and activate the protein kinase Tel1 (ATM in mammals), which coordinates DSB repair with cell cycle progression [7]. Both MRX and Tel1 are also necessary to maintain the length of telomeres, specialized nucleoprotein complexes at the ends of eukaryotic chromosomes [8]. Finally, MRX/MRN also supports DNA replication under stress conditions. In particular, it promotes the recombinational repair of damaged replication forks by resecting nascent DNA strands [9,10]. In mammals, emerging evidence indicates that MRE11-mediated degradation of stalled replication forks is restrained by the recombination proteins BRCA1 and BRCA2 that promote formation of stable RAD51 nucleoprotein filaments [9,10].

At the molecular level, MRX/MRN is a hetero-hexameric ($M_2R_2X_2/N_2$) protein complex, in which the Mre11 subunit dimerizes and interacts independently with both Rad50 and Xrs2/NBS1 [6]. Rad50 is a member of the structural maintenance of chromosomes (SMC) protein family, characterized by ATPase motifs at the N- and C-termini separated by two long coiled-coil domains. The coiled coils fold back on themselves to form two complete ATPase sites on a Rad50 dimer [11,12].

Mre11 displays 3'-5' exonuclease and endonuclease activities [13–18]. In both yeast and mammals, Mre11 catalyzes the endonucleolytic cleavage of the 5'-terminated DNA strand in the vicinity of the DSB end [19–22] (Fig. 1). This endonucleolytic cleavage requires the ATPase activity of Rad50, as well as the Sae2 protein (Ctp1 in *Schizosaccharomyces pombe*, CtIP in mammals) that promotes the Mre11 endonuclease activity within the MRX/MRN complex [23–25]. The MRX-Sae2 initial cleavage is followed by bidirectional resection using the Mre11 3'-5' exonuclease, which proceeds back towards the DSB ends, and the nuclease activities of Exo1 (EXO1 in mammals), or of Dna2 (DNA2 in mammals) in complex with the RecQ-helicase homolog Sgs1 (BLM or WRN in mammals), which degrade DNA in the 5'-3' direction away from the DSB ends [26–32] (Fig. 1).

While orthologs of Rad50 and Mre11 are found in all kingdoms of life, the Xrs2/NBS1 subunit is specific to eukaryotes. In humans, germline hypomorphic mutations in MRE11, NBS1, or RAD50 are

associated with ataxia telangiectasia-like disorder (ATLD), Nijmegen breakage syndrome (NBS), and NBS-like disorder (NBSLD), respectively, which are characterized by cellular radiosensitivity, immune deficiency and cancer predisposition [33]. Aside from germline mutations, all three MRN complex components are mutated in more than 50 types of cancer, as assessed from the International Cancer Genome Consortium projects. Furthermore, in mammals, the MRN complex is essential for cell viability, as deletions of any MRN subunits result in embryonic lethality [34].

2. MR complex architecture

Recent findings have added more and more elements to the complexity of MRX/MRN flexible and dynamic mechanism of action, which evolved in order to integrate protein-protein and protein-DNA interactions, together with Mre11 intrinsic enzymatic activities, with organism specific regulatory networks. A plethora of structural and biophysical data were added to genetic and biochemical characterization of MRX/MRN function, allowing to develop a generally accepted model for Mre11 and Rad50 (MR) heterotetramer assembly, ATP hydrolysis by Rad50 and Mre11 nucleolytic activities [35]. A couple of decades of analyses by X-ray crystallography, small-angle X-ray scattering (SAXS), analytical ultracentrifugation, inductively coupled plasma mass spectrometry, dynamic light scattering, atomic force microscopy (AFM), electron microscopy (EM) and lately cryo-electron microscopy (cryo-EM) were collected and generally agreed on a well conserved architecture for a tetrameric complex constituted by a dimer of dimers of Mre11 and Rad50 (usually indicated as M_2R_2).

Early EM and AFM analyses had revealed a structure characterized by a globular head and a long, straight rod or ring bent projection that can adopt different conformations [11,36–41]. Structural studies allowed to identify the head as deriving from the association of two Rad50 nucleotide binding domains (NBD) and two Mre11 nuclease domains (ND), while the projections are constituted by the about 500 Å anti-parallel coiled-coil (CC) domain in the middle of Rad50 molecule (Fig. 2A) [11,12]. The coiled coils can form large proteinaceous rings or rods, which are joined by a CXXC motif at the apex of the coiled-coils that mediates Rad50 subunits interactions via tetrahedral coordination of a zinc ion [37,39,42–48].

Rad50 NBD contains the ABC ATPase domain, comprising the N-terminal Walker A and the C-terminal Walker B, as well as signature motifs typical of this family (Fig. S1A–C). Binding of a non-hydrolysable ATP analogue (AMP-PNP) to Rad50 induces a dramatic conformational rotation of the C-terminal ATPase subdomains (lobe II) relative to the N-terminal half (lobe I) [11,49–51] that increases the binding affinity of two Rad50 subunits [49,51]. This rotation also allows the formation of a dimer, which adopts a closed conformation with two molecules of ATP shared at the interface (Fig. 2B and C) [49,51].

Mre11 has five highly conserved phosphodiesterase motifs in the N-terminal nuclease domain (Fig. S2A and B) [13–18]. Besides, Mre11 protein is composed by a capping domain and a Rad50 binding domain (RBD). RBD consists in a helix-loop-helix (HLH) domain that takes contact with the base of Rad50 CC portion [50–52] (Fig. 2B and C). Actually, Mre11 embraces Rad50-ATP dimer not only with its RBD, but also with residues in the nuclease and the capping domains as well, resulting in Mre11 being completely inaccessible to double-stranded DNA (dsDNA) [50–56] (Fig. 2B and C). Residues in the nucleolytic catalytic sites are also involved in stabilizing protein-protein interaction [57]. The N-terminal and C-terminal portions of Mre11 are structurally and functionally distinct [58] and connected by a long and largely disordered linker that ensures high flexibility.

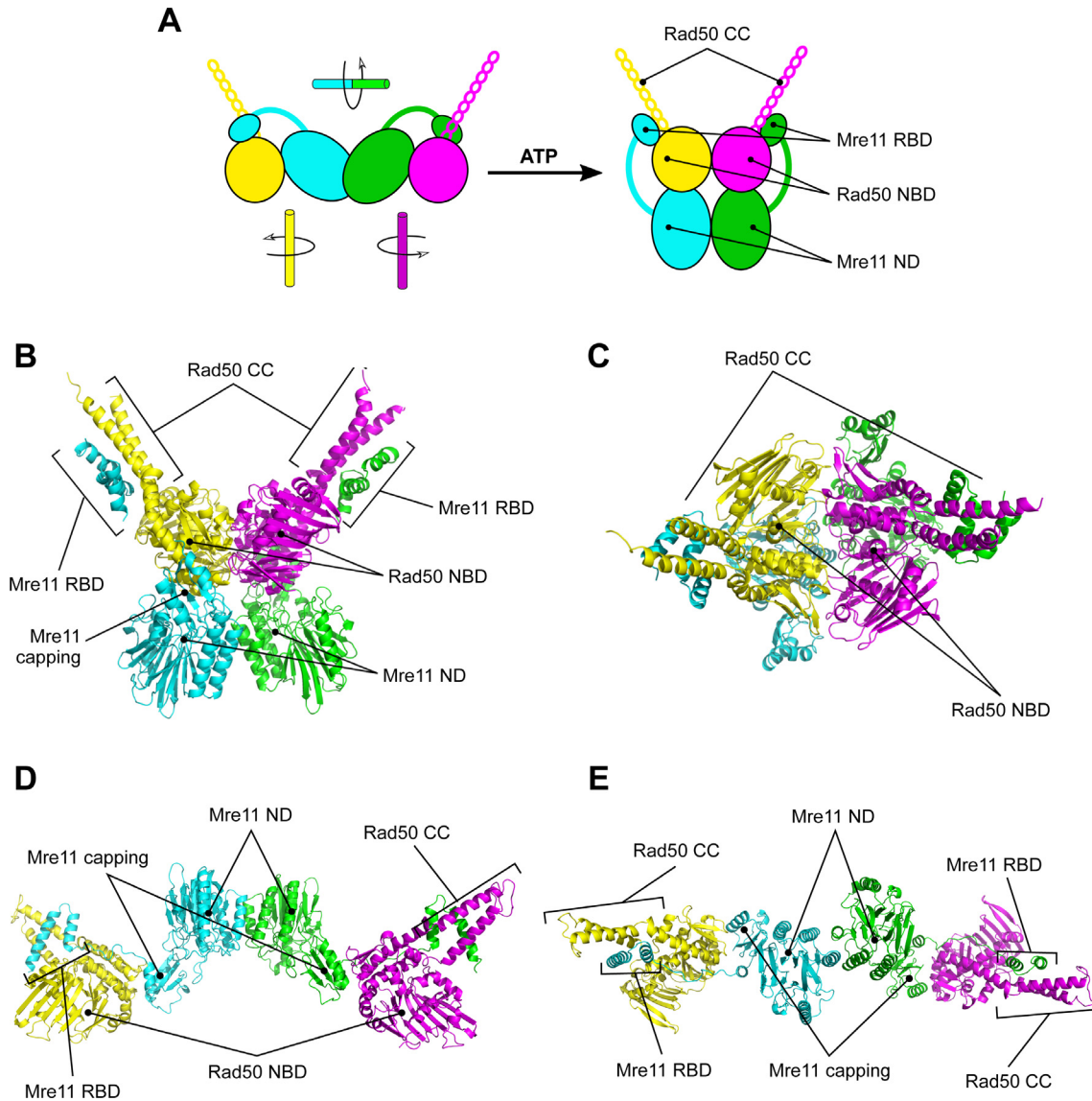


Fig. 2. The Mre11-Rad50 heterotetrameric complex can assume different configurations depending on nucleotide binding. (A) Schematic representation of the ATP-bound and nucleotide-free M_2R_2 complex configurations as characterized by SAXS. (B, C) Structure of the archaean *Methanocaldococcus jannaschii* Mre11-Rad50 heterotetrameric (M_2R_2) complex in presence of an ATP analogue (closed configuration) (B, front view; C, top view; PDB ID: 5DNY). (D, E) Structure of the bacterium *T. maritima* M_2R_2 complex in the absence of ATP (open configuration) (D, front view; E, top view; PDB ID: 3QG5). RBD, Rad50 binding domain; NBD, nucleotide binding domain; ND, nuclease domain; CC, coiled coil. Mre11 subunits are represented in green and cyan; Rad50 subunits are in magenta and yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the absence of ATP, the MR complex was resolved in a configuration where Mre11 only holds the Rad50 ATPase domains near the base of the coiled coils by its RBDs [50], while the NBDs of Rad50 are wide open and available to contact dsDNA (Fig. 2D and E). Other interfaces were proposed to stabilize this open configuration and involve Mre11 capping domain competing with ATP for Rad50 signature motif binding [50]. Mutations preventing this predicted second interface to be settled (such as *S. cerevisiae* Y328A) actually destabilize Rad50 dimer association with Mre11 and partially or completely fail to rescue the sensitivity to DNA damaging agents of *mre11* Δ cells [50], suggesting that this configuration could be a transition state required for correct MR assembly (see Table S1 for complete mutants information).

At the top of the long Rad50 CC domain, conserved and essential CXXC motifs allow formation of two zinc-hooks that are critical for MRX complex function, and whose conformation influences the structural behavior of the head domains [39,44,59–61]. Further-

more, an additional interface is present within the *Homo sapiens* Rad50 distal coiled-coil domains, also validated in a corresponding *S. cerevisiae* hypomorphic *rad50-48* mutant, which is defective in Rad50 dimerization due to loss of CC stabilization of the hook [48].

The third component of MRX/MRN complexes, Xrs2/NBS1, is far less conserved than the previous ones. Apart from the functional similarities, Xrs2 and NBS1 have different structural and functional features (Fig. 3A). They both show a fork-head associated (FHA) domain in the N-terminal and Mre11 and Tel1/ATM interaction domains, as well as nuclear localization signals in the C-terminal that promote the nuclear import of MRN/MRX [62–67]. In *S. pombe*, Nbs1 was revealed to wrap as an extended chain around the Mre11 phosphodiesterase domain with 2:2 (M:N) stoichiometry, but only one of the two Nbs1 completely binds via the NFKXFXK motif to the Mre11 latching loop (Fig. 3B). The stability of this last interaction was reported to be fundamental for at least some of MRX/MRN complex functions, which are compromised when amino acid sub-

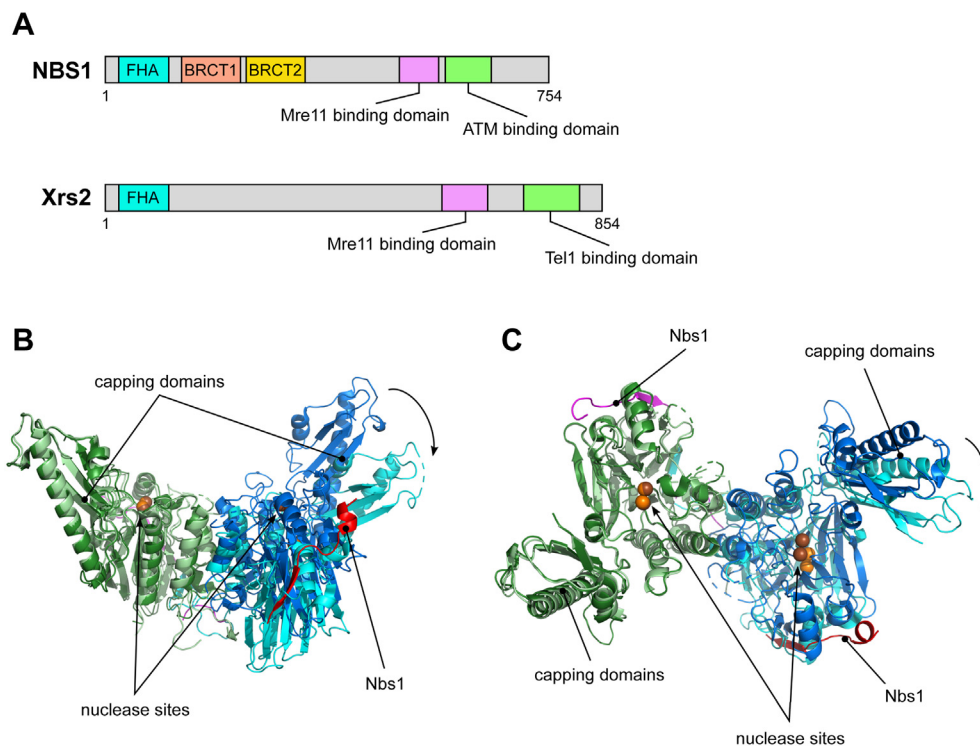


Fig. 3. Effect of Nbs1 binding on *S. pombe* Mre11 dimer conformation. (A) Scheme depicting the conserved domains present in NBS1 and Xrs2. (B, C) Mre11 dimer from *S. pombe* (PDB ID:4FCX) either alone or with an associated fragment of Nbs1 (aa 474–531) (PDB ID:4FBW). Mre11 subunits are represented in dark blue and green; Nbs1 is in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stitutions corresponding to ATLD/NBSLD clinical mutations were introduced in *S. pombe* Mre11 (for example N113S) [68]. It is still unclear if this asymmetric bridging of the Mre11 dimer has any functional meaning: DNA-bound archaeal and *S. pombe* Mre11 structures have different angles in the Mre11 dimer, probably due to the difference in the dimerization interface and thanks to the presence of the latching loop only in eukaryotic orthologs (Fig. S2B). High flexibility of the latching loop confers dynamic properties to eukaryotic dimers suggesting that conformational changes in the Mre11 dimer due to Mre11-Nbs1 interaction could be relevant for MRN function (Fig. 3B). In detail, a variation in Mre11 dimer angle rotation, controlled by Nbs1 on one side and by DNA and/or Rad50 plus ATP on the opposing side of Mre11, might be sensed by effectors of the complex and/or directly influence nuclease activity. Nevertheless, the dimer interface residues of bacterial and archaeal Mre11 also undergo conformational changes upon Rad50 dependent ATP binding [13,50,51,56].

While Xrs2 is largely disordered, NBS1 also contains two BRCT (Breast Cancer Suppressor Protein BRCA1) domains in the N-terminal, after the FHA (Fig. 3A), and they are all involved in the recruitment of NBS1 to phosphorylated histone H2AX at DSBs [69–74]. NBS1 was also recently reported to sense CtIP phosphorylation with its N-terminal domains and activate MRE11 endonuclease activity [75], but this feature does not seem to be shared by Xrs2. In fact, the N-terminal FHA domain of *S. cerevisiae* Xrs2 binds phosphorylated Sae2, although this capacity appears to be partially dispensable for DSB end resection [76].

3. ATP hydrolysis drives a huge MR complex reconfiguration

Several structural data suggested that Rad50-catalyzed ATP hydrolysis would induce a switch between a closed state, in which Mre11 nuclease domain is occluded, to an open configuration with

exposed Mre11 nuclease sites [50,51,55,56], suggesting that this event would be fundamental for the regulation of MR activity and DNA repair. Structural studies on *P. furiosus* (*Pf*) nucleotide-free Rad50 revealed a solvent-accessible channel extending deep into the Rad50 hydrophobic protein core. This channel is substantially remodeled and reduced when the ATPase subdomain rotates in response to ATP binding by concerted movement of R805 and R797 residues, which block and partially fill the remaining cavity upon ATP binding [51].

Structures of Rad50 alternative conformational states were resolved in different organisms [11,49–51]. Some insights, obtained by methyl-based NMR spectroscopy [77] and by molecular dynamics [78], suggest that conformational changes in the $\alpha 1$ - $\beta 4$ loop in Rad50 would be involved in the molecular events driving Rad50 transition from the ATP-bound to the ADP-bound state. Consistently, a shift in $\alpha 1$ - $\beta 4$ loop conformation was previously reported for the *Pf* Rad50^{R805E} mutant variant, which is characterized by high affinity ATP binding and slow hydrolysis rate [55]. Upon ATP-binding, the switch of Rad50 R12 residue towards the nucleotide releases K54 in the $\alpha 1$ - $\beta 4$ loop, which moves to the protein surface gaining solvent accessibility. The R805E mutation induces a conformational rearrangement that alters a neighboring hydrogen bonding network of Y157, R12, and the $\alpha 1$ - $\beta 4$ loop residues D41, D60 and K54, releasing this last and facilitating the transition to the ATP-bound state. Interestingly, deletion of the entire $\alpha 1$ - $\beta 4$ loop ($\Delta 51$ -60) eliminates ATP-stimulated DNA binding of full-length MR [55].

Rad50 ATPase activity is intrinsically low and it is not clear yet which molecular event triggers ATP hydrolysis, although the coiled-coils and hook domains were proposed to influence the catalytic behavior of Rad50 NBD by a long-range allosteric mechanism [48]. Consistently, single amino acid substitutions (e.g. L828F and D829N) in a conserved motif in the D loop (Fig. S1C) of Rad50 NBD were reported to increase the ATP hydrolysis rate, particularly

when they were coupled with CC domain shortening [79]. These mutant variants seem to adopt a hydrolysis competent state in Rad50 dimer head more easily, which would require the two Rad50 NBDs within the dimer at a wider distance, while the wild-type dwells upon a tightly closed conformation that would be recalcitrant to ATP hydrolysis [79]. Surprisingly, these mutants both in *S. cerevisiae* and in mouse embryonic fibroblast cells show a disruption of the DNA damage response, coupled to a loss of Tel1/ATM kinase activation [80]. This behavior was proposed to rely on the scarce persistence of the ATP-bound MRX configuration. Actually, proper Tel1/ATM activation was recently found to require MRX in a tightly closed configuration [81], also according to molecular dynamics simulation of a Rad50 A78T mutation in budding yeast [78]. The Rad50^{A78T} mutant variant is in fact defective in Tel1 activation and it was found to induce a spontaneous switch in Rad50 conformation, albeit bound to ATP, in molecular dynamics simulation of the MR heterotetramer.

In its turn, ATP hydrolysis triggers large changes in MR configuration that lead to the dislocation of Rad50 from the nuclease domain of Mre11 to clear its nuclease site [50,51,53–55]. The C-linker flexibility is mandatory to allow this reorganization in MR complex. In fact, a Ser499 substitution with the more rigid proline residue in the linker of *S. cerevisiae* Mre11 was reported to affect Mre11–Rad50 interaction, although it is not directly involved in any of the described interfaces [78].

The open configuration would allow M₂R₂ complex to contact dsDNA, but the complex would efficiently bind to DSB end only in presence of ATP, suggesting a model where the complex clamps on DNA by adopting the closed configuration [50] (Fig. 2B and C). This model tallies with previous observation by AFM, suggesting that a transition in the orientation of the coiled-coils is dependent on DNA binding by the MRN head [82] and with nucleotide-dependent conformational changes reported at the proximal traits of the coiled-coils in different Rad50 structures [50,51,53,54,56]. Supporting this idea, the affinity of Rad50 to ATP and its hydrolysis are different when coiled-coils are truncated [55]. The conformational change would require both ATP molecules to be either hydrolyzed or recruited in order to promote dimer dissociation or association, which is ensured by the observed cooperativity of ATP hydrolysis and binding which is typical of ABC-ATPases [40].

The early proposed model for dsDNA binding to M₂R₂ [50] has striking analogy to a recently resolved structural model (Fig. 4A) based on cryo-EM analysis of SbcC–SbcD, *Escherichia coli* MR, complex either in presence of ATP (Fig. 4B and C) or of ADP and a short dsDNA (Fig. 4D and E) [83]. In the absence of DNA, the *E. coli* tetramer M₂R₂ (Fig. 4B and C) adopts a similar but not identical configuration to the previously described ‘closed’ state (Fig. 2B and C). Notably, the orientation of the Mre11 dimer with regards to the Rad50 dimer is not exactly identical to the structures obtained by SAXS on crystals (Fig. 5), though this could be attributable to the different organism of origin (archaea vs prokaryotic origin), to the artificial shortening of the coiled-coil traits in the archaea Rad50 or to the different techniques used to obtain the structure (SAXS vs cryo-EM).

Upon DNA binding, *E. coli* MR complex actually forms a clamp around dsDNA through the two complete coiled-coils (CC) (Fig. 4D and E). Though the inability of CC to directly contact dsDNA in previous structural models may be due to the artificial truncation (Fig. 6A), here they zip up into a rod and tightly embrace DNA, together with the Rad50 nucleotide-binding domains (Fig. 6B). Consistently with the most recent finding, one previously reported structure of *Thermotoga maritima* Rad50 dimer together with dsDNA revealed a contact between dsDNA and a single Rad50 subunit strand-loop-helix motif in the proximal CC [84]. However, corresponding mutations in yeast that abolished this

contact were found not to affect DNA double-strand break repair [84].

Upon DNA end binding, Mre11 dimer surprisingly moves to the side of Rad50 dimer, thus reaching the DNA end, which plunges in a channel bordered by Rad50 CC and Mre11 capping domain, and leading to Mre11 nuclease site (Fig. 4D and E). In this configuration, Mre11 and Rad50 share the usual interface between the former RBD and the latter CC domain. A second interface is shared by one of the Rad50 subunits and one of the Mre11 subunits (Fig. 7A), involving in particular the outer β sheet of Rad50 and the 137–149 aa loop in *E. coli* Mre11, the latter, defined as the ‘fastener’ [83], not conserved in eukaryotes (Fig. S2B and Fig. 7A, see below). This interface is claimed to be essential for both endonuclease and exonuclease activity of the complex, since mutants losing this interaction, such as Rad50^{E115K} or Mre11^{K149E}, are defective in both [83]. Actually, this model was demonstrated to be suitable for binding only to DSB ends [83].

It is still to be assessed if this configuration is also topologically compatible with DNA binding far from the DSB end, which is a prerequisite for Mre11 nucleolytic activity, since Mre11 endonuclease cuts from 40 up to 200–300 nucleotides from the DSB end [20–22,85–87]. Furthermore, the abundant Ku heterodimer is generally believed to be the first protein to bind to DSBs at least in mammalian cells [88–92], allowing NHEJ to make the first DSB repair attempt [91]. Experimental data in mammalian cells suggest that the MRN complex can attach to DNA ends that have already been claimed by Ku, which forms a constitutive ring specific for DNA ends and unfit for internal DNA binding [93]. In budding yeast, the absence of Ku weakens the requirement for MRX in DNA end resection to process “clean” DNA ends, suggesting that the two complexes compete for binding to DSB ends [94–98]. The structural traits allowing eukaryotic MR to interact with DNA ends covered by Ku or other associated proteins remain yet to be defined.

4. Interaction of Rad50 and Mre11 with DNA

The MR complex was previously proved to bind to dsDNA efficiently only in presence of ATP [50]. This is consistent with Rad50 interacting primarily with dsDNA in the ATP-dependent dimer conformation [99]. In this closed conformation, Rad50 dimer creates a groove that can host dsDNA, binding it with a patch of several positive residues both on the globular head and on the proximal CC surface (Fig. 6) [50,52,54,83,84].

Nonetheless, the MR complex was previously reported to make contact with DNA even in absence of any nucleotide, as observed by electron microscopy or AFM [39,82]. The large majority of the coiled-coils in DNA-associated MR complex were rod-shaped either in the presence or in the absence of a non-hydrolysable analogue of ATP [82]. Most recently, the same rod-shaped conformation was proposed for the DNA end-bound post-ATP hydrolysis cutting state of MR complex (Fig. 4D and E) [83]. Although it is not clear if the rod-shaped conformation of the coiled-coils has any requirements as for the nucleotide binding in Rad50 dimer, it is clear that it involves a tight contact with nucleosome-free DNA and, likely, intact Zn²⁺-hooked CC. In fact, crystals obtained with shorter versions of Rad50 proteins in presence of dsDNA do not reveal significant contacts between the proximal CC domains and dsDNA (Fig. 6A) [52,54]. Rad50 CC was found to have flexibility properties that could explain its structural dynamic nature [40]. Since the coiled-coils are generally not stiff [37,38], they hardly can transmit changes in conformation of the globular domain to changes in relative orientation of the ends of the coiled-coil, as proposed [39,48]. This would confirm that DNA interaction is a key force required for circular coiled-coils transition to rod-like configuration.

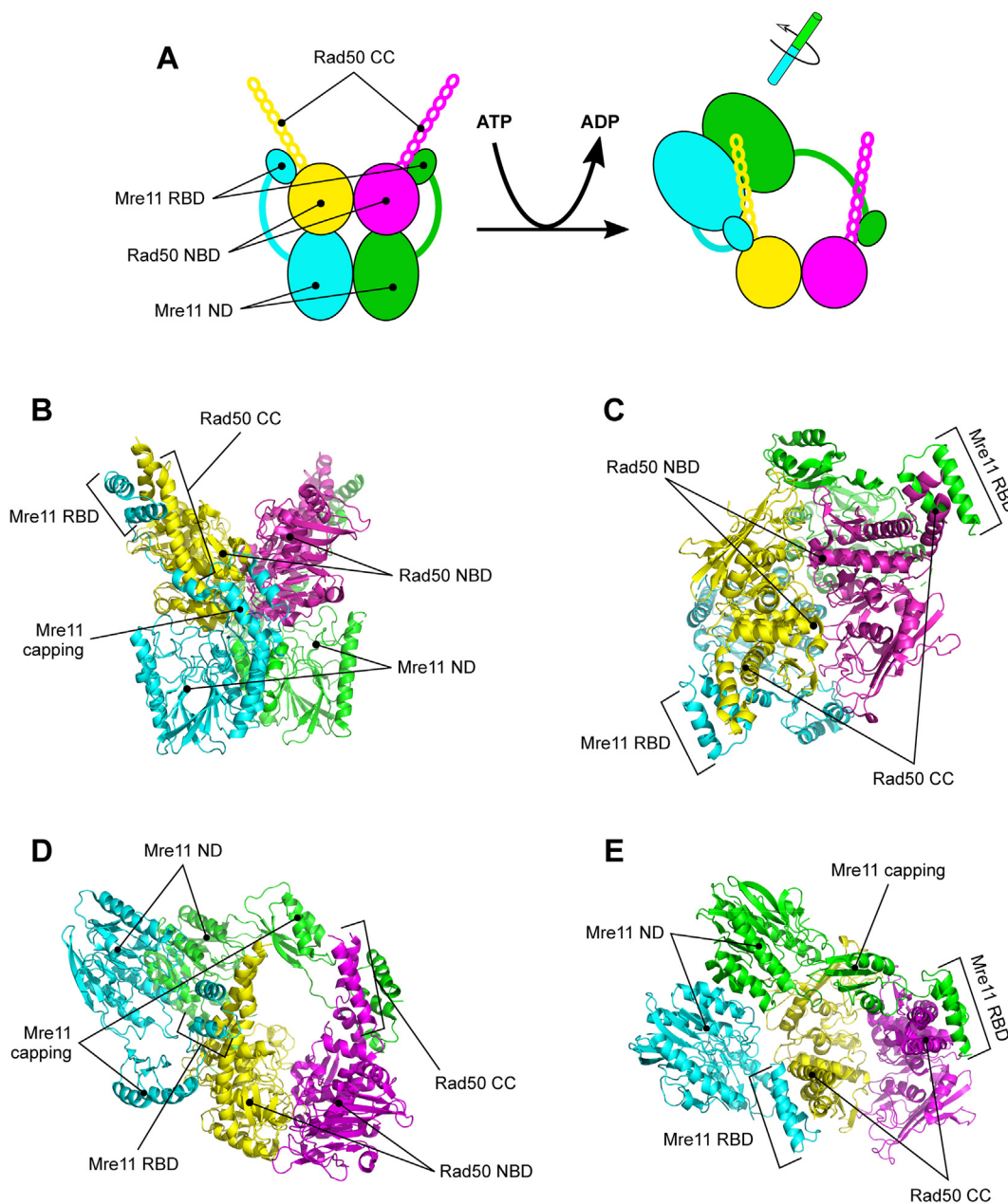


Fig. 4. The Mre11-Rad50 heterotetrameric complex can assume different configurations depending on dsDNA end binding. (A) Schematic representation of the ATP- and DNA-bound *E. coli* M₂R₂ complex configurations as characterized by cryo-EM. (B, C) Structure of bacterial *E. coli* M₂R₂ complex in presence of ATP (closed configuration) (B, front view; C, top view; PDB ID: 6S6V). (D, E) Structure of bacterial *E. coli* M₂R₂ complex in presence of ADP and a 60 bp-dsDNA molecule (cutting state configuration) (D, front view; E, top view; PDB ID: 6S85). RBD, Rad50 binding domain; NBD, nucleotide binding domain; ND, nuclease domain; CC, coiled coil. Mre11 subunits are represented in green and cyan; Rad50 subunits are in magenta and yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DNA access to Mre11 nuclease active sites has long been a major puzzling point, since the appearance of the first tetramer closed configuration structure [53], where Rad50 subunits clearly obstructed Mre11 catalytic sites (Fig. 2A). The Mre11 capping domains were also claimed to be involved in DNA binding, according to structural data obtained with isolated Mre11 dimer together with DNA (Fig. 8A) [13], which was recently confirmed by genetic and molecular dynamics simulations of a Mre11 mutant able to overcome some of *sae2Δ* mutant defects [100]. The clearance of the Mre11 nuclease catalytic sites is apparent in the open configuration structure described by Lammens et al. [50] (Fig. 2D and E), and this would easily allow interaction with dsDNA according to

the reported structure for DNA-bound Mre11 dimer (Fig. 8A). The most recent cutting state model described by Kāshammer et al. [83] (Fig. 4D and E) describes a different configuration for dsDNA end contact with Mre11 catalytic sites, which is actually still in agreement with formerly identified contact sites (Fig. 8B).

Interestingly, high-throughput single-molecule microscopy data showed that MRN, as well as MR, complex tracks the DNA helix for free DNA ends by one-dimensional facilitated diffusion, even on nucleosome-coated DNA [101]. Rad50 would bind homoduplex DNA and promote facilitated diffusion, whereas Mre11 was found to be required for DNA end recognition and nuclease activities [101]. Thus, the ability to bind and track the DNA helix,

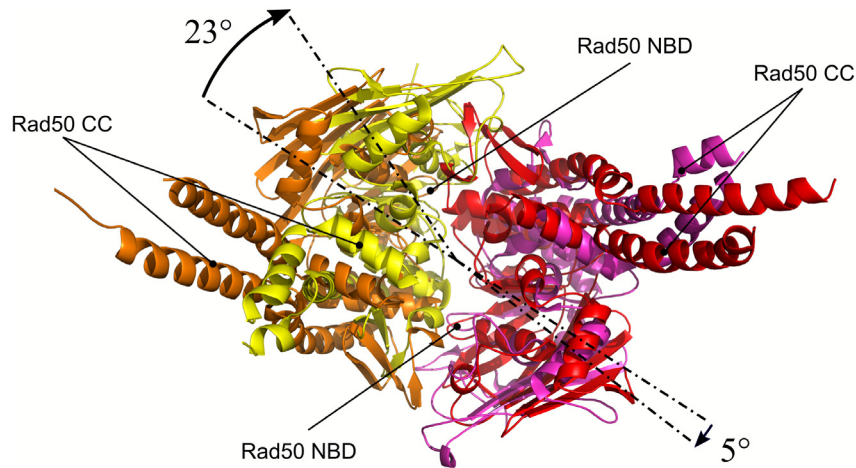


Fig. 5. Differences in Rad50-Mre11 dimer of dimers interaction by comparison between heterotetrameric ATP-bound MR structures. Complexes superposition of the structures of M_2R_2 complex from *M. jannaschii* (PDB ID: 5DNY, red and orange structures) and *E. coli* (PDB ID: 6S6V, yellow and magenta structures) was obtained by structural alignment of Mre11 dimers. Then, only Rad50 dimers were shown, revealing a non-perfect alignment of the Rad50 dimers in the complexes: two subunits are pivoted by 23° (orange and yellow), while the others are rotated by 5° (red and magenta). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

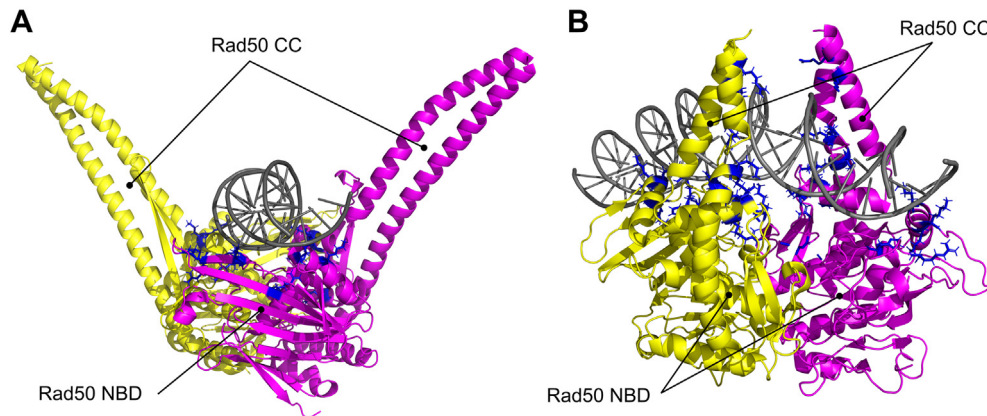


Fig. 6. Rad50 dimer can interact in different configurations with dsDNA. The dsDNA-Rad50 interface in the structure of the filamentous fungus *Chaetomium thermophilum* Rad50 dimer in presence of an ATP analogue and dsDNA (A, PDB ID: 5DAC) and in the structure of *E. coli* M_2R_2 complex in presence of ADP and dsDNA (B, PDB ID: 6S85) reveals that Rad50 can interact with dsDNA with two different interfaces: Rad50 nucleotide binding domain (NBD) groove and proximal coiled-coil (CC) domain. Rad50 subunits are represented in magenta and yellow; dsDNA is in grey. Basic amino acids (Lys, Arg and His) that can be implicated in DNA binding are shown as blue sticks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

whether on naked DNA or on DNA associated to nucleosomes, would rely on Rad50 contacts with dsDNA, while the ability to recognize clean DNA ends would be reliant on Mre11 directly taking contact with the DSB ends.

5. Regulation of Mre11 nuclease activity

Sae2/CtIP promotes the Mre11 endonuclease activity within the MRX complex to initiate DSB resection [23] and this function requires Sae2/CtIP phosphorylation by cyclin-dependent kinases (CDKs) in both mitosis and meiosis [24,25,102–104]. This CDK-dependent regulation of Sae2 activity ensures that resection only takes place in the S/G2 phase of the cell cycle when a sister chromatid is available as repair template [105,106]. Furthermore, it also explains why NHEJ repair predominates in the G1 phase, when CDK activity and therefore DSB resection is low.

In *S. cerevisiae*, CDK-mediated Sae2 phosphorylation promotes MRX nuclease by at least two distinct mechanisms. During the G1 phase of the cell cycle, Sae2 exists in an unphosphorylated state

and is part of an inactive soluble multimeric complex [107]. During S phase in mitosis and prophase of the first meiotic division, Sae2 phosphorylation promotes the formation of Sae2 tetramers, which likely represent the active Sae2 species that promote the Mre11 nuclease within the MRX complex [25,107–110]. Additionally and independently of regulating Sae2 size distribution, phosphorylation of the conserved C-terminal domain of Sae2 is necessary for a direct physical interaction with Rad50 [25], which is crucial to promote the Mre11 endonuclease activity. As ATP hydrolysis by Rad50 is necessary for MRX-Sae2 endonuclease [23], phosphorylated Sae2 may control the Mre11 nuclease by promoting productive ATP hydrolysis.

In vitro, the efficiency of Sae2/CtIP-induced Mre11 endonucleolytic activity is strongly enhanced by the presence of proteins stably bound either internally or at the end of a DNA molecule [86,87,111]. The *E. coli* SbcCD nuclease can cleave dsDNA past protein blocks as well, indicating that this seems to be a conserved mechanism [112]. Such protein blocks include histones, the Ku complex bound at the DSB ends, the RPA complex bound to either

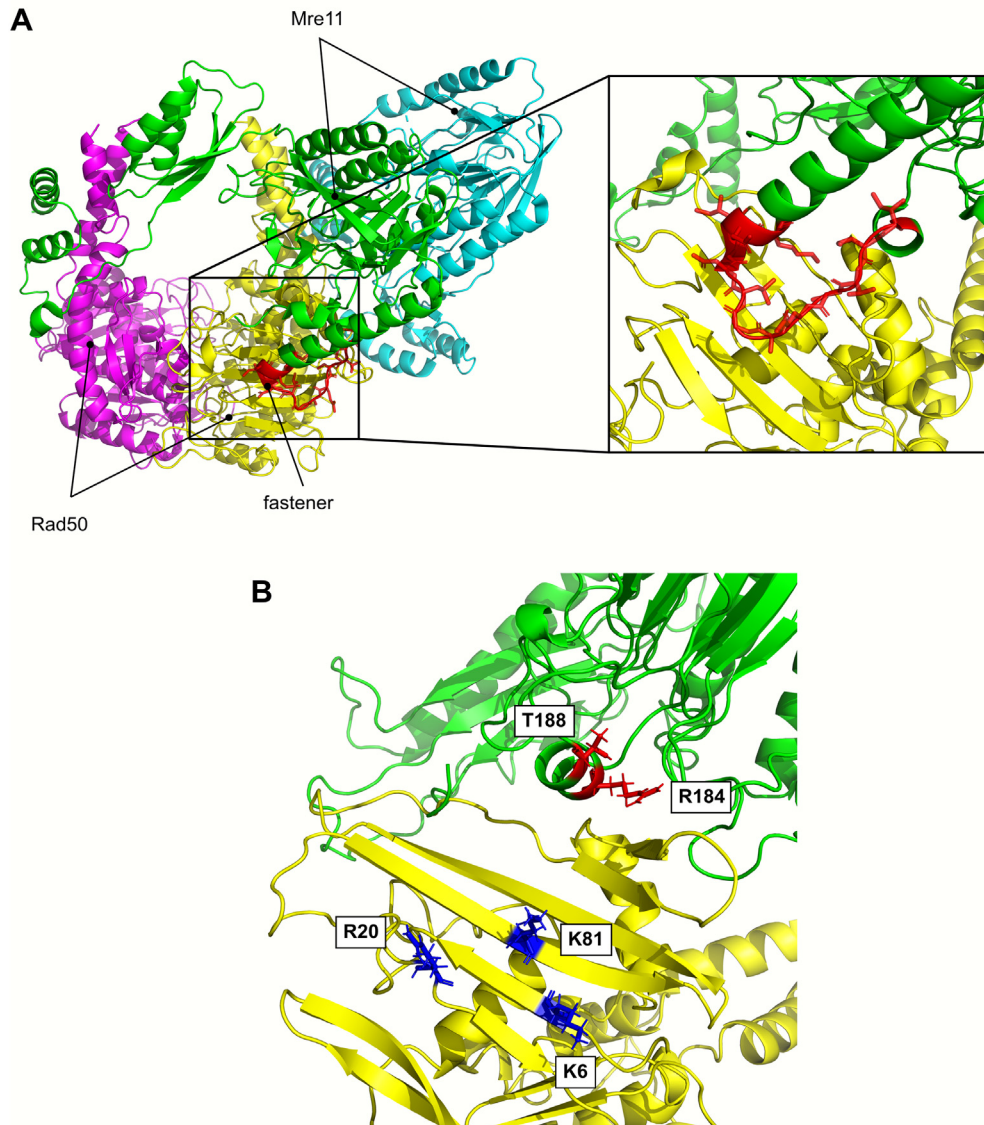


Fig. 7. The interface between Rad50 and Mre11 fastener loop in bacterial cutting state of MR complex is not conserved in eukaryotes, but residues involved in Sae2 interaction with MR complex are localized in these regions. (A) Detail of the Mre11–Rad50 interface in the structure of *E. coli* M₂R₂ complex bound to DNA in the so called ‘cutting state’ (PDB ID: 6S85). (B) Localization of residues affected by the *rad50S* (K6, R20 and K81), *mre11S* (T188) and R184A mutations in *S. cerevisiae*. Mre11 subunits are shown in green and cyan; Rad50 subunits are in magenta and yellow; fastener loop is in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

partially resected DNA ends or terminal hairpin structures, the type II topoisomerase-like Spo11 covalently bound at meiotic DSBs and the MRX/MRN complex itself [22,23,86,87,111,113–117].

During meiosis, formation and repair of programmed DSBs ensures correct alignment and segregation of chromosome homologs in addition to generating diversity [1]. The meiosis-specific Spo11 protein generates DSBs by forming a covalent linkage between a conserved tyrosine residue and the 5' end of the cleaved strand [118,119]. In budding yeast, the MRX complex plays at least two roles during meiotic recombination. First, it is required for Spo11 to generate DSBs. Then, the Mre11 endonuclease activity and Sae2 removes Spo11 from break ends by endonucleolytic cleavage, releasing Spo11 attached to a short oligonucleotide [22,85]. In fact, both the lack of Sae2 or of Mre11 nuclease-defective variants allow Spo11-induced DSB formation, but prevent Spo11 removal and meiotic DSB end resection in both *S. cerevisiae* and *S. pombe* cells [15,18,120,121].

Interestingly, similar to *SAE2* deletion and Mre11 nuclease-defective variants, a group of *rad50* and *mre11* mutants, called S mutants, are proficient in meiotic DSB formation but are unable to remove Spo11 from the DSB ends (Table S1) [15,18,120–124], due to the corresponding mutations impairing MRX–Sae2 interaction not only functionally but also physically [11,25]. Surprisingly, comparison of the structures of *S. cerevisiae* Rad50 and Mre11 (Fig. 7B), generated by homology modeling [58], with *E. coli* cutting state model (Fig. 4D and 7A) shows that these sites are localized exactly at the interface identified between Rad50 β sheet and Mre11 fastener loop in the DNA end binding configuration of *E. coli* MR complex. The fastener loop is hardly conserved in the other eubacterial Mre11 whose structure is available, which is *T. maritima* (PDB ID: 4NZV), while it is not present in archaea or in eukaryotes (Fig. S2B). Indeed, it is not conserved in *S. cerevisiae* as well. However, it is interesting that the T188 residue affected by the *mre11S* muta-

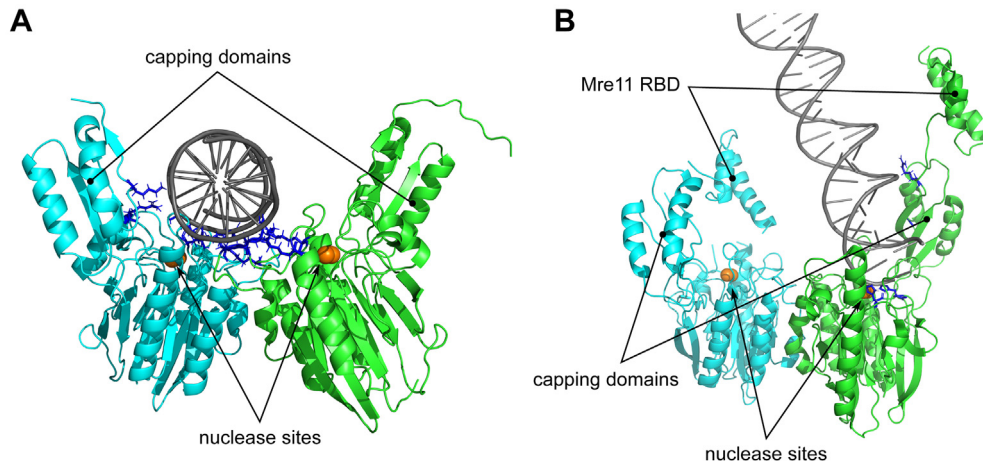


Fig. 8. Structural data agree on Mre11 capping domain contacting dsDNA. (A) Structure of *M. jannaschii* Mre11 dimer in complex with dsDNA (PDB ID: 4TUG). (B) Detail of Mre11 interface with dsDNA end in *E. coli* M₂R₂ complex in cutting state configuration (PDB ID: 6S85). Mre11 subunits are reported in green and cyan; dsDNA in grey. Basic amino acids (Lys, Arg and His) that can be implicated in DNA binding are shown as blue sticks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tion in budding yeast [122] is localized in the Mre11 α helix facing the same region of the fastener loop (Fig. 7B). Moreover, in a *sgs1 Δ* background, the R184A mutant, affecting a residue on the exposed side of the same helix (Fig. 7B), is sensitive to DNA damaging agents as a catalytic dead Mre11 mutant variant (Mre11-H125N) [125]. Thus, it was speculated that a similar configuration could be valid for eukaryotic MR complexes as well, but it could involve the formation of a ternary complex with CtIP/Sae2 [83].

It is not clear yet which structural adjustments allow Mre11 to exert its function as an endo- or an exonuclease. Separation-of-function mutants were isolated, such as H52S in *Pf* Mre11, in one of the conserved Mn²⁺-coordinating motifs, in particular in the phosphoesterase motif II (Fig. S2B), that drove to selective loss of exonuclease activity but retained endonuclease competence [126]. This conserved motif II histidine was proposed to be required for the proper rotation of the 3'-end last nucleotide phosphate bond over the catalytic Mn²⁺ ions prior to its hydrolysis. The H52S mutation was also suggested to affect an allosteric network between the Mre11 active site and capping domain, which is involved in ssDNA binding [13,126].

Another mutant, the Y187C mutant of *Pf*Mre11, was also found to be inactive as an exonuclease but active as an endonuclease [39]. The mutation affects the conserved aromatic residue Tyr187 in *Pf*Mre11 (*S. cerevisiae* F224, *H. sapiens* F227), situated in a loop corresponding to the sealing loop important for *E. coli* MR nuclease activity [83], on the opposite side of the catalytic Mn²⁺ ions. Tyr187 was found by X-ray crystallographic structural studies to interact with the nucleotide of the dAMP released by the cleavage, while the monophosphate interacted with the Mn²⁺ ions in the catalytic site [39]. Structural studies via methyl-based solution-state NMR spectroscopy revealed that both Y187C and H52S mutations in *Pf*Mre11 alter the structural and dynamical interactions with dsDNA [126].

A series of mirin-based inhibitors binding to the same motif II were also designed that could specifically interfere with either one of the nuclease activity [19]. It was hypothesized that the small-molecule inhibitors that differentially prevented endonuclease but not exonuclease activity were able to specifically limit ssDNA binding [19].

6. Regulation of Exo1 recruitment and processivity

MRX/MRN-Sae2/CtIP creates a nick that provides an internal entry site for nucleases capable of degrading DNA in a 5'-3'

direction. These nucleases comprise Exo1 and Dna2, which control two partially overlapping pathways [20,21]. While Exo1 is able to release mononucleotide products from a dsDNA end [127], Dna2 has endonuclease activity that can cleave both 5' and 3' single-stranded DNA overhangs adjoining a duplex region [128]. Reconstitution experiments revealed that Exo1 nuclease requires the support of various factors that promote its nuclease activity. The mismatch recognition complex MutS α was shown to stimulate Exo1 processivity in the presence of a mismatch [129], whereas the proliferating cell nuclear antigen (PCNA) promotes human EXO1 processivity by enhancing its association with DNA [30,130]. Noteworthy, in addition to the end-clipping function, MRX/MRN also stimulates resection by Exo1/EXO1 [28–32,98], thus explaining why *mre11 Δ* cells show a resection defect more severe than *sae2 Δ* or *mre11* nuclease defective mutants.

Human EXO1 is a processive enzyme per se, unless the generated ssDNA is rapidly associated to RPA, as usually happens within the cell, which reduces EXO1 life-time on DNA by ~100-fold [101]. In this condition, physical interaction with MRN [28,30,31], primarily with MRE11 and less strongly with NBS1, is required to maintain EXO1 processivity, retaining the exonuclease on DNA and allowing fully efficient long-range resection [101].

The MRX/MRN complex was early reported to exert an ATP hydrolysis-requiring partial and not processive unwinding of a short DNA duplex [17,131–134]. This limited activity seems to be conserved in prokaryotes as well [54]. Later, it was proposed that Mre11 capping domain could retain a DNA unwrapping activity, ensuring duplex melting at the DNA end, linked to capping domain rotation [13]. This movement was actually reproduced by molecular dynamics simulations of *S. cerevisiae* Mre11, and was reported to be able to cause DNA terminus unwinding [135]. The movement was exacerbated by the presence of a R10T single amino acid substitution in Mre11, which implied an altered orientation of the Mre11 capping domain leading to more persistent melting of the dsDNA end. This hyperactivation allowed a higher exonuclease activity by Exo1, achieving the suppression of the DNA damage hypersensitivity and the resection defect of *sae2 Δ* cells [100]. In fact, although Exo1 is able to degrade a filament in dsDNA *in vitro*, indicating that it does not require the intervention of a helicase, it actually prefers dsDNA bearing a 3' ssDNA overhang [32], and the DNA end unwinding by MR complex could facilitate its access to the DNA 5' terminus.

7. DNA tethering

Besides DNA end processing reaction, a second key requirement of MRX/MRN complexes in DSB repair is the ability to coordinate and bridge DNA ends, achieved through the MRN complex architectural DNA scaffolding activities [39,42–47,55,82,136]. A role in DNA bridging was also proposed for CtIP/Ctp1/Sae2 [137,138],

although it cannot substitute for MRX/MRN deficiency, and it was proposed to supersede after DSB processing.

The DNA tethering function for the MRX/MRN complex is generally ascribed to the extended coiled-coil regions of Rad50 [37,39,45,136] and requires the Rad50 hook domain [39,44,139]. These observations lead to a model describing in trans bridging of DNA molecules through an alternative arrangement of the Zn-

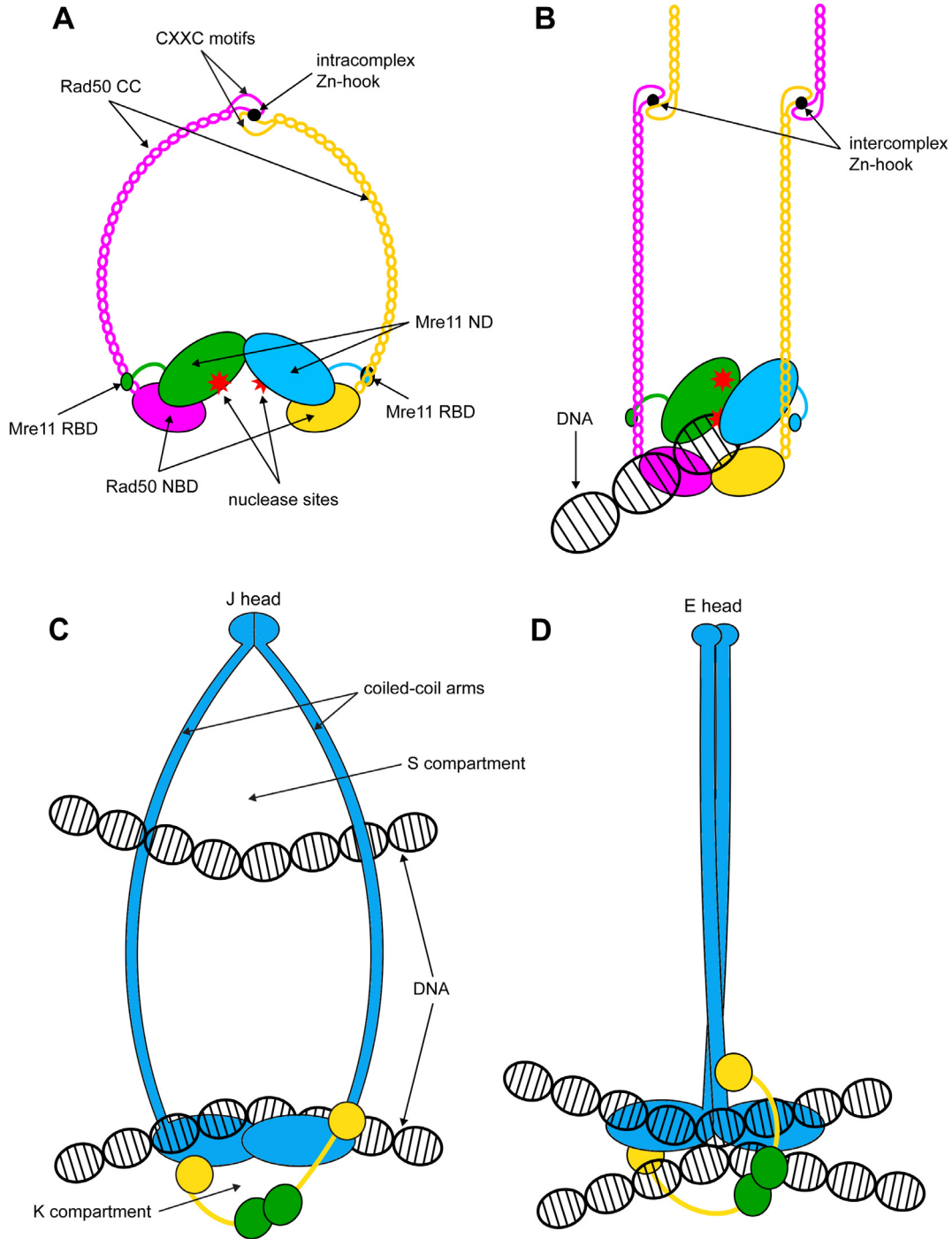


Fig. 9. Comparison among DNA tethering models for MRX/MRN complex and SMC proteins. (A) Open configuration for MR complex. (B) Clamping model of dsDNA binding and DNA tethering by intercomplex Zn-hook dimerization. (C) Schematic representation of the DNA tethering structure observed for the *Bacillus subtilis* SMC (SMC-ScpAB, SMC is in blue and the kleisin family proteins ScpA and B are in yellow and green) complex with J head conformation and overall V shape configuration. (D) Schematic representation of the DNA tethering structure observed for SMC-ScpAB (same colors as in panel C) with E head configuration, leading to the so called I shape configuration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hook interface. In the so-called intercomplex configuration (Fig. S3), the coiled coils of the Rad50 subunits in one heterotetrameric complex connect at the hook with the coiled coils of the Rad50 subunits from another complex, effectively bridging two molecules of DNA (compare Fig. 9A and B).

Other data revealed that the hook alone would not be sufficient for establishing of DNA tethering, which rather involves Rad50 coiled-coils as well. For instance, although mutations in the cysteine residues in the hook actually impair MR complex assembly and chromatin binding in mammals [47,61], this is not sufficient in yeast [44,140], where simultaneous loss of hook and coiled-coil distal interface is required to induce a phenotype similar to *rad50* loss-of-function both in sister chromatid recombination and in NHEJ [48]. Previously, dramatic shortening of the coiled-coils in yeast, though in presence of the hook domain and thus compatible with intercomplex hook-mediated dimerization, was reported to be sufficient to dramatically impair NHEJ, which requires MRX/MRN to tether the two DNA ends together, but not to process them [44].

Moreover, observation by AFM of nucleoproteins formed by incubation of 400 bp-dsDNA with MRX particles revealed that internal and terminal nucleoprotein complexes had similar size, suggesting that the DNA molecules were held together by a single MRX complex [141]. And finally, very recently, high-speed AFM imaging of the human MR complex has shown that the Rad50 coiled-coil arms are stably bridged by the dimerized hooks, while the MR ring rather opens by disconnecting the head domains [142,143].

Taken together, these considerations raise an issue on the possibility that DNA tethering could be achieved in different manners depending on the DNA repair pathway to be undertaken. DNA binding by clamping, as proposed by Lammens et al. [50] (Fig. 9B), and in general dsDNA making contact with Rad50 proximal coiled-coils and DNA binding cleft on its dimer heads presume that DNA is actually protein-free, which would be achieved near the DNA ends by chromatin remodeling and histones eviction [144–146]. Recently, CC clamping on the DNA helix was actually observed by cryo-EM, but only after ATP hydrolysis [83]. Hitherto, no structural evidence allows to predict if DNA molecule actually makes contact with Rad50 proximal CC in the ATP-bound state, although the presence of ATP is necessary to enforce MRN binding to DNA [49,132].

Indeed, the ATP-driven Rad50 dimerization promotes the assembly of MRX/MRN globular domain and has previously been proposed to allow to topologically encircle homoduplex DNA within the ring-shaped CC compartment [52,54], similar to the model envisioned for S compartments defined by cohesin heads in J configuration [147–150] (Fig. 9C). Like its homolog Rad50, cohesin displays two ~30- to 50-nm-long coiled coils as well. Nonetheless, human and *S. pombe* cohesins were reported to fail to overcome diverse roadblocks [151,152]. In contrast, MRX/MRN, unless Rad50 coiled-coils extension is reduced [101], can efficiently diffuse on nucleosome arrays, provided that they are not too dense, which ensures that MRX/MRN can rapidly find DSB ends in euchromatin and nucleosome-depleted genomic regions. Reduced MRN diffusion on highly chromatinized DNA may indeed contribute to scarce HR and delayed repair at heterochromatic DNA breaks (see [5] for a recent review on chromatin context in DSB processing).

Previous cross-linking studies confirmed this topology for DNA capture in the S compartment inside SMC rings in eukaryotic cohesin [153] or prokaryotic condensin [154] in a V shape conformation (Fig. 9C). However, after ATP hydrolysis the yeast cohesin heads adopt a E configuration and the coiled coils seem to be juxtaposed throughout their length, adopting the I shape conformation, for bacterial SMC proteins and eukaryotic cohesin as well [155–157].

The I shape configuration was claimed to hamper DNA entrapment in S compartment and allowing DNA to enter the K compartment, defined by SMC and kleisin heads [142] (Fig. 9D). Consequently, it has been recently proposed that the long, flexible arms of SMC-like proteins would be fundamental to allow DNA translocation by mediating large steps on chromatin, rather than to embrace chromosomal DNA fibers [154], which is a fascinating hypothesis for Rad50 proteins as well. Further experiments will be required to assess if this bimodal DNA tethering mechanism could be adopted by MR complexes.

8. Summary and outlook

DNA damage and DNA repair are fundamental topics in genetics and molecular biology due to their correlation with genomic instability and DNA mutations. Cells possess mechanisms conserved among eukaryotes apt to recognize DSBs and promote their repair. Misrepair of DSBs often leads genomic instability and loss of genetic information that can result in cell death or oncogenic transformation. Consistently, cancer cells have a higher DSB generation rate related to oncogene-induced replication stress and dramatically rely on efficient DSB repair for their survival [158]. To date, a number of inhibitors related to DNA damage repair systems have been developed, particularly for breast cancer [159]. Understanding the mechanism involved in DNA damage repair would be extremely useful in order to identify novel targets for drug discovery efforts.

Events driving DSB-triggered MR-driven ATP hydrolysis and subsequent DNA access to Mre11 endonuclease site have not been clarified yet, despite the joined effort of genetics, biochemical, biophysical, computational, and structural approaches. In particular, the conformational transition recently proposed for *E. coli* MR complex upon recognition of the DNA end poses a novel focus on the molecular rearrangements that can either trigger or allow such transition. Extending this framework to eukaryotic MR complex will be a further and challenging step required to transfer and compare available experimental data on the different molecular systems.

Computational approaches such as molecular dynamics simulations contributed to this issue by providing unique insights on the MR components molecular properties. Indeed, further structural insights in eukaryotic complexes, which display specific traits totally absent in archaea and prokaryotic homologs, will be required to experimentally validate predictions obtained in these homologs by advanced structural analysis techniques. Future investigation will enable to determine whether the novel conformational rearrangement proposed for bacterial MR complex could be envisioned for eukaryotic MR complexes as well, allowing to exploit the available structural insight and transfer the novel concept to other organisms systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.csbj.2020.05.013>.

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