Using time-resolved crystallography and cryo-EM to investigate human DNA repair nucleases

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Human Exonuclease I (hExo1) plays key roles in DNA repair, replication, and recombination. Defects in hExo1 are linked to various cancers. It exposes large single-stranded regions by processively excising nucleotides from 5' ends of nicks or breakage points; it also removes 5' flaps by endonucleolytic cleavage. Control of these activities is critical for proper hExo1 function. Time-resolved crystallographic studies have shown that processive cleavage involves a carefully orchestrated sequence of protein conformational changes and DNA motions that iteratively place scissile bonds into the active site for hydrolysis catalyzed by two Mg^{2+} ions, release the excised nucleotide or flap, and reset the protein for the next reaction cycle by translocating the enzyme along the DNA **(1,2)** . Here we expand our time-resolved X-ray crystallography studies to define reaction intermediates and describe a novel, inhibited state observed in several exo- and endonucleolytically cleaved (processed) and uncleaved (initiation) DNA complexes. Structures of hExoI complexes with mismatch repair proteins Human Msh2-Msh6 and MSH2-Msh3 heterodimers define critical interaction interfaces essential for repair.

References

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