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Envisioning the dynamics and flexibility of Mre11-Rad50-Nbs1 complex to decipher its roles in DNA replication and repair

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ABSTRACT

The Mre11-Rad50-Nbs1 (MRN) complex is a dynamic macromolecular machine that acts in the first steps of DNA double strand break repair, and each of its components has intrinsic dynamics and flexibility properties that are directly linked with their functions. As a result, deciphering the functional structural biology of the MRN complex is driving novel and integrated technologies to define the dynamic structural biology of protein machinery interacting with DNA. Rad50 promotes dramatic long-range allostery through its coiled-coil and zinc-hook domains. Its ATPase activity drives dynamic transitions between monomeric and dimeric forms that can be modulated with mutants modifying the ATPase rate to control end joining versus resection activities. The biological functions of Mre11's dual endo- and exonuclease activities in repair pathway choice were enigmatic until recently, when they were unveiled by the development of specific nuclease inhibitors. Mre11 dimer flexibility, which may be regulated in cells to control MRN function, suggests new inhibitor design strategies for cancer intervention. Nbs1 has FHA and BRCT domains to bind multiple interaction partners that further regulate MRN. One of them, CtIP, modulates the Mre11 excision activity for homologous recombination repair. Overall, these combined properties suggest novel therapeutic strategies. Furthermore, they collectively help to explain how MRN regulates DNA repair pathway choice with implications for improving the design and analysis of cancer clinical trials that employ DNA damaging agents or target the DNA damage response.

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Abbreviations: AFM, atomic force microscopy; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; BRCT, BRCA1 C-terminus domain; DSB, DNA double strand break; DSBR, double strand break repair; EM, electron microscopy; FHA, fork head-associated domain; HRR, homologous recombination repair; MRN, Mre11-Rad50-Nbs1; NHEJ, non-homologous end-joining; SAXS, small-angle X-ray scattering.

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1. Introduction

DNA double strand break repair (DSBR) is a fascinating process that involves a plethora of proteins in a complex choreography of dynamic events in which the Mre11-Rad50-Nbs1 complex (MRN) plays critical roles. Double strand breaks (DSB) are created by exposure to ionizing radiation or chemicals, and by endogenous cellular events, including DNA replication and V(D)J recombination. For more than 40 years, scientists have strived to determine how DSBR works. By combining results piece by piece and by developing new methods, they are revealing the integrated mechanisms of this crucial phenomenon. We know there are many other interacting components for homologous recombination such as those that promote homologous DNA pairing in meiotic recombination (Zhao et al., 2014); however here we focus on the MRN complex, which acts in the first stages of DSBR. MRN has emerged as one of the main protein complexes at multiple stages in the process, impacting pathways for homologous recombination repair (HRR) and both classical and alternative non-homologous end-joining (NHEJ) (Hammel et al., 2011; Hopfner et al., 2002b; Shin et al., 2004; G. J. Williams et al., 2014, 2010; Zha et al., 2009). It detects DSBs, tethers the ends of the broken chromosomes, activates DNA damage response pathways and nucleolytically processes the DNA ends. A fascinating characteristic of MRN is that these diverse functions are possible due to its flexibility and dynamic properties that are finally being unveiled.

In 2002 an article in Nature revealed multiple conformations of the MR complex by electron microscopy (EM) (Hopfner et al., 2002a) and in 2005 a second Nature article presented a video captured by atomic force microscopy (AFM) of the MRN complex interacting with DNA (Moreno-Herrero et al., 2005; Williams and Tainer, 2005), allowing the implications of the dynamics and flexibility to be examined in the context of Mre11 and Rad50 crystal structures. These papers opened the door for the scientific community to visualize MRN in its dynamic splendor. In the solution AFM video, the long coiled-coils of Rad50 are moving between two different states upon DNA binding. In their DNA-bound form, the two 500 Å-long coiled-coils of an MRN dimer seem to be straight and rigid. When the complex leaves the DNA, the coiled-coils adopt a relaxed, bent position, similar to what is seen by EM (de Jager et al., 2001). These observations revealed that the complex is highly dynamic over a large spatial area.

In the last 10 years, tremendous efforts have been done to better understand the dynamic nature of MRN, its structure and its cellular functions. Indeed, MRN has diverse and critical functions: DSB detection, DNA end tethering, ATM activation, DNA end processing and replication fork protection or resection (reviewed in (Paull and Deshpande, 2014; Schiller et al., 2014; Symington, 2014; Williams et al., 2010)). The emerging picture is that MRN uses flexible and dynamic structures to integrate its ATPase and nuclease enzymatic activities with protein–protein and protein-DNA interactions to control biological outcomes at a DSB. To study dynamic Mre11-Rad50 complexes, we and other researchers in the field have used the power of optimal model systems including hyperthermophiles (reviewed in Shin et al., 2014). Furthermore, multiple and combined technologies have been employed, which prompted the design of our synchrotron beamline to combine crystallography and X-ray scattering (Classen et al., 2013). Indeed, the MRN complex has provided problem-driven development of multiple biophysical metrics and methods, which is noted along with the biological results.

In this review we cover the most recent conceptual advances about MRN in the context of foundational results. We first explore the current models on its roles in different pathways. Then we examine how the flexibility and dynamics of MRN's components and partners are linked with their function. Rad50 is capable of long-range allostery and its activities are controlled by its ATPase functions, which impacts the nucleolytic activities of Mre11. Mre11 sculpts dsDNA ends and ssDNA differently and inhibitors were therefore able to separately block either endonuclease or exonuclease activities by blocking Mre11-mediated DNA conformational changes. Also, Nbs1 and CtIP are responsible for important dynamic protein–protein interactions with the Nbs1 C-terminus helping to connect MRN to ATM activation and signaling. Finally, there is growing evidence that MRN can be targeted for cancer therapeutics and knowing its dynamics and molecular mechanism is thus crucial for developing new therapeutic strategies. Overall, we aim to help the reader appreciate the dynamic and structural aspects of MRN functions. This knowledge includes growing evidence that structural dynamics can regulate repair pathway selectivity and control biological outcomes. This appreciation of the MRN complex has general biological implications, especially for the Rad50 ABC ATPase superfamily (Hopfner and Tainer, 2003), for structure-specific nucleases (Tsutakawa et al., 2014), and for phosphorylated protein binding by FHA and BRCT domains (Reinhardt and Yaffe, 2013).

2. Functional architecture of the MRN complex

The structure of the whole MRN complex or of its individual components has been examined by multiple techniques: X-ray crystallography, small-angle X-ray scattering (SAXS), analytical ultracentrifugation, inductively coupled plasma mass spectrometry (ICP-MS), dynamic light scattering (DLS), atomic force microscopy (AFM) and electron microscopy (EM). Initially, proteins from archaea and bacteria were used, since they are more stable in their purified form, but recently eukaryotic Mre11 and Nbs1 have also been studied, strengthening the previous structural findings. Before going into further details with the MRN complex, it is useful to consider a structural overview of each of its components (Fig. 1).

Rad50 is a member of the ABC ATPase superfamily and has provided insights of broad interest across the superfamily (Hopfner and Tainer, 2003). It is the largest protein of the MRN complex, with 1312 amino acids in the human enzyme (Fig. 3). Both the N- and C-terminal ends of the protein form the “head” ABC-ATPase domain, which contains the Walker A/B motifs and signature motif characteristic of this family of ATPases (Moncalian et al., 2004). It has both ATPase and adenylate kinase activities *in vitro* (Bhaskara et al., 2007; Paull and Gellert, 1999), but its major biological activity seems to be as an ATPase. Between the two termini of the protein, anti-parallel coiled-coil domains extend the length of the protein up to ~500 Å (Hopfner et al., 2000). Opposite to the head and at the tip of the coiled-coil, a CXXC motif creates a zinc-hook that facilitates dimerization of Rad50 (Cahill and Carney, 2007; Hopfner et al., 2002a). Indeed, two zinc-hooks (a total of four cysteines) can be attached together by coordinating a Zn^{2+} atom. But this is

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