DNA Repair by the MRN Complex: Break It to Make It

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Genomic instability in ataxia telangiectasia-like disorder and Nijmegen breakage syndrome is due to disruption of the Mre11-Rad50-Nbs1 complex. Buis et al. (2008) and Williams et al. (2008) now reveal the importance of the nuclease activity of Mre11 for mammalian genome maintenance and present a molecular view of its active site.

Human syndromes that predispose individuals to developing cancer have furthered our understanding of DNA damage response and repair pathways. Examples include Xeroderma pigmentosum, a syndrome instrumental in piecing together the multistep pathway of nucleotide excision repair, and certain forms of hereditary breast cancer that highlight the important role of homologous recombination in ensuring genome stability. Similarly, the importance of the Mre11-Rad50-Nbs1 (MRN) complex in the cellular response to DNA double-strand breaks (DSBs) was initially revealed by ataxia telangiectasia-like disorder and Nijmegen breakage syndrome. The MRN complex is an assembly of two Mre11 subunits, two Rad50 subunits, and Nbs1 protein (Figure 1). This remarkably versatile protein assembly merges a number of activities that are essential for genome maintenance and duplication. It has an arsenal of biochemical talents: ATP hydrolysis, DNA binding by multiple subunits, incision of the DNA phosphodiester backbone through its single-stranded DNA (ssDNA) endonuclease and 3′ to 5′ exonuclease activities, and tethering of DNA molecules through interactions between MRN complexes. In addition to these activities that affect its behavior on DNA, the complex is involved in setting up a cell cycle checkpoint response in reaction to DNA damage, particularly by promoting the activation of the ATM kinase. Not surprisingly, it is a formidable challenge to sort out the relevant mechanistic roles of individual subunits and domains of the MRN complex.

Two new papers about the MRN complex in this issue provide some clear answers, reveal new possibilities, and bring up new questions (Buis et al., 2008; Williams et al., 2008). The two achievements presented here are in different experimental realms—in one case, mouse and tissue culture tests of in vivo function (Buis et al., 2008), and in the other, X-ray crystallography and structural analysis, whose biological implications were then tested by mutational analyses in fission yeast (Williams et al., 2008). From these diverse approaches, it is clear that the nuclease activity of Mre11 is essential. In addition, we now see that Mre11 organizes bound DNA in different ways to promote genome stability.

The early embryonic lethality of *Mre11* deletion in mice shows that it functions in an essential process, likely one underpinning DNA replication. Buis et al. (2008) gambled that mice expressing nuclease-deficient Mre11 would be viable. Although they lost that bet, they were not deterred. By applying virtuoso cell engineering, Buis et al. isolated cells that could be triggered to lose one *Mre11* allele and were then left with either no Mre11 or only the nuclease-deficient version. Cells with only nuclease-deficient

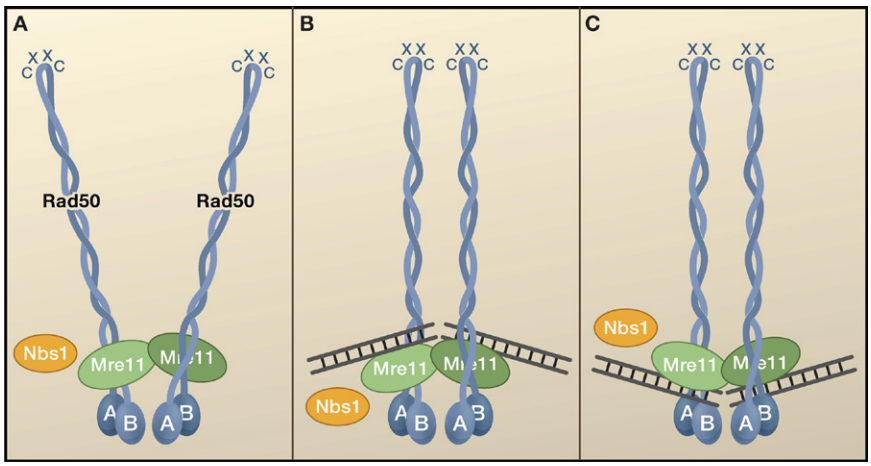

Figure 1. Architecture of the MRN Complex and Its Interaction with DNA
(A) The MRN complex consists of a globular domain that contains the Walker A and B ATPase domains of both Rad50 molecules and the Mre11 dimer. The Nbs1 protein is part of the globular domain, but its stoichiometry is uncertain. Emanating from the globular domain are the 50 nm flexible coiled coils of Rad50, which carry the intercomplex interaction zinc hook domain (CxxC) at their apex.
(B and C) DNA binding induces a large conformational change resulting in a parallel orientation of the coiled-coil arms. The crystal structure of Williams et al. (2008) provides detailed insight into the interaction of Mre11 with DNA and sets the stage for solving the question as to where the DNA is bound in the context of the complete complex. Two possibilities are indicated.

Mre11 suffer growth defects and chromosomal abnormalities and are sensitive to DNA-damaging agents. Thus, the function of MRN in genome stability requires the nuclease activity of Mre11.

Other complexities await resolution. In particular, it is unclear which steps of DSB repair and which problems in replication require the nuclease activities associated with MRN. Indeed, DSB repair may require a variety of nuclease activities depending on the nature of the ends to be repaired (Wyman and Kanaar, 2006). DSB repair by homologous recombination requires exposure of ssDNA with a 3′ overhang. This is most easily created by a 5′ to 3′ exonuclease. Because the described Mre11 exonuclease activity has the opposite polarity, MRN is unlikely to fulfill this role directly. However, many DNA ends are not suitable exonuclease substrates, either because they are blocked by a covalently bound protein or because of their chemical structure. These "dirty" ends first need processing by an endonuclease. Interestingly, in fission yeast, an *Mre11* mutant lacking only exonuclease activity was only slightly sensitive to DNA-damaging agents, whereas the mutant lacking both exo- and endonuclease activities were very sensitive (Williams et al., 2008). Thus, a role in cleaning "dirty" DNA ends may be MRN's essential function. Ends not resected in the absence of Mre11 nuclease activity could reflect the frequency of blocked ends that remain inaccessible to further processing.

In contrast to its importance in DNA processing during repair, the Mre11 nuclease activity is not required for ATM activation, which is an essential step in DNA damage signaling. Separating the ATM activation and nuclease activities of Mre11 is intriguing in light of other recently published information. In frog egg extracts, ATM can be activated by ssDNA oligonucleotides that bind to MRN. Moreover, the production of these ssDNA oligonucleotides depends on MRN (Jazayeri et al., 2008). The data of Buis et al. indicates that if ATM activation in mouse cells also depends on ssDNA oligonucleotides, these ssDNAs do not have to be produced by Mre11 itself. Perhaps an associated factor, such as CtIP (or Sae2 and Ctp1 in *S. cerevisiae* and *S. pombe* cells) (Takeda et al., 2007) could promote the formation of activating oligonucleotides in an MRN-dependent manner. Roles for these partner proteins in this process cannot be ruled out, given that they still interact with the nuclease-deficient MRN complexes.

The binding of the MRN complex to DNA is an essential prerequisite for its function in genome maintenance. This potentially simple interaction influences function in complex ways. Williams et al. (2008) have made great progress toward understanding where DNA binds and what happens after DNA binding by solving the structure of an archaeal Mre11 homolog bound to two different DNAs. The two DNAs were designed to resemble intermediates in DSB repair (a synaptic complex of two 7 bp double-stranded DNAs with 2 nucleotide 3′ overhangs) and DNA replication (a branched DNA with a 7 bp stem and non-complementary 4 nucleotide extensions). In both structures, double-stranded DNA binds across the Mre11 dimer interface, and they provide an example of reciprocal structural effects; protein dimerization stabilizes DNA binding, and DNA binding stabilizes the protein dimer. As expected, changes at the dimer interface prevent or diminish dimerization and DNA binding. However, nuclease activity is not affected, indicating that dimerization, and by implication stable DNA binding, is not critical for the DNA-processing activity of Mre11. Tests for in vivo function of the Mre11 mutants in fission yeast establish a correlation, albeit imperfect, between dimerization and sensitivity to DNA-damaging agents. Williams et al. suggest that interaction with the other components in the MRN complex might promote a specific dimeric arrangement for Mre11 and that a precise self-association of Mre11, not simply a dimer, is needed for proper assembly of the MRN complex.

That DNA binding will control MRN architecture and function is known, but new wrinkles and new possibilities are now revealed. Now we know that specific changes in Mre11 structure depend on what sort of DNA is bound. Both the DNA and protein are arranged differently in the two structures determined by Williams et al., providing evidence that Mre11 is a multivalent DNA-binding platform with diverse DNA scaffolding functions for DSB repair and genome maintenance. The synaptic complex has the two DNAs symmetrically bound with their open ends nearly aligned at the center of the complex. In contrast, the DNA with a branched or frayed end binds asymmetrically across the dimer. Given that the endonuclease activity of MRN may also help resolve cruciform or hairpin structures formed during replication of palindromic sequences (Lobachev et al., 2002), one intriguing alternative possibility for the DNA in the synaptic complex is to view it as a cruciform substrate for nuclease activity. Furthermore, how the DNA binding activity of Rad50 contributes to or modifies DNA scaffolding in the MRN complex will depend on the relative positions of the Mre11 and Rad50 DNA-binding sites, which are still unknown. The conformation of Mre11 is also different between the two structures. Branched DNA binds a subdomain of Mre11, known as the nuclease capping domain, which changes position to make contact with the ssDNA overhang and occludes the other DNA-binding site in the dimer. In the MRN complex, an Mre11 dimer contacts two Rad50 molecules near the globular end of this very elongated coiled-coil protein (Figure 1) (Wyman and Kanaar, 2006). This architecture allows relatively small changes in the orientation of Rad50 at one end to produce large changes in the arrangement of the zinc hooks, which mediate interactions between MRN complexes (de Jager et al., 2001; Hopfner et al., 2002; Moreno-Herrero et al., 2005; Wiltzius et al., 2005). MRN also interacts with and activates ATM only after it is engaged with damaged DNA, in this case DSBs. The conformation changes in MRN induced by DNA binding (and which actives ATM) should require DNA ends, but the exact structure responsible has not yet been determined.

The structures determined by Williams et al. provide a starting point from which the aspects of the Mre11-DNA interactions that are important for ATM activation can be determined. In addition, the architectural changes in the whole MRN complex after DNA binding and the structure of Rad50-Mre11 interface are important missing pieces of the puzzle. The MRN complex remains a fascinating molecular machine capable of both subtle and dramatic conformational flexibility in performing its multiple functions in preserving genome stability.

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Spectraplakins: The Cytoskeleton's Swiss Army Knife

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Spectraplakins are multifunctional proteins that interact with all three types of cytoskeletal filaments. In this issue, Wu et al. (2008) demonstrate a new actin-dependent ATPase activity for the spectraplakin ACF7 that allows it to guide microtubule ends along actin stress fibers to focal adhesions, promoting disassembly of adhesion contacts and hence cell movement.

The shape and movement of cells is produced by a large number of proteins that collectively form the cytoskeleton. The main structural elements are three protein filaments: actin filaments, microtubules, and intermediate filaments. These filaments work in concert with a large number of accessory proteins that contribute in a variety of ways to regulate filament assembly and turnover, to alter the configuration or arrangement of filaments by bundling or crosslinking, to link the cytoskeleton to other structures in the cell such as membranes and junctions, and to transport cargo along the filaments. In general, accessory proteins only work with a single type of cytoskeletal filament. Although these three cytoskeleton systems are able to operate independently, it is clear that communication between them is required to coordinate cellular activities. However, relatively few proteins have been identified that work with more than one filament type. The study by Wu et al. (2008) in this issue of *Cell* now uncovers how ACF7/MACF1, a spectraplakin protein, plays an essential role in microtubule-mediated focal adhesion turnover, a process that uses the coordination of both actin filaments and microtubules.

Spectraplakins are giant, multifunctional cytoskeletal proteins that are grand masters of coordination between different types of cytoskeletal filaments—they are able to bind to all three types of cytoskeletal filaments. As their name reflects, spectraplakins contain domains found in two cytoskeletal families—the spectrins and plakins (Röper et al., 2002, Sonnenberg and Liem, 2007). Spectraplakins are encoded by genes that produce diverse polypeptides by using alternative transcription start sites and alternative splicing. Different spectraplakins contain combinations of a variety of protein domains that bind to different filament types and filament-associated proteins, investing the proteins with a diverse range of functions in cytoskeleton regulation. As a result, spectraplakins are very large proteins—the largest isoform, in the fly *Drosophila melanogaster*, is 8842 amino acids with an estimated length of approximately 400 nm. Intriguingly, despite the separation of the many protein domains over the long length of the protein, activities of the domains are coordinated. For example, activity of the N-terminal actin-binding domain of ACF7/MACF1 is affected by the C-terminal microtubule-binding domain (Karakesisoglou et al., 2000).

Formation and regulation of the cellular adhesions that bind to the extracellular matrix (focal adhesions) and enable movement makes use of coordination of actin filaments and microtubules (Rodriguez et al., 2003). The depolymerization of microtubules leads to larger, more stable focal adhesions (Bershadsky et al., 1996, Enomoto, 1996), and single microtubules appear to seek out focal