

Assembled Structure of the Mre11/Rad50/DNA complex from X-ray Solution Scattering and Crystallography

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The 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 non-homologous end joining. MRN acts as a DNA damage sensor, an enzymatic effector in DNA damage repair, and as a transducer of critical damage response signals to the cell-cycle checkpoint apparatus. To clarify architectural and enzymatic functions of the MR core complex and understand mechanisms of chemo-mechanical communication between Mre11 and Rad50, we describe crystal structures of Mre11 bound to DNA and Rad50. We further present analysis of conserved determinants of MR complex quaternary assembly in solution using Small angle X-ray Scattering (SAXS), and *in vivo* using structure guided mutagenesis and functional analyses.

The structure of the *P. furiosus* Mre11 bound to a small DNA hairpin highlights a Mre11 dimeric cleft that houses two opposed terminal DNA ends. This DNA bound orientation clarifies the role of Mre11 in mediating both nuclease-dependent and independent functions of the MRN complex. The DNA bridging Mre11-Mre11 interface is essential for MRN function, because charge substitution mutations that disrupt the hydrophobic homo-dimeric surface *in vivo* and *in vitro* also confer radiation and clastogen sensitivity in *S. pombe*. Remarkably, the restoration of wild type *S. pombe* phenotypes is achieved by reconstituting the Mre11 hydrophobic dimer interface with complimentary salt-bridging interactions.

The structure of a minimal Mre11-Rad50 complex reveals that a Mre11 C-terminal helical bundle tethers the Mre11 catalytic dimer to the Rad50 coiled-coils. Crystallographically observed flexibility at the Rad50 coiled-coil roots suggests nucleotide and DNA induced conformational rotations within Rad50 may communicate chemo-mechanical signals directly to Mre11, and the more distal Rad50 hook domain some 500-600 Å away, through modulation of Rad50 coiled-coil twisting.

Solution X-ray scattering suggests the (Mre11)₂(Rad50)₂ catalytic heterotetrameric DNA binding head assembles in a manner analogous MutS, revealing convergent evolution of quaternary assembly modes in the ABC-ATPase superfamily. Collectively, our SAXS and EM observations suggest M₂R₂ likely assembles as two juxtaposed half-rings, with dimeric Mre11 wrapped by the two Rad50 coiled-coils, and the Rad50 ATPase domains capping the opposite end of the ring. Together, biological, crystallographic, biochemical and SAXS data provide the basis for construction of composite working Mre11/Rad50/DNA structural models, define functional roles for MR complex assembly in DNA tethering and exonucleolytic processing *in vitro* and *in vivo*, and suggest novel roles for RAD50 ATP driven conformational controls in DNA double strand break repair.