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Mre11-Rad50 complex crystals suggest molecular calisthenics

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Abstract

Recently published crystal structures of different Mre11 and Rad50 complexes show the arrangement of these proteins and imply dramatic ligand-induced rearrangements with important functional consequences.

Keywords

Genome maintenance; DNA double-strand breaks; DNA repair

The Mre11-Rad50 (MR) complex, an essential early component of DNA double-strand break repair, is a protein machine with many parts and many purposes [1-3]. Genetics tell us that the components are essential and cell biology indicates that MR is one of the first responders to DNA breaks. The available biochemical and structural information suggests that the MR machine works to organize and process DNA ends, but major mechanistic mysteries remain. Detailed structural information on parts of the complex, though valuable, was insufficient to explain how the parts are combined for biological function. Now new data does just this with some surprising revelations. Structural work from three groups converges in exciting ways to expose working details of the MR molecular machine while providing curious clues that make us wonder about the movements implied but not yet seen [4-6].

It is well established that the MR complex includes two Rad50 protein molecules and two Mre11 protein molecules (in eukaryotes the complex contains a third component, NBS1 or Xrs2, which will not be considered here). This may be expected since both Rad50 and Mre11 are able to dimerize. The different possible dimer interfaces would allow multiple alternative arrangements, dynamic transitions and create mechanisms for conformational and thereby functional control [7]. Mre11 alone is a stable dimer and has been assumed to have a similar structure / interface in the MR complex [3, 8]. Rad50 has dimer interfaces that are likely more dynamic. Notably Rad50 is a very elongated protein with a globular nucleotide-binding domain at one end of an extended intramolecular coiled coil that can be up to 50 nm long [9, 10]. The apex of the coiled coil is a CXXC motif that can dimerize around a bound Zinc ion [11]. This interaction is presumably relatively weak as dynamic association and dissociation is common [12]. The globular nucleotide-binding domains of Rad50 associate as a dimer with ATP bound; two ATP molecules can bind in the closed or

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engaged dimeric form. Mre11, which contain nuclease domains important for processing DNA during repair [13], contacts Rad50 in the complex along the Rad50 coiled coils near their base at the globular ATPase domains [14]. For this reason the complex has often been illustrated with a globular Mre11 dimer sitting between and bridging Rad50 coiled coils, atop the Rad50 ATPase domains (Figure 1A and B). That arrangement accurately represented the available information but also had to include aspects for which there was no data. Due to the recent publications the picture can now be revised as discussed below.

All three of the recent papers describe the interface of Mre11 along the Rad50 coiled coils [4-6], which is essentially the same among the MR complexes of the three organisms investigated (two different Archaea and a Bacterium). The Rad50 binding domain of Mre11 is a neat helix loop helix (HLH) bundle that binds across, almost orthogonal to, the Rad50 coiled coil. The details of this interface are discussed particularly well in Williams et al. [6]. Moving just a bit away from the interface amino acids along Mre11 things get very interesting. This C-terminal HLH Rad50 binding domain is connected to the rest of Mre11 by a flexible linker. Two such tethered Rad50 binding domains project from each end of an oblong Mre11 dimer (Figure 1C). The overall structure of an MR complex (without ATP bound), determined by SAXS and X-ray crystallography by Lammens et al., is an elongate crescent shape with an Mre11 dimer between the two Rad50 nucleotide-binding domains, thereby keeping them apart [4]. This flexible connection between Mre11 and Rad50, in principle, allows multiple relative orientations and arrangements of the Rad50 (ATPase) and Mre11 (nuclease) globular domains. The two Rad50 ATPase domains, tethered to each end of an Mre11 dimer, are notably nowhere near each other in the complex (Figure 1C). The bipartite Rad50 ATP binding sites, half on each globular head, are separated by over 100 Å. ATP binding must therefore be accompanied by large conformational changes to bring the globular domains together. Also an additional interface, discovered in the open structure, between Mre11 and Rad50 would inhibit ATP binding. So an Mre11 dimer can grab two Rad50s at the base of their coiled coils, potentially holding the Rad50 nucleotide binding domains far apart.

All this changes, in some expected and some very unexpected ways, when the Rad50 globular domains are engaged with ATP bound. The expected result is that in the MR complex, two ATP molecules bind at the interface of two Rad50 nucleotide-binding domains, similar to the structure obtained from isolated Rad50 catalytic domain [15]. This results in compaction of the catalytic head detected in the solution structure derived from SAXS data [4]. The surprising details of this arrangement are revealed in by X-ray crystallography of Lim et al. [5]. They present the most complete structure to date of full length Mre11 in the complex with an ATP bound Rad50 (having shortened coiled coils as also used in the other reports). Here the Rad50 nucleotide-binding domains are engaged with two bound ATP_S molecules. This structure could also match the SAXS envelope determined by Lammens et al. but differs in an important aspect from their presented model and from common cartoons of the MR complex prevalent in the literature. The Mre11 dimer is not sitting between and bridging the Rad50 coiled coils. Instead, Lim et al. reveal that the Mre11 HLH domains are bound to the “outside” of the Rad50 coiled coils, the Mre11 flexible linker lies along the outside of the coiled coils. The flexible linker connects to the Mre11 nuclease domain dimer that associates with the Rad50 nucleotide-binding domains on the surface opposite to the coiled coils, not between them. So the Mre11 globular domain is “outside” of the coiled coils, not “inside” (Figure 1D). These relative positions of Mre11 and Rad50 preclude the suggestion from some MR cartoons that the coiled coils form part of a protein ring that closes when Rad50 binds ATP (Figure 1B). The crystal structure has another surprise, in this arrangement the DNA binding and nuclease sites of Mre11 are located at the interface with Rad50 and are not accessible to solution. Access to the Mre11

active site, in the ATP bound form of MR, is limited and may only accommodate single-stranded DNA (Figure 1D and 2B).

These structural insights have interesting implications for MR function. Biochemical activities of the parts can now be seen in the context of a cooperating whole. The many roles of MR in DNA break recognition and processing exploit a variety of DNA binding options. The structure of the MR complex nicely explains some aspects of DNA binding and modulation by nucleotide but also makes this seemingly simple activity quite complicated. Mre11 DNA binding is a given prerequisite for its nuclease activity [16]. Crystal structures of Mre11 orthologs bound to DNA have identified the DNA binding and nuclease active sites but also revealed variation and complexity [8, 17]. Different DNA structures bound in different ways to two sites symmetrically arranged on the dimer. This lead to the suggestion that blunt DNAs could be aligned when bound and that forked DNA would be positioned for nucleolytic processing of one strand [8]. The existence of two distinct DNA bound forms suggests others are possible. Both Lammens et al. and Lim et al. report variation in the Mre11 dimer in the MR complex that could create different DNA interactions and control nuclease activity [4, 5]. In fact overlay of different Mre11 structures, Mre11 alone bound to DNA, MR complex in the “open” form (Figure 1C) and MR in the ATP bound “closed” form (Figure 1D), shows that the relative position of the nuclease active sites across the dimer interface varies significantly (Figure 3). If we assume that these changes between the Mre11 dimers do not reflect species differences but ligand-induced conformational changes, this means that the rotation of one Mre11 nuclease domain with respect to the other results in a displacement of the catalytic histidine as large as 12.2 Å, comparing the position between the open MR complex and DNA bound Mre11. But binding of ATP to Rad50, in the absence of DNA, also changes Mre11 such that the histidine is displaced over 8.4 Å. This indicates that the nucleotide-bound state of Rad50 could control nuclease activity directly by inducing conformational changes in the Mre11 dimer. Given the variation seen among the bacterial and archaeal forms so far crystallized it will be important to determine the structure of human MRE11 active site to understand its, perhaps different, regulation.

Rad50 alone can also bind DNA in some circumstances. Though available evidence suggests that Rad50 in cells is only present in the MR complex, purified Rad50 or parts of the protein bind DNA when engaged as an ATP-bound dimer [15, 16, 18, 19]. Crystal structures of Rad50 orthologs and related proteins predict a site for DNA binding based on the location of positive charged patches in a surface groove [15, 20-22]. The importance of different DNA binding sites and possible preference for specific DNA structures will ultimately depend on the relative orientation of the DNA binding sites of Mre11 and Rad50 within the functional MR complex. None of the current MR structures have been determined with DNA. However, they reveal important constraints to DNA binding and new possibilities. The most obvious new information is a dramatic change in accessibility of the DNA binding face of Mre11 in the ATP-bound MR complex. In the absence of bound ATP, MR forms an “open complex” with the Rad50 nucleotide binding domains far apart and an accessible DNA binding face of Mre11. There is nothing preventing access of DNA, even DNA damaged at its end by bulky additions would be able to bind to Mre11. However with ATP bound to Rad50, the nucleotide-binding domains are engaged having apparently rotated and moved over the Mre11 DNA binding face, toward each other resulting in a “closed complex” (Figure 1D and 2B). ATP-bound Rad50 now occludes the Mre11 DNA binding face. Access would only be possible for single-stranded DNA. Considering the observed changes in Mre11 dimerization (Figure 3), nuclease activity towards this single-stranded DNA might be different in the open and closed complexes. These characteristics are consistent with the effects of ATP on nuclease activity of the MR complex [23, 24]. But here, and in some other assays, adding ATP to MR stimulates DNA binding. Lim et al. remark that ATP engaged nucleotide-binding domains can create DNA binding sites on proteins similar to Rad50.

They provide some mutational analysis to suggest that nucleotide-engaged Rad50 dimers in the MR complex also create a DNA binding cleft, on the surface between the coiled coils, away from the Mre11 interface (Figure 2B). Taken together, this suggests that sandwiching two ATPs between Rad50s in the MR complex restricts DNA binding to Mre11 and creates a DNA binding surface on Rad50 (Figure 2).

Nucleotide-induced engagement of Rad50 in the MR complex is, however, not required for double-stranded DNA binding as significant association occurs in the absence of added nucleotide [10, 12, 25, 26]. In fact DNA induces a dramatic conformational change in the MR complex, indicating that DNA binding also stabilizes an altered, possibly closed or engaged, form of the Rad50 dimer [12]. Cross-linking data by Lammens et al. suggest that ATP and DNA cooperate to stabilize the “closed” Rad50s engaged form of the complex. However, cross-linking did not occur to completion, even in the presence of ATP and DNA indicating that this Rad50 interface is dynamic or has a variable arrangement. The simultaneous occlusion of one binding site and creation of another is an obvious complication to a simple two body, DNA-protein, binding reaction. The dramatically different arrangements of Rad50 in these two MR complex structures (comparing the “open” -ATP form determined by Lammens et al. [4] to the “closed” +ATP forms determined by Lim et al. [5]) suggest that other arrangements are also possible. For starters, to get from one conformation to the other Rad50 nucleotide-binding domains have to move on the order of 50 angstroms each. Positions in between must exist and transiently stable forms may contribute additional biochemical function or specificity. The complexes crystallized have ATP bound at each of the two ATP binding sites. This symmetry at the ATP binding sites need not be the only relevant form. An intriguing comparison can be made to a structurally similar DNA repair protein, the DNA mismatch recognition factor MutS. MutS can also bind nucleotides at an interface between monomers closely related to the Rad50 nucleotide-binding domain [27, 28]. Interestingly, MutS in the mismatch recognition state has ATPase sites that are asymmetric with respect to the nucleotide form bound. This asymmetry is crucial in relaying long range allosteric communications towards multiple DNA binding domains to enhance specificity for DNA mismatches and to correctly initiate repair [29-31]. Could MR be similar? It certainly seems possible, and could contribute an important element of control to interactions with DNA.

There is one important interface in the MR complex that was not considered in these new structures, the Rad50 coiled-coil apex. All of the current Rad50 proteins analyzed have truncated or shortened coiled coils, longer than those studied before and revealing new dynamics possibilities but still far from the 30-50 nm long complete structures. Flexibility of the coiled coils makes them unlikely to be amenable to crystallography [12, 32, 33]. These long floppy bits with sticky ends however may constrain the movements and orientation of the globular nucleotide-binding domain at its other end. For instance coiled-coil apexes bound together within one complex will almost certainly place constraints on the position and movement of the nucleotide-binding domains. Indeed in the “open” structure the coiled coils within the complex are projecting in almost opposite directions. Would the apexes be able to contact each other in such an arrangement? Would the nucleotide-binding domains be able to assume this orientation if the coiled-coil apexes engage? The long flexible bits can probably accommodate these variations but at what cost we cannot yet determine. Comparing the coiled-coil position in 3-D in the “open” conformation with Rad50s apart and the “closed” conformation with Rad50s engaged, shows that one of the coiled coils has rotated about 180 degrees in this transition. Tantalizing details of coiled-coil flexibility and arrangement are however now clear. One strand of the coiled coil is attached at its base by a flexible hinge, thus allowing some rotation of even a relatively stiff structure. In addition, the interface of Mre11 helices packing across the Rad50 coiled coils can serve to keep the Rad50 coils in register or fix their association at this point. All in all there is a lot of

movement suggested from the very static crystal structures. How this movement occurs, how it is controlled or constrained as well as the importance of intermediate structures remain to be discovered. The pleasure in science of answering some questions well is making new questions ripe to explore.

These three recent articles have a wealth of fascinating structural details on the working of the MR molecular machine. We have only been able to consider a few aspects here. Have a look at the originals for their vision of protein mechanochemistry and the fine working of this molecular machine. A good read of the data and discussion in all three of these papers is highly recommended.

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Figure 1.

Schematic representation of the MR complex. Rad50 molecules are depicted in orange with a globular ATPase domain and part of the coiled coil. ATP is represented by a blue pentagon. The ATP binding domains of Rad50 are depicted in dark orange. Mre11 is shown in green. Its globular dimerization domain is connected through a linker to its HLH domain, which interacts with the Rad50 coiled coils. The Mre11 DNA binding domain is depicted in dark green. All complexes are oriented with respect to a fixed Mre11 orientation. **(A, B)** In previous models (indicated by “Old”) Mre11 was imagined to be located between the Rad50 coiled coils. The ATPase binding pockets of the Rad50 monomers are pointing towards each other and their dimerization upon ATP binding can occur with minimal relative movement. **(C, D)** In the “New” model, in the absence of ATP, the Mre11 globular domains including its DNA binding and nuclease active sites are located between the Rad50 ATPase domains, whose ATP binding pockets are facing away from each other in an “open” complex. Upon ATP binding a dramatic rearrangement within the MR complex is required to dimerize the ATPase domains resulting in a “closed” complex.

Figure 2.

Interaction of the MR complex with DNA. Mre11, Rad50 and ATP are depicted as in Figure 1. DNA strands are shown in red. **(A)** In the “open” complex arrangement, in the absence of ATP, the DNA binding site of Mre11 can accommodate double-stranded DNA, while Rad50 is unlikely to bind DNA. **(B)** In the “closed” complex conformation upon ATP binding, Rad50 can engage double-stranded DNA, while the Mre11 DNA binding site is more constrained and now possibly limited to binding single-stranded DNA.

Figure 3.

Nucleotide binding to Rad50 modulates the Mre11 dimer interface and relative orientation of nuclease active sites. Overlay of the Mre11 globular domains (including the nuclease and capping domains) as observed in different crystal structures [4,5,8]. The structures are superimposed by fixing the position of one dimer-interface helix from one subunit. Mre11 bound to DNA (3DSD.pdb) is depicted in green, Mre11 in the MR complex with open Rad50 (3QG5.pdb) is depicted in blue and Mre11 in the MR complex with closed Rad50 (3AVO.pdb) is depicted in yellow. The nuclease active site histidine involved in transition state stabilization is indicated with a colored sphere. The displacement of this histidine between the different structures is indicated. The three structures clearly also differ with respect to a rotation of one subunit relative to the other.