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A Nanomachine for Making Ends Meet: MRN Is a Flexing Scaffold for the Repair of DNA Double-Strand Breaks

In a recent study (Moreno-Herrero et al., 2005), atomic force microscopy (AFM) imaging of the human Mre11/Rad50/Nbs1 (MRN) complex engaging substrate DNA revealed large-scale, DNA binding-induced propagation of conformational change to the distal ends of the Rad50 coiled coils and erection of a 1000 Å scaffold to productively bridge DNA ends.

The phylogenetically conserved Mre11/Rad50 complex (Mre11/Rad50/Nbs1 or MRN in higher eukaryotes) plays essential roles in the biologically critical processes of telomere maintenance and of DNA double-strand break repair by homologous recombination repair and nonhomologous end joining. Biological, biochemical, and structural data furthermore argue that MRN plays multifaceted roles in the following diverse activities: MRN acts as a DNA-damage sensor, as an enzymatic effecter in DNA damage repair, and as a transducer of critical damage-response signals to the cell cycle checkpoint apparatus (reviewed in Stracker et al., 2004). The search for a unifying basis for these roles is generating growing evidence that the MRN complex acts in part as a multipurpose tether that bridges severed DNA ends (de Jager et al., 2001; Chen et al., 2001; Hopfner et al., 2002).

The emerging visualization of the MRN molecular machinery derives from the detailed crystallographic structures of components of the Mre11-Rad50 (MR) core plus the overall anatomy from both electron- and atomic force-microscopic imaging of the intact MR(N) homologs (de Jager et al., 2001; Chen et al., 2001; Hopfner et al., 2000, 2001, 2002). The MR core complex exists as a heterotetrameric assembly (M_2R_2) whose morphology is divided into distinct head, coil, and hook domain regions (Figure 1A). Each Rad50 polypeptide assembles with the intramolecular collapse of an expansive antiparallel coiled coil, which conspicuously emanates from the head domain and measures ~500 Å long for eukaryotic Rad50 homologs. The extreme Rad50 N- and C-terminal ends coalesce to form a bipartite ATP binding cassette (ABC)-ATPase, and the central region of Rad50 abruptly kinks and reverses directionality of each coil, thereby capping the distal end of the coiled coils with a CXXC Zn-hook motif. The integrity of the hook is required for Zn(II)-mediated Rad50-Rad50 homodimeric interactions and MRN function in vivo (Wiltzius et al., 2005). The four-lobed, globular DNA binding head is likely comprised of two Rad50-ATPase domains and dimeric Mre11 that is physically bound to the base of the Rad50 coiled coils and to Nbs1 (Hopfner et al., 2001). Microscopic observations underscore the flexible nature of the coils, and widely varying models for MRN-mediated DNA bridging involve extended or folded coiled coils in combination with intercomplex, hook-hook-mediated oligomerization. All of these models hint that structural changes affecting directionality, dynamics, or flexibility of the Rad50 coiled coils may regulate MRN architecture and function. Now, exciting new evidence (Moreno-Herrero et al., 2005) reveals a surprisingly large-scale reorganization of MRN upon DNA binding. AFM imaging of the MRN complex caught in the act of engaging and migrating along DNA substrates extends previous ideas, supports some models and eliminates others, and posits new roles for MR(N) complex DNA-ligand-induced conformational change.

Moreno-Herrero et al. (2005) provide us with rare, time-resolved video footage of a biological nanomachine in solution to reveal dynamic motions of MR and MRN in their native, DNA bound, and nucleotide-liganded states. As both MR and fully reconstituted MRN show similar behavior, the motions derive from the MR core. Imaging of free M_2R_2 complexes confirms the coiled coils are flexible and suggests that the intramolecular Zn-hook interaction at the apex of the joined coiled coils can intermittently dissociate and rejoin. An intriguing idea developed by the authors is that formation of intracomplex M_2R_2 hook-hook interactions would inhibit long-range DNA tethering involving an octameric $(M_2R_2)_2$ by competing with and preventing intercomplex hook-hook interactions (Figure 1A). Moreover, the AFM imaging of DNA bound MR suggests an elegant solution to this dilemma that involves large-scale restructuring of the complex. DNA binding causes the Rad50 coils to become more rigid and parallel to one another, disables intracomplex hook-hook dimer formation, and increases the number of observed octameric $(M_2R_2)_2$ intercomplex forms in solution that are able to participate in long-range DNA tethering. In contrast to the M_2R_2 coils of the DNA-free form, which appear to flex independently from one another, the DNA bound M_2R_2 coils sway in unison. Furthermore, M_2R_2 imaged as it spontaneously dissociates from duplex DNA suggests the conversion is reversible and that flexibility can be restored.

The resulting view of MRN-DNA interactions includes two new steps: (1) MRN loading onto DNA via the head domain that is coupled to a concomitant unhooking and extension of the coiled coils, and (2) consequently productive intercomplex tethering between extended M_2R_2 hooks to maintain opposing DNA strands in close nuclear proximity, thereby erecting a scaffold for subsequent DNA-break processing. Thus, DNA-promoted extension of the coiled coils is coupled to an interface exchange of the Zn-hook domains that switch from intracomplex to intercomplex tethering (Figure 1). Another informative observation is that once loaded onto DNA, the MR complex may diffuse freely along DNA,

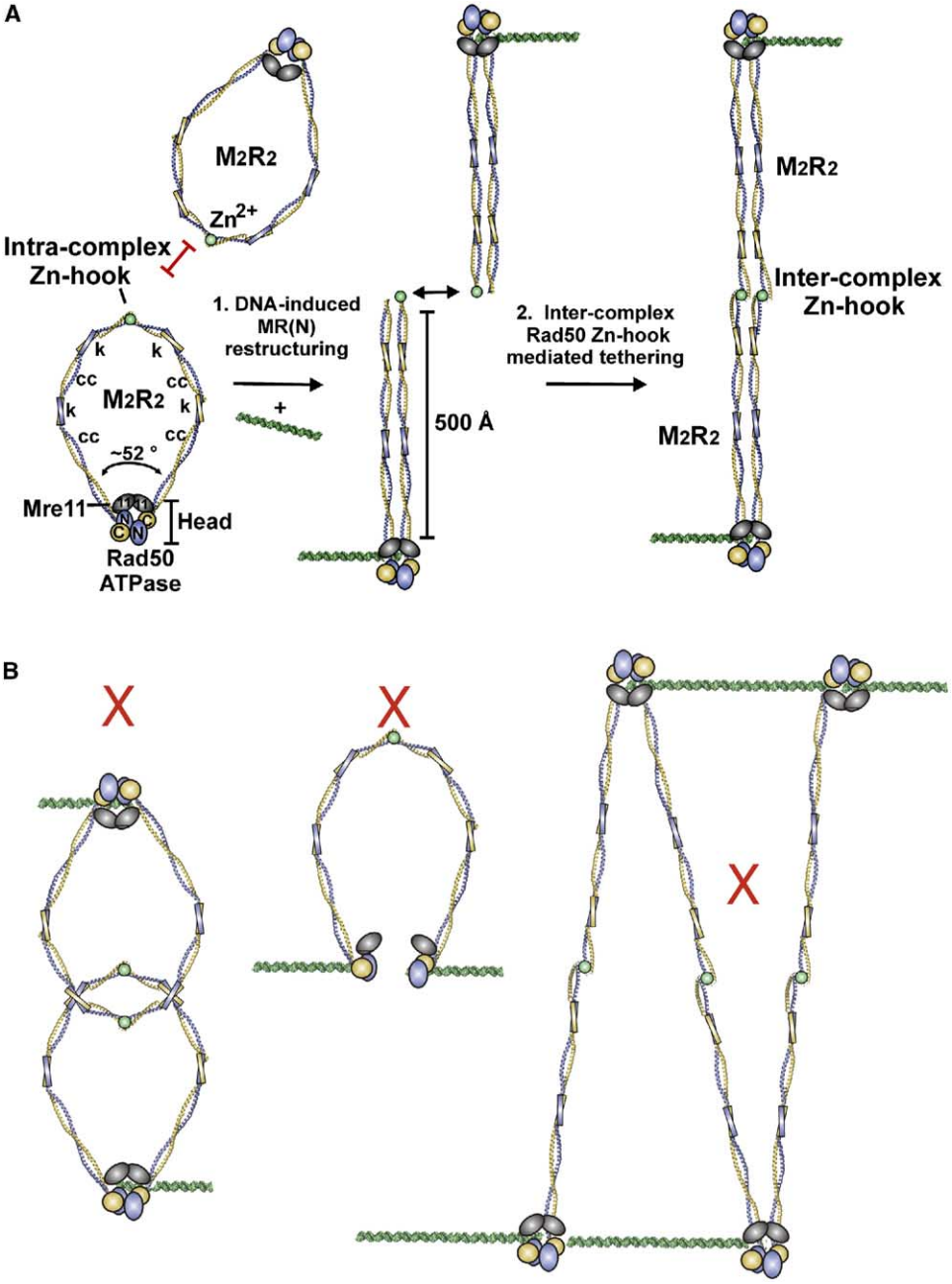

Figure 1. Dynamic Conformational Changes and DNA Tethering by the M_2R_2 Core Complex

(A) Intracomplex Zn-hook minimizes unproductive intercomplex interactions in the absence of DNA. DNA binding straightens the Rad50 coiled coils, favoring inter-complex tethering via Rad50 Zn-hooks with extended and parallel coils. "k" indicates kink regions that intersperse regions with strong coiled coil-forming potential "cc."

(B) DNA tethering architectures that are incompatible with the observed MRN DNA bound extended-coil structure.

thereby suggesting a sliding mechanism whereby MRN may scan for and clear broken DNA ends of debris. Together, these observations of a distinct DNA binding-induced conformational state preclude previously proposed models for DNA bridging by MRN that utilized curved coils and mechanisms with V shaped combinatorial coil-hook interactions (Figure 1B).

Overall, this stunningly dynamic MRN anatomy underscores the need to characterize the molecular basis for these conformational changes underlying MRN biological functions. How DNA binding mediates the striking MR conformational change, how it allows sliding to scan for and clear broken DNA ends, and how rigidity is propagated to the distal ends of the Rad50 coils

some 500 Å away are all mysteries. Direct measurements of the angular trajectories of the coils as they exit from the MR head suggest that the mean intercoil angle is sharply reduced from ~52° to being near parallel in the DNA bound state (Figure 1A). Yet, the intrinsic coil flexibility appears localized to separate, specific kink regions characterized by degeneration of the coiled coil heptad repeat sequence pattern (van Noort et al., 2003), therefore there must be some long-distance communication mechanism between the MR head and these kink regions (Figure 1). Curiously, at this low resolution, coil directionality in the DNA free and bound states appears unaltered by nucleotide binding, which is surprising because binding of ATP or AMP-PNP to the Rad50 ATPase domain closes the ATPase dimer and induces a 30° rotation of the N-terminal region relative to the C-terminal region of the bipartite Rad50 ATPase (Hopfner et al., 2000). What then are the functional roles of ATP-driven Rad50 conformational changes? Conformational switching in the MR(N) DNA binding head appears positioned to mediate localized DNA-end metabolism in conjunction with Mre11 DNA binding and nuclease activities and to transmit vital information to the cell cycle signaling apparatus via association of the MRN head with the ATM protein kinase (Lee and Paull, 2005). Thus, armed with these visualizations, we not only see answers to key questions about overall MRN architectures but also the need for high-resolution views of the MR complex with its bound DNA substrates and nucleotide cofactors to address intriguing new questions about how this multifaceted nanomachine functions in biology.

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